NEUROTRANSMITTER RECEPTOR PLASTICITY AND ITS FUNCTIONAL CONSEQUENCES IN RELATION TO ALZHEIMER'S DISEASE

BY

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TABLE OF CONTENTS

CHAPTER II

METHODS

APPENDICES

LIST OF TABLES

PAGE

LIST OF FIGURES

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SUMMARY

Neurotransmitter receptor sites have been examined in both human postmortem tissue and a lesioned polysynaptic pathway in rat brain using quantitative ligand binding autoradiography.

Human Postmortem Studies

The putative involvement of glutamatergic mechanisms in the pathophysiology of Alzheimer's disease (A.D.) was investigated by determining the distribution and density of Na^+-dependent glutamate uptake sites and glutamate receptor subtypes; kainate, quisqualate and N-methyl-D-aspartate (NMDA), in adjacent sections of frontal, temporal and cerebellar cortex from six patients with A.D. and six age-matched controls. The number of senile plaques in each region was determined in adjacent sections to those used for receptor autoradiography. Binding of [^{3}H]-D-aspartate to Na^+-dependent uptake sites was reduced by approximately 40% throughout A.D. frontal cortex relative to controls, indicating a general loss of glutamatergic presynaptic terminals. [^{3}H]-Kainate binding was significantly increased by approximately 70% in deep layers of A.D. frontal cortex compared to controls, but unaltered in superficial laminae. Scatchard analysis of this response indicated an increase in kainate receptor numbers with no change in receptor affinity. [^{3}H]-Kainate binding and senile plaque numbers were positively correlated (r=0.914) in deep layers of A.D. frontal cortex, but unrelated in superficial laminae (r=0.089). There was a small reduction (25%) in NMDA-sensitive [^{3}H]-glutamate binding only in superficial layers of A.D. frontal cortex relative to controls, although [^{3}H]-glutamate binding in A.D. subjects was

unrelated to senile plaque numbers in these cortical layers (r=0.104). Quisqualate receptors, as assessed by [^{3}H]-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H]-AMPA) binding were unaltered in A.D. frontal cortex compared to controls. There was no significant alteration in any glutamate binding sites in A.D. temporal cortex relative to control brains, despite the presence of senile plaques in comparable numbers to those found in A.D. frontal cortex. However, in the molecular layer of cerebellar cortex from A.D. subjects, there was a significant reduction (40%) in the number of [^{3}H]-AMPA binding sites indicating a loss of quisqualate receptors in this region. [^{3}H]-D-aspartate, [^{3}H]-kainate and NMDA-sensitive [^{3}H]-glutamate binding were unaltered in either the molecular or granule cell layers of A.D. cerebellar cortex. Within the cerebellum, senile plaques were found in very small numbers in only two A.D. brains.

These results indicate anatomically-selective alterations in glutamatergic sites within the A.D. brain. The association of the kainate response in frontal cortex with the level of local neuropathology and the loss of quisqualate receptors in the cerebellum in the absence of gross neuropathological change suggests that the mechanisms of glutamatergic dysfunction in A.D. are heterogeneous with respect to anatomical locus.

Rat Visual System Studies

The rat visual system was employed as a model polysynaptic pathway in which to examine neurotransmitter receptor alterations under conditions of functional deficit. Within the visual system, glutamate is the major excitatory transmitter, although serotonin, noradrenaline, acetylcholine and GABA (γ-aminobutyric acid) are also involved in visual processing. Unilateral orbital enucleation experiments were undertaken to examine:

(1) the response of glutamate receptor subtypes under conditions of reduced glutamatergic input;

(2) receptor regulation in separate but functionally related systems, by simultaneously quantifying specific serotonergic, noradrenergic, GABAergic and cholinergic receptors post-lesion; and

(3) the relevance of alterations in neurotransmitter receptors to changes in local cerebral function, by combining the [^{14}C]-2-deoxyglucose technique for the measurement of cerebral glucose use with in vitro receptor autoradiography. Experimental parameters were measured at four time points up to 20 days after the lesion.

There were no significant alterations in glutamate receptor subtype binding up to 10 days post-enucleation. At this time point, however, [^{3}H]-AMPA binding was significantly reduced (30%) in the visually-deprived superior colliculus, but unaltered in other visually-deprived areas. In contrast, at 20 days post-lesion, a 15% increase in [^{3}H]-kainate binding was evident only in the visually-deprived superior colliculus. Both responses were due to changes in B_{max} values with no

change in receptor affinities. NMDA-sensitive [^{3}H]-glutamate binding was unaltered at any time point post-enucleation. Results are likely to be indicative of a postdenervation up-regulation of kainate receptors in the superior colliculus and a loss of presynaptic quisqualate receptors on degenerating retinal fibres.

In a separate series of experiments [^{3}H]-5-hydroxytryptamine (5HT), [^{3}H]-ketanserin, [^{3}H]-dihydroalprenolol (DHA), [^{3}H]-quinuclidinylbenzylate (QNB) and [^{3}H]-muscimol binding to $5HT_1$, $5HT_2$, β-adrenergic, muscarinic and $GABA_A$ receptors was examined post-enucleation. The functional deficit was assessed in the same animals using quantitative [^{14}C]-2-deoxyglucose autoradiography. At 1 day post-enucleation, there were no significant alterations in ligand binding although local cerebral glucose use was reduced in both primary and secondary visual structures in the visually-deprived hemisphere. At 5 days post-lesion, however, [^{3}H]-5HT and [^{3}H]-DHA binding were significantly reduced in both the visually-deprived superior colliculus (by 17% and 23% respectively) and dorsal lateral geniculate nucleus (by 33% and 30% respectively). There were similar alterations in the binding of these two ligands in these primary retinal projection areas at 10 and 20 days after orbital enucleation, but there were no changes in secondary areas (e.g. visual cortex) at any time point. [^{3}H]-Muscimol binding was significantly reduced in the visually-deprived dorsal lateral geniculate (30%) and visual cortex (21%) only at 20 days post-lesion, whilst [^{3}H]-ketanserin and [^{3}H]-QNB were not altered in any region in the visually-deprived hemisphere at any time point post-enucleation. AT 10 and 20 days post-enucleation, the degree of [^{3}H]-5HT, [^{3}H]-DHA and [^{3}H]-muscimol binding deficits in visually-deprived structures correlated significantly with the level of reduced metabolic activity in

these areas (r=0.700, r=0.757 and r=0.543 respectively).

The specificity and regional and temporal heterogeneity of neurotransmitter receptor binding alterations provides evidence of selective adjustments within visual system circuitry in response to orbital enucleation.

The results presented in this thesis highlight the applicability of quantitative receptor autoradiography in studies of receptor dynamics in both rat polysynaptic systems and human neurodegenerative disease. The novel alterations in glutamatergic sites in the A.D. brain uncovered by the present investigations provide new insight into the role of glutamatergic dysfunction in the pathophysiology of the disease.

PREFACE AND DECLARATION

This thesis primarily presents results from ligand binding studies in human postmortem material and rat brain using quantitative autoradiographic methods. Local cerebral glucose utilisation was measured in rat visual system studies using the [^{14}C]-2-deoxyglucose technique.

Investigations have been conducted in three broad areas:

(1) the integrity of glutamate receptors and uptake sites in postmortem tissue from patients dying with Alzheimer's disease;

(2) post-lesion studies of glutamate receptors in the rat visual system, and

(3) post-lesion studies of non-glutamatergic receptors and cerebral function in the rat visual system. Results from these studies are presented and discussed separately.

In the final discussion I have attempted to highlight the insight provided by ligand binding autoradiography in studies of brain receptors and some limitations of the approach.

This thesis comprises my own original work and has not been presented previously as a thesis in any form.

CHAPTER I

INTRODUCTION

1. NEUROTRANSMITTER RECEPTORS AND CEREBRAL FUNCTION

Before the pioneering work of Santiago Ramón Y Cajal in the late nineteenth century, the central nervous system (CNS) was regarded as a complex network of neurons having cytoplasmic continuity with each other ("reticular theory"). Using Golgi staining techniques to visualise individual cells, Cajal conceptualised and presented much of the empirical support for the "neuron theory" which posits that the CNS is composed of many individual signalling elements - neurons (Cajal, 1906). At this time, whilst trying to rationalise the physiological differences between conduction in nerve trunks and reflex arcs, Sherrington concluded that a "surface of separation" must exist between neurons and introduced the term "synapse" (Sherrington, 1906). Today, the neuron is recognised as the structural and functional unit in the brain and the synapse as the essential means by which neurons communicate in order to co-ordinate the multiplicity of brain functions.

Communication across the vast majority of synapses involves the use of a chemical transmitter, "bridged" or "gap" junctions are regarded as electrical synapses (Kandel and Siegelbaum, 1985). In addition to the classical neurotransmitters, such as acetylcholine and noradrenaline, there is growing evidence that amino acids, such as glutamate, and certain peptides may act as chemical transmitters at CNS synapses. However, regardless of the neurotransmitter employed, neurotransmission across all chemical synapses involves the interaction of transmitter molecules and specific postsynaptic receptors. Neurotransmitter receptors thus represent the primary recognition sites by which extracellular signals may be transduced and

amplified to postsynaptic biological response.

The receptor concept is accredited to the physiologist, Langley, who whilst studying the effects of pilocarpine and atropine on salivary secretion in 1878 concluded that there was some "receptive substance" with which the cholinergic compounds could interact (Langley, 1906). We now recognise that the neurotransmitter receptor is a macromolecule, probably a glycoprotein, which traverses the lipid bilayer of the cell membrane and possesses one or several recognition sites which will bind the neurotransmitter. Interaction of the neurotransmitter with the membrane-bound receptor may induce either a direct alteration in membrane ion permeability (ionotropic) or an alteration in membrane-bound enzymes which subsequently catalyse intracellular activity (metabolotropic) (Schwartz, 1985). It is the transduction properties of the neurotransmitter receptor, and not the neurotransmitter, which determines whether a synapse is inhibitory or excitatory. In addition to mediating trans-synaptic neurotransmission, it is now evident that neurotransmitter receptor activation may induce a spectrum of events on neuronal structure, ranging from neurite sprouting and synaptic plasticity to neuronal death (Lipton and Kater, 1989). Neurotransmitter receptors are, therefore, not only important in the homeostatic control of neurotransmission but may be the primary sites for neuro-degenerative or neuro-regenerative changes in the CNS.

As evidence continues to emerge to indicate that the most remarkable of brain activities, such as memory and learning, are directly related to the

properties of neurotransmitter receptors, (Collingridge and Bliss, 1987; Morris et al. 1986), our need for understanding receptor regulation becomes more fundamental to our understanding of cerebral function. It is the question of neurotransmitter receptor behaviour and how this may relate to functional activity within the brain which is the central theme of the studies presented in this thesis. Investigations in Alzheimer's disease have focused on receptors for the excitatory amino acid transmitter, glutamate, in view of the receptor-mediated neurotoxic properties of this transmitter (Rothman and Olney, 1987) and the putative involvement of cortical glutamatergic fibres in the pathophysiological progression of the disease (Pearson et al. 1985). Studies in the rat visual system have examined the post-lesion regulation of both glutamate receptors, implicated in mediating retino-fugal activity (Crunelli et al. 1987), and receptors for non-glutamatergic systems involved in modulating visual processing. In this way it has been possible to examine a number of neurotransmitter receptors at multiple synapses under conditions of functional deficit.

2. GLUTAMATE AS A NEUROTRANSMITTER

The ability of glutamate and a number of related amino acids to activate CNS neurons (Curtis et al. 1959), formed the basis for proposing a transmitter role for these compounds. However, initial electrophysiological studies on spinal cord neurons (Curtis and Watkins, 1960a) indicated that the excitatory action of glutamate was widespread and effected by both the D and L forms of the amino acid. In addition, the widespread metabolic roles of glutamate as a Krebs cycle intermediary (Krebs, 1935), in protein and peptide synthesis (Meister, 1979), as a precursor for the inhibitory neurotransmitter γ-aminobutyric acid GABA (Roberts and Frankel, 1950), and in the detoxification of ammonia (Weil-Malherbe, 1950) led to initial scepticism in regarding glutamate as a neurotransmitter.

However, over the last 20 years a large body of evidence has emerged to support the concept that glutamate is a transmitter in the brain (for reviews see Roberts et al. 1981; Di Chiara and Gessa, 1981) and this amino acid is now widely accepted as the major excitatory transmitter in the CNS.

The four main criteria for classification of any compound as a neurotransmitter are:

1. It is pre-synaptically localised in specific neurons;
2. It is specifically released by physiological stimuli in concentrations high enough to elicit a postsynaptic response;

3. It has identity of action with the endogenous transmitter, e.g. response to antagonists;

and 4. Uptake mechanisms exist which terminate transmitter action rapidly.

Glutamate satisfies each of these requirements to varying degrees.

A sodium-dependent high affinity uptake system is a common requirement for transmitter amino acids and amines (Fonnum et al. 1980). Such a system exists for glutamate in the CNS. L-Glutamate and D- and L-aspartate, to the exclusion of all other amino acids, are taken up by this uptake carrier (Logan and Snyder, 1971; Balcar and Johnston, 1972). The uptake of these compounds at this site shows an absolute requirement for sodium (Bennet et al. 1973), two sodium ions being required for the uptake of one glutamate molecule (Stallcup et al. 1979). Inhibitors of this uptake process, L-glutamate dimethylester (Haldeman and McLennan 1973) and L- and D-threo-3-hydroxyaspartate (Johnston et al. 1980), prolong the excitatory action of L-glutamate confirming the importance of this system in terminating the effects of this amino acid.

Ca^{2+}-dependent release of glutamate/aspartate has been demonstrated in both slices and synaptosome preparations by several different depolarisation methods (Fonnum, 1984). In addition, specific nerve pathway stimulation has been shown to increase the release of glutamate/aspartate in vitro

(Skrede and Malthe-Sorenssen, 1981) and in vivo (Crawford and Connor, 1973) experiments. Glutamate release, evoked by such physiological stimuli is sufficient to elicit postsynaptic responses (Granata and Reis, 1983).

As no precursor or synthetic enzyme is known to be specific for the transmitter pool of glutamate, no reliable enzyme marker is available for glutamatergic nerve terminals. However, the visualisation of glutamate-like immunoreactivity in some synaptic vesicles (Otterson and Storm-Mathisen, 1984) and the similarity of this histochemical localisation with results obtained with a high-affinity glutamate uptake site marker (Fonnum, 1984) indicates that glutamate is pre-synaptically localised in specific neurons. Moreover, Naito and Ueda (1983) have demonstrated a selective ATP-dependent uptake of glutamate into synaptic vesicles.

As D-aspartate has a high affinity for the glutamate uptake carrier, this metabolically stable compound has been employed in uptake and release studies allowing identification of numerous glutamatergic pathways (Fonnum, 1984). With the development of antagonists of glutamate receptors, blockade of synaptic excitation has now been demonstrated in a number of specific pathways (Watkins and Evans, 1981). Taken together these data at least partially satisfy the identity of action criterion for neurotransmitter actions and support the postulated role of acidic amino acids as excitatory neurotransmitters.

The many different roles assigned to glutamate, in addition to neurotransmitter, necessitates that its synthesis and metabolism are compartmentalised within the CNS. Lesions of glutamatergic pathways to different brain regions are accompanied by 20% - 45% reductions in endogenous glutamate content, indicating the relative size of the transmitter glutamate pool (Lund-Karlsen and Fonnum, 1978; Walaas and Fonnum, 1980). However, determination of the synthetic pathway specific for transmitter glutamate has not been possible and, as alluded to above, enzymes known to be involved in glutamate synthesis (for review, see Hertz, 1979) do not provide specific markers of glutamatergic neurons. Although the precise synthetic pathway is unclear, glutamine, 2-oxo-glutarate and aspartate are immediate precursors for glutamate synthesis. Their relative contribution to transmitter glutamate synthesis <u>in vivo</u> remains undefined.

3. GLUTAMATE RECEPTOR CLASSIFICATION AND BINDING STUDIES

In 1960, the molecular requirements for amino acids to possess excitatory activity were defined as being "*the presence of an amino group optimally situated alpha to a carboxyl group and spaced two or three carbon atoms distant from a second acidic site*" (Curtis and Watkins, 1960b). This suggested that acidic amino acids interacted with a single three point attachment site on the neuronal membrane. However, with the development of excitatory amino acid antagonists, the concept of a single receptor site became less tenable and the electrophysiological effects of glutamate and its congeners were subsequently found to be mediated by several distinct receptor types (for reviews see McLennan, 1981 and Watkins and Evans, 1981).

The most common glutamate receptor classification describes three distinct receptor subtypes (Watkins and Evans, 1981). These are named N-methyl-D-aspartate (NMDA), quisqualate and kainate, after the most specific agonist compounds at each receptor. A fourth class is defined by the antagonist L-2-aminophosphonobutyric (L-AP4). The precise electrophysiological function of this receptor, however, is uncertain.

(1) NMDA Receptors: These receptors are defined by their high selectivity for NMDA, NMLA, aspartate and ibotenate. Pharmacological progress has been particularly rapid in relation to the NMDA receptor subtype and both competitive and non-competitive antagonist compounds are now available, one of the most potent

competitive antagonists being 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) (Davies et al. 1986). A number of chemically different compounds are classed as non-competitive NMDA receptor antagonists including "dissociative anaesthetics" (phencyclidine and ketamine) and the benzomorphan sigma opiates (e.g. SKF 10047, N-allyl-normetazocine) (Kemp et al. 1987). However, the dibenzo-cycloheptene-imine, MK-801, has been identified as the most potent member of this class of compounds (Kemp et al. 1987). Antagonism of NMDA-receptor mediated responses by these compounds is voltage and use-dependent (Kemp et al. 1987), indicating that their site of action is at the level of the NMDA receptor-operated ion channel. Mg^{2+} and other divalent cations also act to produce a voltage-dependent block of this ion channel (Mayer et al. 1984), although most probably at a different locus of action from the above mentioned compounds (Kemp et al. 1987). Conversely, NMDA receptor action has been shown to be enhanced by both glycine (Johnson and Ascher, 1987) and spermidine (a polyamine found in high concentrations in synaptosomal fractions) (Ransom and Stec, 1988), suggesting an overall complex modulation of NMDA receptor function in vivo.

(2) Quisqualate Receptors: These receptors are activated selectively by quisqualic acid, L-glutamate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). In contrast to NMDA receptors, no highly selective quisqualate receptor antagonists are available. However, L-glutamic acid-diethyl-ester (GDEE) shows some selectivity

for quisqualate receptor responses (Watkins and Evans, 1981). More recently, two potent competitive non-NMDA receptor antagonists, 6,7-dinitro-quinoxaline-2,3-dione(DNQX)and6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), have been developed (Honoré et al. 1988). These compounds block the excitatory action of quisqualate and kainate in spinal neurones with little or no effect on that of NMDA (Honoré et al. 1988).

(3) Kainate Receptors: Kainate-induced excitatory responses are relatively insensitive to antagonism by NMDA receptor antagonists or by GDEE, suggesting that kainate acts at a third class of glutamate receptors (McLennan and Lodge, 1979). At these sites, kainate and domoate are potent agonists while AMPA and glutamate are moderately effective (Davies et al. 1982).

There is now substantial evidence from electrophysiological studies to indicate that NMDA , quisqualate and kainate receptors mediate excitatory neurotransmission in the CNS (Foster and Fagg, 1984; Meldrum, 1985). Glutamate is considered a mixed agonist (Watkins and Evans, 1981) interacting with each of the physiologically defined receptor subtypes and is a strong candidate as the endogenous transmitter at these CNS receptors.

As discussed in section 1 of this chapter, the neurotransmitter/receptor interaction is the fundamental basis of synaptic function in the CNS. Although electrophysiological and biochemical experiments provide essential information relating to receptor function, ligand binding techniques provide the only means of probing the neurotransmitter/receptor interface in the absence of cellular influences. These techniques have been used successfully in the study of an extensive number of different CNS receptors and, in this respect, glutamate receptors are not exceptional.

The first ligand binding studies of glutamate receptors were published by Michaelis et al. and Roberts in 1974. Using [^{14}C]-glutamate, these investigators found that the binding was saturable of relatively high affinity and displaced by other acidic amino acids. The development of L-[^{3}H]-glutamate with higher specific activity initiated a number of investigations of [^{3}H]-glutamate binding to brain homogenates using a variety of technical approaches (for review see Foster and Fagg, 1984).

Ligand binding studies are only of value if the sites labelled correspond to the physiological/pharmacological receptors of interest. In this respect, autoradiographic receptor binding techniques are superior to those using brain homogenates as both the pharmacological specificity of binding and its regional localisation can be compared to electrophysiological data. The first reports of high affinity [^{3}H]-glutamate binding using quantitative autoradiography were published in 1983 (Greenamyre et al. 1983; Halpain et al. 1983; Monaghan et al. 1983). These studies indicated that [^{3}H]-

glutamate bound to all three subtypes of glutamate receptor and revealed a heterogeneous distribution of these sites in the CNS. Subsequent autoradiographic studies indicated that the distribution of glutamate-binding sites correlated well with the projection areas of putative glutamatergic pathways and provided pharmacological evidence to suggest that the NMDA, quisqualate- and kainate-sensitive glutamate binding sites corresponded to the physiologically defined NMDA, quisqualate and kainate receptors (Greenamyre et al. 1984a; Greenamyre et al. 1985; Monaghan and Cotman, 1985).

As [^{3}H]-glutamate was a "mixed agonist" radioligand, more accurate studies of the three glutamate receptor subtypes required more selective ligands. From electrophysiological data (Davies et al. 1979; Watkins and Evans, 1981), AMPA, kainate and NMDA were the most appropriate agonists for selective radioligands. However, attempts to label the NMDA receptor using [^{3}H]-NMDA were relatively unsuccessful with only low levels of specific binding being obtained (Foster and Fagg, 1987). Competitive and non-competitive antagonists have provided more successful and selective NMDA receptor labelling. The distribution of [^{3}H]3-((±) 2-carboxypiperazin-4-yl) propyl-1-phosphonic acid ([^{3}H]-CPP), [^{3}H]-1-(1-(2-thienyl)-cyclohexyl) piperidine ([^{3}H]-TCP) and [^{3}H]-MK-801 corresponds remarkably well with that found for NMDA-sensitive glutamate binding sites (Jarvis et al. 1987; Cotman et al. 1987), although more recent evidence suggests that [^{3}H]-CPP may label a subpopulation of NMDA sites (Monaghan et al. 1988), albeit the most substantial receptor subtype. In addition, [^{3}H]-glutamate binding under the

appropriate binding conditions and in the presence of selective blockers provides highly selective labelling of NMDA receptors (Monaghan and Cotman, 1985).

[^{3}H]-AMPA binding to rat brain membranes was found to be saturable and reversible (Honoré et al. 1981) and to possess the appropriate pharmacological profile and anatomical distribution for quisqualate receptor labelling (Monaghan et al. 1984). The discovery that chaotropic ions (e.g. SCN^-) markedly increase the affinity of quisqualate receptors for [^{3}H]-AMPA (Honoré and Nielson 1985) has made accurate autoradiographic studies of quisqualate receptors possible. Similary, binding of [^{3}H]-kainate to rat brain membranes was saturable and reversible (Simon et al. 1976) and identical to kainate-sensitive [^{3}H]-glutamate binding sites (Greenamyre et al. 1985). [^{3}H]-Kainate binding has been shown to have a synaptic localisation (Foster et al. 1981) and the anatomical distribution of [^{3}H]-kainate binding in the CNS suggests that kainate sites are associated with select terminal fields (Monaghan and Cotman, 1982).

In view of the above data, the autoradiographic labelling of glutamate receptors presented in this thesis has relied on the use of [^{3}H]-AMPA, [^{3}H]-kainate and [^{3}H]-glutamate as selective radioligands for quisqualate, kainate and NMDA receptors respectively.

4. GENERAL NEUROPATHOLOGICAL AND NEUROCHEMICAL ASPECTS OF ALZHEIMER'S DISEASE

Alzheimer's disease (A.D.) is a dementing disorder of insidious onset characterised by memory loss and general progressive cognitive decline (Huppert and Tym, 1986). The disease is believed to affect some 10-15% of the population of 65 years and over (Henderson, 1986). As other neuropathologies cause similar clinical features, (e.g. multi-infarct dementia), clinical diagnosis of the disease is one of exclusion and definitive diagnosis requires neuropathological confirmation. The histological criteria for diagnosis are the presence of large numbers of senile plaques and neurofibrillary tangles in certain areas of the neocortex and hippocampus, as first described by Alzheimer in 1907. In addition to these microscopic features, gross morphological changes in Alzheimer's disease have been described in terms of cortical atrophy in which there is a shrinkage of the gyri and widening of the sulci, usually within the frontal and temporal lobes (Perry, 1986). Plaques and tangles may, however, be found in the absence of any distinct cortical atrophy (personal observations; Perry, 1986).

Senile plaques are the microscopic features upon which definitive diagnosis of A.D. depends (Khachaturian, 1985). The general histological features of the plaques are usually described in terms of a central spherical amyloid "core" surrounded by numerous degenerating neurites (presynaptic nerve terminals) and glial cells (astrocytes and microglia) (Brun, 1983). Wisniewski and Terry (1973) have, however, subdivided plaques into three categories related to their stage of development: (1) a "primitive" plaque, consisting of

a small number of neurites and small amount of amyloid; (2) a "mature" plaque, consisting of a dense core of amyloid and numerous neurites and (3) a "burnt-out" plaque, consisting almost entirely of amyloid. The primitive plaque is typically found in younger Alzheimer patients while the mature plaque is present in relatively greater numbers in older patients (Mann, 1988). The principal protein component of plaque amyloid is a low molecular weight hydrophopic protein (β-protein) which is also present in cerebrovascular amyloid (Selkoe, 1987). It is of interest that the β-protein gene has been located on chromosome 21 (Selkoe, 1987), providing a direct explanation for the development of plaques in virtually all adult trisomy 21 (Down's Syndrome) patients. X-ray and NMR analysis further suggests the presence of a form of aluminosilicate in the plaque core, strengthening the hypothesis that mineral deposition may be a stimulus for plaque formation (Bertholf, 1987). Plaque neurites appear to derive from a variety of neuron types including cholinergic, noradrenergic and somatostatinergic (Walker et al. 1988) and possibly glutamatergic (Perry, 1986).

Plaques accumulate as spherical lesions in the neuropil and are found in high numbers in frontal and temporal cortex, hippocampus and amygdala and very low numbers in primary visual, auditory, somatosensory and motor cortices (Mann, 1988). The distribution of plaques in cortex does not appear to be random but may be associated with specific intracortical fibres (Pearson et al. 1985; Rogers and Morrison, 1985).

Neurofibrillary tangles, like plaques, are also present in high numbers in the cerebral cortex and hippocampus, however, they are also found in subcortical structures such as the nucleus basalis of Meynert, raphé nuclei and the locus coeruleus (Wilcock & Esiri, 1982; Mann et al. 1985; Perry, 1986). Within the association areas of cerebral cortex, tangles are arranged in definite "clusters" (Pearson et al. 1985), particularly in cortical layers III and V, which are associated with cortico-cortical projections.

At the light microscopic level, tangles appear as an accumulation of filamentous material within the neuron perikarya (Brun, 1983). Under the electron microscope, tangles are seen to be composed of numerous pairs of filaments, wound in a helical manner, leading Kidd (1963) to propose the term "paired helical filaments" for these structures. Whether paired helical filaments are composed of normal cytoskeletal elements or some new abnormal protein remains unknown. However, using immunohistochemical techniques, a number of microtubule-associated phosphoproteins (MAP2 and tau proteins) have been identified as antigenic constituents of paired helical filaments and tangles (Kosik et al. 1984 and 1986).

The formation of tangles within neuronal perikarya has been implied to be the cause of cell loss in A.D. (Saper et al. 1985). Consistent with this hypothesis, high correlations between neuronal counts and estimates of neurofibrillary change have been reported in both frontal and temporal cortex in the A.D. brain (Mountjoy et al. 1983).

It is likely that the accumulation of tangles progressively disrupts essential intracellular functions (protein synthesis, oxidative metabolism) (Sumpter et al. 1986) subsequent to neuronal death.

Although plaques and tangles are the characteristic microscopic features of A.D., it is unclear to what extent these neuropathological changes relate to the clinical expression of the disease. Blessed et al. (1968) and Wilcock and Esiri (1982) report significant correlations of postmortem plaque scores and degree of dementia while Neary et al. (1986), using biopsy material from A.D. patients, found that both plaques and tangles were unrelated to dementia ratings. As Mann (1988) has suggested, it is likely that methodological problems, such as ceiling effects at postmortem, may preclude achievement of statistical significance in correlations of neuropathological and cognitive indices. Measurement of pyramidal cell loss (Neary et al. 1986) or synaptic density (Davies et al. 1987) in the A.D. brain appears to be a more sensitive indice of cognitive function.

Perhaps the most consistent neurochemical changes in A.D. are related to the cholinergic system. Numerous investigators have reported reductions in choline acetyltransferase (ChAT) activity, both in postmortem and biopsy material, in cerebral cortex and hippocampus of Alzheimer patients (White et al. 1977; Perry et al. 1977; Davies and Terry, 1981; Mountjoy et al. 1984). The loss of this presynaptic cholinergic marker in these regions probably relates to the loss of ascending cholinergic fibres whose cell bodies lie in the nucleus basalis of Meynert, diagonal band of Broca and the septal

nuclei (Whitehouse et al. 1982; Saper et al. 1985; McGeer et al. 1984) of the basal forebrain. The reported correlation of ChAT activity with both mean plaque count and the extent of intellectual impairment in a group of Alzheimer patients (Perry et al. 1978) precipitated the "cholinergic hypothesis" of A.D. This posits that the cognitive impairments of the disorder, particularly memory, are directly related to the level of disturbance in cerebral cholinergic neurotransmission. However, attempts to improve cognitive functions in Alzheimer patients with acetylcholine precursors, acetylcholinesterase inhibitors or cholinergic agonists have been, for the most part, unremarkable (Hollander et al. 1986), and it is now apparent that multiple neurotransmitter systems are affected in A.D.

Biochemical markers for noradrenergic, serotonergic, peptidergic, GABAergic and glutamatergic neurotransmission are all found to be altered in A.D. (Hardy et al. 1985; Rossor and Iversen, 1986 and Quirion et al. 1986). Furthermore, a large body of literature indicates, for the most part, reductions in cortical neurotransmitter receptors associated with each of these systems. These include reductions in: α_1, α_2, β_1 and β_2-adrenergic receptors (Shimohama et al. 1986a and 1987); $5HT_1$ and $5HT_2$ receptors (Cross et al. 1984 and 1988); somatostatin and CRF (corticotropin-releasing factor) receptors (Beal et al. 1985; De Souza et al. 1986); $GABA_A$ and $GABA_B$ receptors (Chu et al. 1987); benzodiazepine receptors (Shimohama et al. 1988); and glutamate receptors (Greenamyre et al. 1987). The status of cortical cholinergic muscarinic receptors is unclear with reports of normal or reduced non-selective antagonist binding, normal M_1 binding and reduced

M_2 binding (Perry et al. 1986). Reduced M_2 receptor binding has been proposed to reflect a loss of presynaptic autoreceptor sites (Mash et al. 1985). In a similar fashion to muscarinic receptors, cortical cholinergic nicotinic receptors have been reported to be either unaltered (Shimohama et al. 1986b) or reduced (Whitehouse et al. 1986) in A.D. Consistent losses in presynaptic ChAT activity in the cerebral cortex of A.D. brains is thus not associated with any consistent postsynaptic receptor response.

To date, much has been discovered in relation to both neuropathological and neurochemical alterations in A.D. Which of these neurochemical and neuropathological changes are fundamental to the disease process and which are epiphenomenal is, at present, unclear. The central question of how neurochemical, neuropathological and functional changes are inter-related, if at all, remains unanswered and, as yet, no definitive statements can be made regarding the aetiology of the disease. Resolving these basic issues clearly requires a greater understanding of the disease process. In this respect, a growing body of evidence indicates that the cerebral cortex is the site of the primary "lesion" in A.D. and that projections utilising the excitatory neurotransmission glutamate, may be selectively involved.

5. GLUTAMATE AND ALZHEIMER'S DISEASE

Neuropathological changes and nerve cell loss in A.D. occurs both in the cerebral cortex (including hippocampus and amygdala) and selective subcortical nuclei including the nucleus basalis, locus coeruleus and various raphé nuclei (Mann, 1988; Perry, 1986). The significance of this widespread pattern of neurodegeneration for the pathogenesis of A.D. has remained obscure. However, recent neuroanatomical studies suggest that the areas typically involved in A.D. are characterised by neuronal connections (afferent or efferent) with other areas involved (Hardy et al. 1986; Saper, 1988) and that the disease process may progress primarily along selective cortical pathways (Pearson et al. 1985), with subcortical changes occurring secondarily. The cerebral cortex, therefore, may be the primary lesion site in the A.D. brain. The involvement of glutamate as the major excitatory transmitter within the cerebral cortex and the neurotoxic properties of this amino acid have been the basis of hypotheses implicating the glutamatergic system in the pathophysiology of cortical dysfunction in A.D. (Maragos et al. 1987a; Greenamyre et al. 1988).

In 1957, Lucas and Newhouse demonstrated that subcutaneous injection of glutamate was toxic to the immature mouse retina. It was subsequently discovered that oral or subcutaneous administration of glutamate destroyed neurons in brain areas which lacked a blood-brain barrier (Olney, 1969). Further studies examining the molecular specificity of glutamate neurotoxicity, using a range of glutamate analogues, revealed that only agents possessing glutamate-like excitatory properties could mimic its neurotoxicity

and that a close correspondence existed between the neurotoxic potency of a given agent and its ability to depolarise neurons (Olney et al. 1971). From these findings, Olney introduced the term "excitotoxin" to emphasise that glutamate's neurotoxic action is mediated by a prolonged depolarising action at its postsynaptic receptor sites. The ability of specific glutamate receptor anatagonists to block the toxic effects of glutamate and its analogues (Rothman & Olney, 1987), further suggests that toxicity is a receptor-mediated phenomenon. The precise mechanism of excitatory amino acid neurotoxicity is unclear. However, based on in vitro experiments, it is currently believed that two, possibly additive, mechanisms are responsible for neuronal death: an acute Na^{2+}- and Cl^{-}-dependent process and a delayed Ca^{2+}-dependent process (Rothman & Olney, 1987). If excitotoxicity is the mechanism of neuronal death in A.D., we may expect that the distribution of neuropathological changes in cerebral cortex should correspond to the terminal fields of glutamatergic pathways.

Glutamate has been proposed to be the transmitter of cortical pyramidal cells (Fonnum et al. 1981). Uptake, release and immunocytochemical studies in rodent brain (Fonnum, 1984) and, more recently, immunocytochemical localisation of glutamate in pyramidal cells of primate brain (Conti et al. 1987), forms a convincing body of evidence in support of this neuro-transmitter identification. Pyramidal cells constitute the principal efferent cells of the cerebral cortex, mediating both cortico-cortical (associational) and cortico-fugal neurotransmission (Jones, 1981). Pearson and colleagues have proposed that the distribution of histopathological changes in

Alzheimer's cerebral cortex is not random and correlates with the terminal fields of cortical association pathways (Pearson et al. 1985). The authors suggest that neurofibrillary tangles are found in the cell bodies of pyramidal cells giving rise to these fibres and that senile plaques may be at the ends of fibres and their collaterals. Consistent with this hypothesis, senile plaques are most dense in zones of association fibre termination and neurofibrillary tangles are particularly prominent in cortical layers III and V, the layers of origin for cortico-cortical pathways (Pearson et al. 1985). The suggestion that the neuropathological changes in A.D. may propagate along cortico-cortical association pathways is further strengthened by the reported induction of paired helical filaments, similar to those which make up neurofibrillary tangles, in cultured human neurons incubated with glutamate (De Boni and Crapper-McLachlan, 1985). In addition, application of NMDA to the cortical surface in rats produces retrograde degeneration of cholinergic neurons in the nucleus basalis (Sofroniew & Pearson, 1985), indicating that cortical excitotoxicity can produce subcortical changes characteristic of A.D. Taken together, the above data form a strong case for the involvement of glutamatergic dysfunction in the pathophysiology of A.D. However, an inevitable consequence of any excitotoxic hypothesis is the loss of glutamate receptors and glutamate terminals as the postsynaptic cells and cells of origin of glutamatergic pathways are destroyed. In this respect, the evidence is less clear cut.

In general, assessments of postmortem glutamate receptor densities in A.D. have given conflicting results. For the most part, these studies have concentrated on the hippocampal formation where glutamate is implicated as the transmitter of entorhinal input fibres, intrahippocampal pathways and hippocampal efferent pathways (Cotman et al. 1987). In this region, Greenamyre et al. (1987) have reported losses of both NMDA and quisqualate receptors while Geddes and colleagues have found no change in these sites (Geddes et al. 1986; Geddes et al. 1987). Using the non-competitive antagonist [^{3}H]-TCP as a ligand, Maragos et al. (1987b) found a deficit in hippocampal binding, whereas Monaghan et al. (1987) and Simpson and colleagues (1988) reported no change. Some of these contradictions may be related to methodological differences in binding procedures (discussed in Bridges et al. 1988). However, the status of glutamate receptors in this region of the A.D. brain is clearly far from certain.

Less attention has been directed towards the integrity of glutamate receptors in the neocortex in A.D. and almost all of these studies have focused on the NMDA receptor. Greenamyre et al.(1984b) first reported a loss of "low affinity" quisqualate sites in temporal cortex which they later interpreted as a loss of NMDA receptors and supportive of excitotoxic cell loss in this region (Maragos et al. 1987a). Subsequent investigations however, using both [^{3}H]-glutamate and non-competitive antagonist ligands ([^{3}H]-TCP and [^{3}H]-MK-801) found no alterations in the NMDA receptors in this region (Cowburn et al. 1988; Simpson et al. 1988; Mouradian et al. 1988).

The Na^+-dependent, high-affinity uptake system for glutamate provides a simple marker of glutamatergic terminals in brain. D-Aspartate is a substrate for this glutamate transport system (Balcar & Johnston, 1972) and has been employed extensively in uptake and release studies in relation to glutamatergic pathways. [^{3}H]-D-Aspartate has been proposed to be a suitable marker for glutamatergic terminals in human postmortem brain (Cross et al. 1986) and a number of investigators have examined the binding of this ligand in A.D. brain. Using membrane preparations, reductions in [^{3}H]-D-aspartate binding have been reported in frontal, parietal and temporal cortex of brains from Alzheimer subjects. (Palmer et al. 1986; Cross et al. 1987; Cowburn et al. 1988) This suggests a loss of glutamatergic terminals in those cortical regions of the A.D. brain most severely affected neuropathologically. However, in the absence of defined postsynaptic glutamate receptor alterations, this presynaptic deficit may be interpreted as indicative of either preceeding glutamatergic hyperfunction or chronic glutamatergic hypofunction in A.D.

Although it seems likely that the glutamatergic neurotransmission is disrupted to some degree in A.D., the question arises as to the possible functional significance of this alteration to the clinical symptoms of the disease. In relation to this, the putative role of glutamate in learning and memory processes is of great significance. NMDA receptor activation has been shown to be required for the development of long-term potentiation (LTP) (an activity-dependent change in synaptic efficacy), a model for learning and memory storage in the brain (Collingridge et al. 1983).

Furthermore, NMDA receptor antagonists impair spatial discrimination and learning in vivo (Morris et al. 1986). The amnesic effects of dissociative anaesthetics in humans is further evidence for the involvement of glutamate in memory function as these compounds are non-competitive NMDA receptor antagonists (Kemp et al. 1987). Glutamatergic dysfunction in the cerebral cortex or hippocampus could thus form a neurochemical basis for the learning and memory deficits which are prominent in A.D. In addition, any disruption (pre- or postsynaptic) of glutamatergic association pathways may affect higher cortical processing and be responsible for the signs of cortical disconnection (agnosia, aphasia) which are common in A.D. (Huppert and Tym, 1986). In this respect, it is of interest that cognitive test scores in A.D. patients have been reported to correlate with CSF glutamate levels (Smith et al. 1985).

In summary, there is a strong neuroanatomical case for the involvement of glutamatergic neurotransmission in the pathogenesis of A.D. In those anatomical regions most severely affected pathologically, there is good evidence for a loss of presynaptic glutamatergic markers, however, the pathophysiological significance and functional interpretation of such alterations is dependent on the status of postsynaptic glutamate receptors which has, to date, remained undefined.

6. AIMS OF HUMAN POSTMORTEM STUDIES

The primary aim of the human postmortem studies presented in this thesis was to investigate the integrity of glutamatergic transmission in selective regions of the Alzheimer brain, using quantitative receptor autoradiography. By using ligands specific for the high affinity Na^+-dependent uptake site and glutamate receptor subtypes respectively, it was hoped to image and quantify both presynaptic glutamatergic input and postsynaptic glutamate receptors in adjacent brain sections from all regions of interest. The use of autoradiography would allow quantification of these parameters in single cortical layers and aid a functional interpretation of any receptor alterations in relation to the circuitry of the regions examined.

A central theme of the present postmortem studies was how any alterations in glutamatergic sites may relate to the neuropathological severity of the disease in any anatomical locus. This question was addressed by quantifying senile plaque numbers in adjacent brain sections to those used for receptor autoradiography, thus allowing an intimate comparison of glutamatergic parameters and local neuropathology. The putative involvement of glutamatergic dysfunction in relation to neuropathological changes was further explored by examining three separate anatomical regions (frontal, temporal and cerebellar cortex) in which glutamatergic transmission was of equivalent importance but in which the extent of neuropathological changes in A.D. was differential.

7. THE RAT VISUAL SYSTEM AS A POLYSYNAPTIC MODEL

In the CNS, "receptor plasticity" may be defined as the ability of neurons to alter the number and sensitivity of neurotransmitter receptors in response to changes in their functional input. As outlined above, multiple neurotransmitter receptors are altered in A.D. as a consequence of dysfunction in a number of neurotransmitter systems. This complicates the analysis of this receptor plasticity as any receptor response may be as a result of a primary neuronal degeneration or as a consequence of a dysfunction in a separate but functionally related system. The rat visual pathway provides a simple model system in which to study receptor behaviour as a consequence of specific neuronal degeneration. This polysynaptic neuronal pathway has the advantages of being anatomically well-defined, containing significant quantities of receptors for a number of neurotransmitters and being easily and completely reproducibly lesioned. In addition, as most retinal ganglion cells in the rat (97-98%) project to the contralateral hemisphere (Jeffery, 1984), a unilateral lesion of this pathway allows the ipsilateral hemisphere to act as a reference, against which, changes in the visually-deprived contralateral hemisphere can be contrasted.

The main anatomical components of the rat visual pathway are illustrated schematically in Figure 1. It has been estimated that the optic nerve in the adult hooded rat contains 120,000 axons (Hughes, 1977). The superior colliculus is the main target of these axons, receiving 65% of retinal project fibres (Toga and Collins, 1981). This structure has a horizontally laminated organisation, the layers being: zonal; superficial gray; optic; intermediate

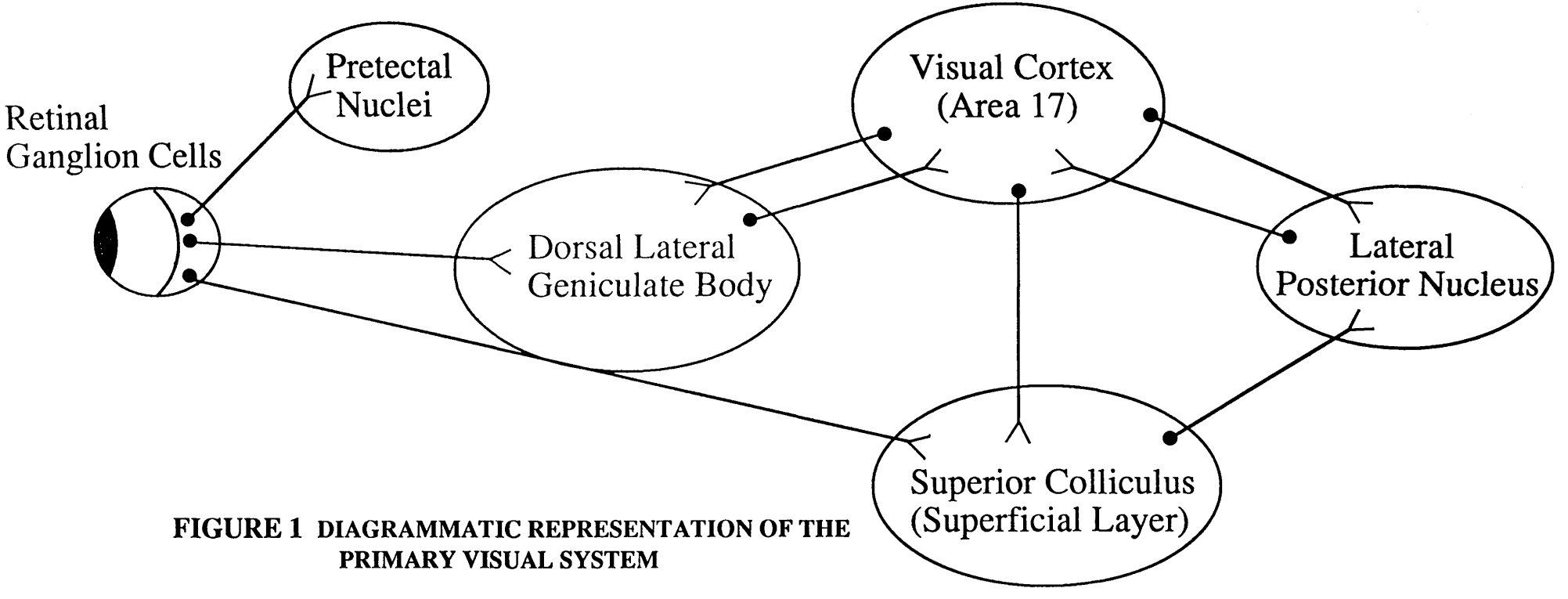

FIGURE 1 DIAGRAMMATIC REPRESENTATION OF THE PRIMARY VISUAL SYSTEM

gray and white, and deep gray and white (Paxinos and Watson, 1986). Only the superficial layers are innervated by retinal axons whilst other sensory systems are represented in deep layers (Stein, 1981). There are no direct projections to visual cortex from the superior colliculus. However, cells in the superficial layers of the superior colliculus do project to other subcortical visual structures including the dorsal lateral geniculate nucleus (Pasquier and Villar, 1982), the lateral posterior nucleus and pretectal nuclei (Takahashi, 1985).

The dorsal lateral geniculate nucleus is found in the dorsolateral part of the thalamus and acts to relay information from the retina to visual cortex. It has been estimated that the dorsal lateral geniculate nucleus receives 15% of retinal projection fibres (Toga and Collins, 1981), corresponding to estimates of the number of relay cells in the nucleus (Martin et al. 1984). The projection to layer IV of primary visual cortex (area 17) constitutes the major output of the dorsal lateral geniculate nucleus (Peters and Feldman, 1976), although thalamocortical axons also branch to supply the reticular thalamic nucleus (Hale et al. 1982). Unlike the superior colliculus, the dorsal lateral geniculate nucleus does not project to other subcortical visual structures, but does receive afferents from pretectal nuclei (Pasquier and Villar, 1982), and the superior colliculus (Takahashi, 1985).

The pretectum is found in the most rostral plane of the midbrain bordering the thalamus and is composed of four major nuclei: the nucleus of the optic tract; the olivary pretectal nucleus; the anterior pretectal nucleus and the

posterior pretectal nucleus (Scalia, 1972). Approximately 13% of retinal projection fibres terminate in the contralateral pretectum (Toga and Collins, 1981), although the anterior pretectal nucleus does not appear to be retinorecipient (Sefton and Dreher, 1985). Pretectal nuclei project to the superior colliculus, dorsal lateral geniculate nucleus and the lateral posterior nucleus (Sefton and Dreher, 1985; Mackay-Sim et al. 1983).

In contrast to the superior colliculus, dorsal lateral geniculate nucleus and pretectum, the lateral posterior nucleus receives only a minimal retinal input (Perry and Cowey, 1982). However, this thalamic nucleus, which lies medial and caudal to the dorsal lateral geniculate nucleus, receives a significant projection from the superior colliculus (Takahashi, 1985) and in turn sends afferents to area 17 of visual cortex (Perry, 1980). Although only the dorsal lateral geniculate nucleus and lateral posterior nucleus project directly to visual cortex, all of the above mentioned subcortical visual structures receive afferents from primary visual cortex (Sefton and Dreher, 1985).

Using both light and electron microscopy, the neuronal composition of area 17 of rat visual cortex has been described in some detail by Peters and colleagues (Peters and Kara, 1985a and b; Peters and Kara, 1987). From these studies it has been estimated that 85% of neurons in this area of cortex are pyramidal cells, their cell bodies occurring throughout layers II-VI. The vast majority of thalamic afferents to visual cortex, principally from the dorsal lateral geniculate nucleus, terminate in cortical layer IV (Peters and Feldman, 1976). Most thalamic terminals synapse with the

dendritic spines of apical and basal dendrites of pyramidal cells whose cell bodies are situated in cortical layers III-VI, although some synapse on sparsely spined stellate cells and multipolar cells (Sefton and Dreher, 1985). Pyramidal cells in cortical layers V and VI are responsible for projections to subcortical visual structures (Olavarria and Van Sluyters, 1982; Sefton et al.1981), whilst those in cortical layers II/III participate in cortico-cortical projections (Cusick and Lund, 1981).

Biochemical, electrophysiological and immunological evidence indicate that glutamate is the major excitatory transmitter within this sensory system. The excitatory postsynaptic potentials evoked by electrical stimulation of the optic tract have been shown to be reversibly inhibited by a glutamate receptor antagonist (Crunelli et al. 1987), whilst glutamate induced ionic currents have been demonstrated in cultured neurons from the superior colliculus and visual cortex (Grantyn et al. 1987; Huettner and Baughman, 1988). Ablation of visual cortex results in selective marked reductions in L-glutamate and D-aspartate uptake in the superior colliculus, dorsal lateral geniculate nucleus and lateral posterior nucleus (Lund-Karlsen and Fonnum, 1978; Fosse and Fonnum, 1987). In addition, large pyramidal neurons in neocortex which are likely to include cortico-collicular and cortico-geniculate cells, possess high immunoreactivity for glutamate (Ottersen and Storm-Mathisen, 1984). All of the above data indicates that glutamate is likely to be the transmitter employed in retino-fugal and cortico-fugal projections and within visual cortex.

In addition to the glutamatergic system, a number of other neurotransmitter systems are involved in visual processing. Within visual cortex, cells containing the γ-aminobutyric acid (GABA)-synthesising enzyme glutamic acid decarboxylase, are found in many types of interneurons throughout the visual cortex (Houser et al. 1984). GABAergic terminals are found on the cell bodies, axon segments and dendrites of both pyramidal and non-pyramidal neurons (Peters and Proskauer, 1980; Somogyi et al. 1982) where they make symmetric inhibitory synapses. Furthermore, the release of GABA during inhibition in visual cortex (Iversen et al. 1971) and the production of altered firing patterns in visual cortical cells consequent to administration of the GABA antagonist bicuculline (Daniels and Pettigrew, 1973), indicates that GABA plays an important role in information processing within visual cortex. In subcortical visual structures, GABAergic neurons are present in both the superficial layers of the superior colliculus and the dorsal lateral geniculate nucleus (Mize et al. 1982; Fitzpatrick et al.1984) whilst GABAergic input to the dorsal lateral geniculate nucleus from the reticular thalamic nucleus provides local inhibitory control of relay neuron activity (Kayama, 1985). Noradrenergic and serotonergic projections, from the locus coeruleus and dorsal raphé nucleus, respectively facilitate and depress the spontaneous or synaptically evoked activity of dorsal lateral geniculate cells (Rogawski and Aghajanian, 1980). In contrast, superior collicular neurons are inhibited by both noradrenergic and serotonergic input (Sato and Kayama, 1983; Lai et al. 1978). Visual cortex also receives an extensive monoaminergic innervation (Parnavelas et al. 1985) and a number of studies suggest that noradrenaline is an important modulator of visual

cortical plasticity (Kasamatsu and Pettigrew, 1979; Kasamatsu et al. 1979; Bear and Singer 1986). Finally, visual centres such as the superior colliculus, dorsal lateral geniculate nucleus and visual cortex exhibit choline acetyltransferase and acetylcholinesterase activity (Fibiger, 1982). Cholinergic innervation of these areas originates in the nucleus basalis and nucleus cuneformis (Fibiger, 1982; Vincent and Reiner, 1987), acting to modulate both geniculo-cortical activity and intracortical transmission (Singer, 1977; Bear and Singer, 1986).

The [^{14}C]-2-deoxyglucose technique (Sokoloff et al. 1977) provides a means of measuring functional alterations in the visual pathway in response to lesions of the optic nerve. The conceptual basis of this approach derives from three features of brain biochemistry. First, the energy requirements of cerebral tissue under normal conditions are derived, almost exclusively, from the aerobic catabolism of glucose (Sokoloff et al. 1977). Second, functional activity within any region of the central nervous system is intimately and directly related to energy consumption within that region (McCulloch, 1982). Third, function-related energy requirements must be met from the oxidative catabolism of glucose, continuously supplied by cerebral blood flow because of low levels of carbohydrate stored in the CNS. Although, in the strictest sense, the [^{14}C]-2-deoxyglucose technique provides only a measure of the rate at which glucose is phosphorylated in any region of the brain, almost 80% of all energy generated is destined for the maintenance of ionic gradients (Astrup et al. 1981), predominantly at neuronal terminals (Schwartz et al. 1979), thus allowing mapping of

alterations in neuronal activity. It is, however, important to recognise that increases and decreases in glucose use cannot be simply equated with neuronal excitation and inhibition respectively. Indeed, there is no evidence to suggest that the energy requirements of synaptic inhibition should be dissimilar to those associated with synaptic excitation.

The sensitivity of the [^{14}C]-2-deoxyglucose technique is perhaps best exemplified in simple sensory stimulation and deprivation experiments. Auditory stimulation or occlusion of the external auditory canals is associated with increases and decreases in glucose utilisation throughout the primary auditory pathway (Sokoloff et al. 1977), whilst discrete areas of enhanced glucose utilisation can be demonstrated in specific areas of the olfactory bulb in response to stimulation with different odours (Sharp et al. 1975). Previous investigations in the visual system have shown that deprivation of visual stimuli results in reductions of glucose use, in neuroanatomical components of the pathway, whereas stimulation with diffuse light results in intensity-related increments in their glucose use (Kennedy et al. 1975; McCulloch et al. 1980; Toga and Collins, 1981). It is thus apparent that glucose utilisation within components of well defined functional systems is related to the processing of information in those systems. In view of this, studies of functional and receptor alterations within the simple circuitry of the primary visual system provides a powerful analogy for the more complex circuitry of the CNS.

8. AIMS OF VISUAL SYSTEM STUDIES

Studies in the rat visual system were primarily designed to examine the plasticity of CNS neurotransmitter receptors within a polysynaptic system. Taking advantage of the almost complete cross-over of retinal projection fibres at the optic chiasma, unilateral lesions of this system have allowed comparisons of receptor responses in anatomical components of the functionally-deprived visual pathway with those in the contralateral but functionally-intact system.

To date, studies of post-denervation regulation of glutamate receptors have been limited and have focused, for the most part, on single projection pathways (mostly cortico-striatal). The prominent role of glutamatergic neurotransmission throughout the rat visual system (retino-fugal, cortico-fugal and intracortical transmission) has permitted post-lesion studies of glutamate receptors at multiple synapses, under conditions of reduced glutamatergic input. The plastic capabilities of glutamate receptor sybtypes under these conditions may be relevant to the regulation of these sites in A.D.

In addition to the glutamatergic system, serotonergic, noradrenergic, GABAergic and cholinergic projections are implicated in visual processing. By simultaneously examining specific serotonergic, noradrenergic, GABAergic and cholinergic receptors post-lesion, it was hoped to gain some understanding of the importance of receptor regulation in separate but functionally related systems.

Finally, the question of the relevance of alterations in neurotransmitter receptors to changes in local cerebral function was addressed by combining the [^{14}C]-2-deoxyglucose technique, for the measurement of cerebral glucose use, with in vitro receptor autoradiography. The combination of these techniques, for the first time fully quantitatively in the same animal, allowed simultaneous measurements of cerebral function and receptor dynamics with a high degree of anatomical resolution and permitted an exploration of the inter-dependence of these parameters within the same brain.

CHAPTER II

METHODS

1. AUTORADIOGRAPHIC TECHNIQUES

1.1 IN VITRO LIGAND BINDING AUTORADIOGRAPHY

1.1.1 Theory

In vitro ligand binding autoradiography is based on the principle that the spatial distribution of radiolabelled substances within biological tissue can be detected by the blackening of radiation-sensitive film apposed to the material. The pattern of optical density in resulting autoradiograms is then quantified by comparison to a calibrated radioactive scale. Hence, the use of a radiolabelled ligand, highly selective for a particular neurotransmitter receptor, allows high resolution anatomical mapping of this site in CNS tissue. Autoradiographic ligand-receptor binding studies follow classical ligand-receptor kinetics:

$$a[L] + b[R] \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} c[LR] \qquad (1)$$

describes a reversible binding phenomenon where,

[L] concentration of free ligand,

[R] concentration of unoccupied receptor sites,

[LR] concentrations of ligand-receptor complex and a, b and c represent the stoichiometry of the reaction. At equilibrium:-

$$K_{+1} [L]^a [R]^b = K_{-1} [LR]^c \qquad (2)$$

The equilibrium dissociation binding constant (K_D) is then defined:

$$K_D = \frac{K_{-1}}{K_{+1}} = \frac{[L]^a [R]^b}{[LR]^c} \qquad (3)$$

Ligand-receptor interactions are also saturable, i.e. a finite number of receptor sites exist per unit mass of tissue, designated B_{max}:-

$$B_{max} = [LR] + [R]$$

Multiplying by [L] and substituting equation (3) with a = b = c = 1 yields:-

$$[LR] = \frac{B_{max}[L]}{[L] + K_D} \qquad (4)$$

If we define LR as bound ligand = B, and L as free ligand = F

$$B = \frac{B_{max} F}{F + K_D}$$

$$BF + BK_D = B_{max} F$$

Dividing by F and transferring fields yields:-

$$\frac{B}{F} = \frac{B_{max} - B}{K_D} \qquad (5)$$

The Scatchard equation (Scatchard, 1949). This equation allows the determination of both the equilibrium binding constant (K_D) and maximum number of binding sites (B_{max}) if the concentrations of ligand bound and free at equilibrium are known.

1.1.2 Practice

Figure 2 illustrates the general procedure for quantitative in vitro receptor autoradiography. Briefly, slide-mounted tissue sections are preincubated in an appropriate buffer in order to remove endogenous ligands which may interfere with radiolabelled ligand-receptor interactions. Tissue sections are then incubated with the radioligand in the presence and absence of a competitive displacer in order to determine specific binding. Once the receptor sites are labelled, unbound radioactivity is washed from the sections which are then dried in a stream of cold air. Labelled sections are finally apposed to radiation-sensitive film which, after the appropriate exposure time, is developed to produce receptor autoradiograms. These films are then analysed quantitatively using computer-assisted densitometric analysis. The precise receptor protocols employed in both visual system and human postmortem studies and the published methods from which they are derived are listed in the following tables.

RECEPTOR AUTORADIOGRAPHY

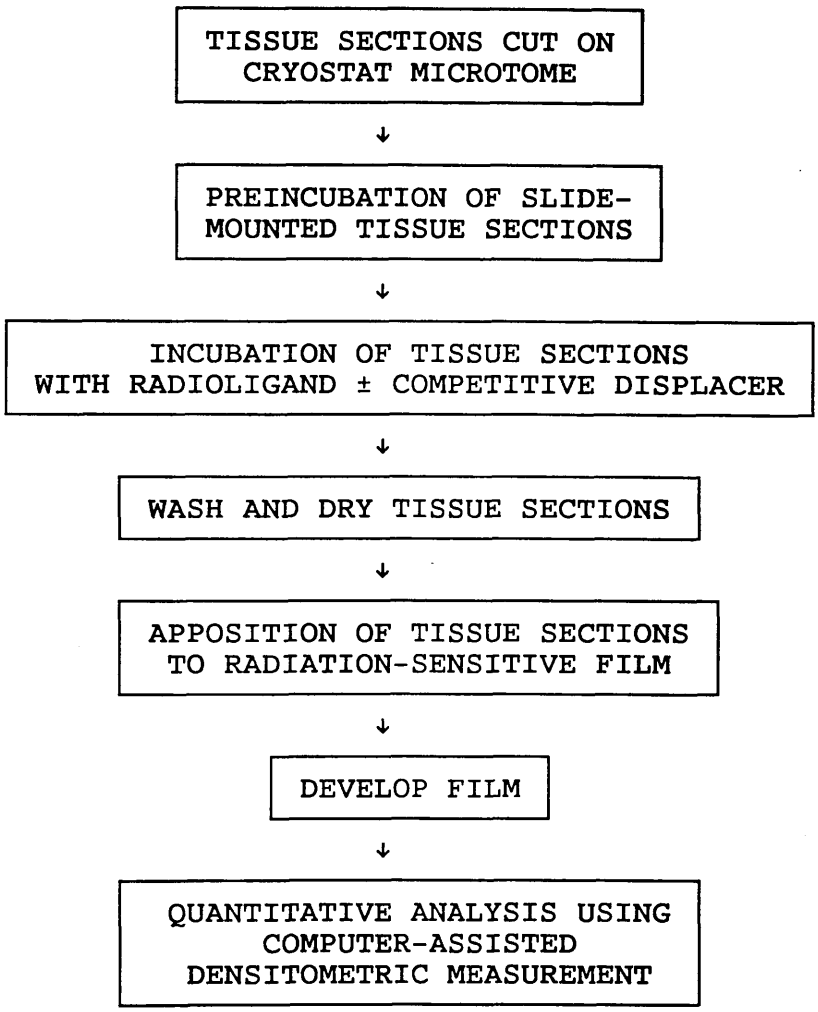

FIGURE 2 GENERAL PROCEDURE FOR QUANTITATIVE IN VITRO RECEPTOR AUTORADIOGRAPHY

TABLE 1 - SUMMARY OF LIGANDS AND BINDING PROTOCOLS

RECEPTOR	LIGAND	DISPLACER	PRE-INCUB.	INCUBATION	WASH	BUFFER	REFERENCE
$5HT_1$	[^{3}H]-5HT (2nM)	5HT	30 Min. R.T.	60 Min. R.T.	1 x 5 Min. (4°C)	0.17M Tris/HCl, pH 7.6 +4mM $CaCl_2$ +0.01% Ascorbic Acid	Pazos et al. 1988
$5HT_2$	[^{3}H]-Ketanserin (2nM)	Methysergide	15 Min. R.T.	120 Min. R.T.	2 x 10 Min. (4°C)	0.17M Tris/HCl, pH 7.7	Pazos et al. 1988
$GABA_A$	[^{3}H]-Muscimol (10nM)	GABA	20 Min.(4°C)	30 Min. (4°C)	3 x 5 Sec. (4°C)	50mM Tris/Citrate Buffer, pH 7.1	Pan et al. 1985
Muscarinic M_1 & M_2	[^{3}H]-QNB (2nM)	Atropine	20 Min. R.T.	120 Min. R.T.	2 x 5 Min. (4°C)	50mM phosphate Buffer, pH 7.4	Walmsley et al. 1984
β-adrenergic	[^{3}H]-DHA (2nM)	Propanolol	20 Min. R.T.	40 Min. R.T.	2 x 10 Min. (4°C)	0.17M Tris/HCl, pH 7.7 +10mM $MgCl_2$	Palacios and Walmsley 1984

R.T. room temperature.

TABLE 2 - SUMMARY OF EXCITATORY AMINO ACID RECEPTOR LIGANDS AND BINDING PROTOCOLS

RECEPTOR	LIGAND	DISPLACER	PRE-INCUB.	INCUBATION	WASH	BUFFER	REFERENCE
Glutamate Uptake Site	[^{3}H]-D-aspartate (1. 250nM)	D-aspartate	1 Hour 0°C 15 Min. 30°C	30 Min. (4°C) +300mM NaCl	4 x 5 Sec. Buffer 1 x 5 Sec. H_2O (4°C)	50mM Tris/HCl pH 7.4	Parsons and Rainbow, 1983
Kainate	[^{3}H]-Kainate (1,2. 50nM)	Kainate	1 Hour 0°C 15 Min. 30°C	30 Min. (4°C)	4 x 5 Sec. Buffer 1 x 5 Sec. H_2O (4°C)	50mM Tris/Citrate pH 7.0	Westerberg et al. 1987
Quisqualate	[^{3}H]-AMPA (1. 130nM, 2. 50nM)	Quisqualate	1 Hour 0°C 15 Min. 30°C	30 Min. (4°C) +100mM KSCN	4 x 5 Sec. Buffer 1 x 5 Sec. H_2O (4°C)	50mM Tris/Acetate pH 7.2	Westerberg et al. 1987
NMDA	[^{3}H]-Glutamate (1.150nM, 2.100nM)	NMDA	1 Hour 0°C 15 Min. 30°C	10 Min. (4°C) + 5µM Quis-qualate + 100µm SITS	4 x 5 Sec. Buffer 1 x 5 Sec. H_2O (4°C)	50mM Tris/Acetate pH 7.2	Monaghan and Cotman, 1985
NMDA-related channel	[^{3}H]-MK-801 (2.10nM)	MK-801	1 Hour 0°C 15 Min. 30°C	20 Min. R.T.	2 x 20 Sec. Buffer 1 x 5 Sec. H_2O	50mM Tris/HCl, pH 7.4	Bowery et al. 1988
Adenosine A_1	[^{3}H]-CHA (2.3nM)	R-PIA	20 Min. R.T.	90 Min. R.T. + 0.5 i.u./ml Adenosine Deaminase	2 x 5 Min. Buffer 1 x 5 Sec. H_2O	0.17M Tris/HCl pH 7.4	Fastbom et al. 1987

1. Human Postmortem Studies 2. Visual System Studies R.T. room temperature.

SITS - 4-acetamido-4'-iosothiocyanatostilbene-2,'2-disulfonic acid. R-PIA - R-phenylisopropyladenosine.

1.1.3 Kinetic Studies

From the Scatchard equation defined in section 1.1.1 (equation 5), we can see that knowledge of the concentration of a ligand bound and free at equilibrium will allow determination of both the equilibrium binding constant (K_D) and the maximum number of binding sites (B_{max}) for that ligand. A plot in which the ordinate shows the moles of ligand bound divided by the concentration of free ligand, $\frac{B}{F}$, and the abscissa shows moles of bound receptor, B, defines line of gradient equal to $-\frac{1}{K_D}$ and intercept on the abscissa equal to B_{max}. In both visual system and human postmortem studies, determination of K_D and B_{max} values were made by applying Scatchard analysis to auto-radiographic images. Scatchard plots were fitted using a least squares linear regression programme or a Statgraphics programme, IBM.

1.1.4 Methodological Problems

All of the ligand protocols employed in these studies were drived from well established methods (Tables 1 and II) and, for the most part, provided reliable means of quantifying the desired neurotransmitter receptor. However, some ligand binding problems were encountered in relation to the binding of excitatory amino acid receptor sites. [^{3}H]-Glutamate labels NMDA receptors with high affinity (Olverman et al. 1984) and under the appropriate binding conditions provides a suitable ligand for an autoradiographic assay. However, agonist affinity is associated with rapid dissociation from the receptor site,

necessitating rapid postincubation washing with this ligand.

The discovery of the high affinity NMDA receptor antagonist CPP (3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) and its reported suitability as a selective, high affinity ligand for NMDA receptors in membrane preparations (Murphy et al. 1987) prompted investigation of this ligand as an alternative to [^{3}H]-glutamate for autoradiographic studies. However, using the autoradiographic method of Jarvis et al. (1987), the degree of anatomical resolution obtained with [^{3}H]-CPP was very much inferior to that produced with [^{3}H]-glutamate. In addition, signal/noise ratios obtained with [^{3}H]-CPP, using L-glutamate as a displacer, were very low (~10%). For these reasons, [^{3}H]-CPP was not suitable as an NMDA receptor ligand in autoradiographic studies. Comparatively low signal/noise ratios (excluding hippocampus) were also a feature of [^{3}H]-MK-801 autoradiograms, although average specific binding levels of ~30% were much higher than for [^{3}H]-CPP, allowing quantification of [^{3}H]-MK-801 binding. The high lipophilicity of [^{3}H]-MK-801 (Woodruff et al. 1988) may account for the relatively high levels of non-specific binding obtained with this ligand.

[^{3}H]-D-Aspartate is a substrate for the Na^+-dependent, high affinity glutamate uptake site (Balcar and Johnson, 1972) and a number of investigators have employed [^{3}H]-D-aspartate binding as a marker of glutamatergic terminals (see Chapter I). [^{3}H]-D-Aspartate binding in postmortem human cerebral and cerebellar cortex was of a high degree of anatomical resolution (Chapter III, Section 1), allowing quantification of binding within distinct cortical laminae. However, [^{3}H]-D-aspartate binding in rat brain was not as reproducible as in human. Subcortical [^{3}H]-D-aspartate binding was of a smeared, patchy appearance, preventing accurate determination of binding in distinct anatomical regions. The reasons for this binding artefact in rat but not human brain is unclear. Possible explanations include a relatively greater susceptibility of Na^+-dependent uptake sites in rat brain to freezing, (studies in rat brain indicate that freezing results in a 50% decrease in D-asartate uptake compared to fresh tissue (Hardy et al. 1987)), or an influence of the relatively higher degree of cell packing in rat brain on the accessibility of presynaptic Na^+-dependent glutamatergic binding sites.

Evidence from mutant mice and lesion studies indicate that adenosine A_1 receptors may be located on the terminals of glutamatergic neurons (Goodman et al. 1983). [^{3}H]-CHA (cyclohexyladenosine) is a selective adenosine A_1 receptor ligand and provided an alternative to [^{3}H]-D-aspartate as a presynaptic marker of retinal fibres in the rat visual system studies presented in this thesis.

1.2 [^{14}C]-2-DEOXYGLUCOSE AUTORADIOGRAPHY

1.2.1 Theory

Under normal physiological conditions the energy requirements of cerebral tissue are derived almost exclusively from the aerobic catabolism of glucose (Sokoloff, 1981). As alterations of cerebral function are intimately associated with alterations in cerebral metabolic rate (Sokoloff,1981) measurement of local rates of glucose phosphorylation provides direct insight into the level of local functional activity.

2-Deoxyglucose, a structural analogue of glucose, crosses the blood-brain barrier and is metabolised through part of the pathway of glucose metabolism at a definable rate relative to that of glucose (Figure 3). However, unlike glucose-6-phosphate, deoxyglucose-6-phosphate is not a substrate for glucose-6-phosphate dehydrogenase and remains essentially "trapped" in cerebral tissues. Thus, the quantity of deoxyglucose-6-phosphate accumulated in any cerebral tissue at any given time following the introduction of deoxyglucose-6-phosphate into the circulation, is equal to the rate of deoxyglucose phosphorylation by hexokinase during that time. This is in turn related to the amount of glucose phosphorylated over the same time.

Using the theoretical model in Figure 3, these relationships can be mathematically defined in an operational equation which defines the rates of cerebral glucose utilisation (Ri) in terms of the concentration

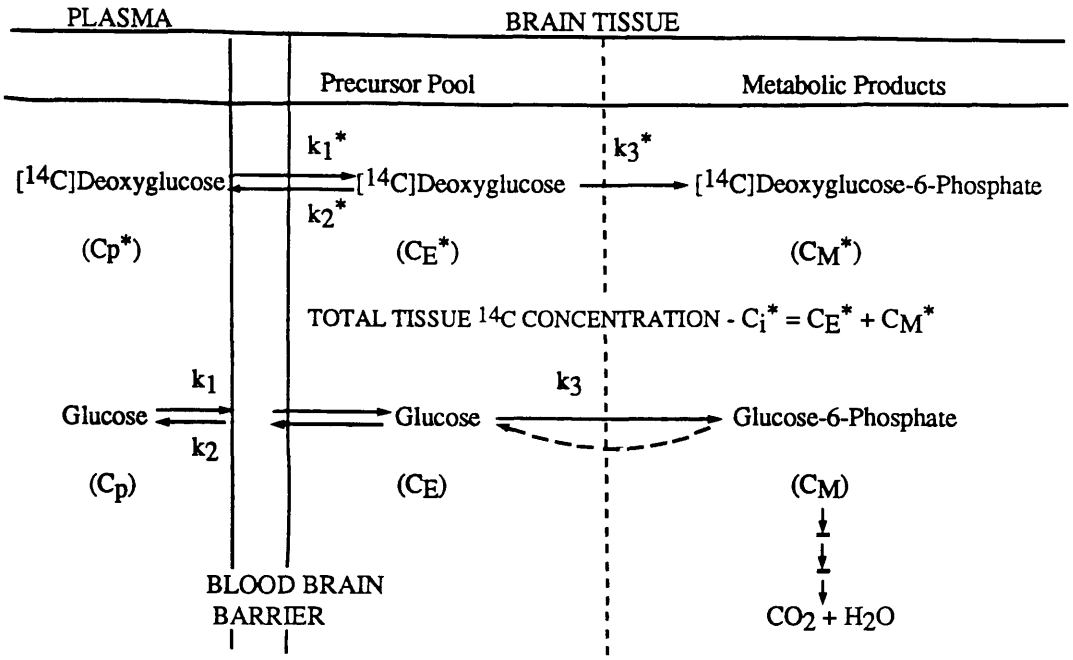

FIGURE 3. The Theoretical model.

C_p^* and C_p represent the concentrations of [^{14}C]-2-deoxyglucose and glucose in arterial plasma, C_E^* and C_E represent their respective concentrations in the tissue precursor pool. C_M^* represents the concentration of [^{14}C] deoxyglucose-6-phosphate in the tissue. The total ^{14}C concentration in a homogeneous tissue of the brain is represented by C_i^*. The constants, k_1^*, k_2^*, and k_3^*, represent the rate constants for carrier-mediated transport of [^{14}C]-2-deoxyglucose and subsequent phosphorylation by hexokinase; k_1, k_2 and k_3 are the equivalent rate constants for glucose. The dashed arrow represents the possibility of glucose-6-phosphatase activity.

(Sokoloff et al, 1977.)

of [^{14}C]-2-deoxyglucose and glucose in arterial plasma during the experimental period (Cp* and Cp) and the concentration of tracer found within cerebral tissue (Ci*) (Figure 4). In deriving this equation, the following assumptions are made:

(1) constant plasma glucose concentration and constant rate of glucose consumption throughout the period of the procedure,

(2) homogenous tissue compartment within which the concentrations of [^{14}C]-2-deoxyglucose and glucose are uniform and exchange directly with plasma,

and,

(3) [^{14}C]-2-deoxyglucose is present in tracer concentrations.

The rate constants, k_1^*, k_2^* and k_3^*, which define the distribution of tracer between plasma and brain tissue compartments and the lumped constant, K, which "corrects" for the relative preference of the glucose transport and enzyme system for glucose as opposed to 2-deoxyglucose are not measured in each experiment. The constants used are those which have been determined in other groups of animals as reported by Sokoloff and colleagues in the original description of the technique (Sokoloff et al. 1977). Although the lumped constant does not appear to change significantly over a wide range of physiological conditions (Sokoloff et al. 1977), it is conceivable that the rate constants, k_1^*, k_2^* and k_3^* may not be applicable in all

FIGURE 4 The Operational Equation

$$Ri = \frac{Ci^*(T) - k_1^* e^{-(k_2^*+k_3^*)T} \int_0^T Cp^* e^{(k_2^*+k_3^*)t}\, dt}{K \bullet \left[\int_0^T (Cp^*/Cp)\, dt - e^{-(k_2^*+k_3^*)T} \bullet \int_0^T (Cp^*/Cp)\, e^{(k_2^*+k_3^*)t}\, dt \right]}$$

where the rate of glucose consumption (Ri) is calculated in terms of the total [^{14}C] in tissue at the termination of the experiment period (Ci^* (T)) and the concentrations of [^{14}C]-2-deoxyglucose and glucose in plasma (Cp^* and CP respectively); k_1^*, k_2^* and k_3^* are the rate constants for carrier-mediated transport of [^{14}C]-2-deoxyglucose from plasma to tissue and for phosphorylation by hexokinase respectively. The lumped constant K, is composed of the ratio of the distribution space for deoxyglucose in the tissue relative to that of glucose, a factor which corrects for the fraction of glucose which, once phosphorylated, continues down the glycolytic pathway and the Michaelis-Menten kinetic constants of hexokinase for deoxyglucose and glucose (Sokoloff et al. 1977).

physiological states. However, potential errors in calculations of glucose utilisation arising from uncertainty in the values of these constants are minimised in the operational equation by allowing sufficient time for the clearance of [^{14}C]-2-deoxyglucose from plasma and the exponential terms containing the rate constants to fall to levels too low to influence the result. An experimental time course of 45 minutes limits the influence of exponential factors in the operational equation whilst ensuring minimal loss of [^{14}C]-2-deoxyglucose 6-phosphate from the tissues due to glucose-6-phosphatase activity (Sokoloff et al. 1977).

1.2.2 Practice

The design of the experimental procedure for the [^{14}C]-2-deoxyglucose technique was based on the theoretical considerations discussed above. Measurements of local cerebral glucose use were performed on conscious rats prepared for the experiment by the insertion of polyethelene catheters in the femoral vessels.

Each experiment was initiated with an intravenous infusion of [^{14}C]-2-deoxyglucose (125μCi/kg) injected at a constant rate over 30 seconds. A total of 14 samples of arterial blood (~75μl/sample) were withdrawn from the femoral cannula according to a pre-determined schedule over the subsequent 45 minutes. These samples were immediately centrifuged to separate the plasma from the cell fraction

and minimise haemolysis. The concentrations of [^{14}C]-2-deoxyglucose (Cp*) and glucose (Cp) were determined in each arterial sample by using scintillation counting and a standard glucose enzyme assay (Beckman glucose analyser) respectively. After 45 minutes the animal was killed by decapitation and the brain was frozen and processed for quantitative autoradiography (3.4.2). Rates of local cerebral glucose utilisation within anatomically discrete regions of the brain were calculated from the local tissue concentrations of [^{14}C] (Ci*) and the plasma [^{14}C]-2-deoxyglucose (Cp*) and glucose (Cp) concentrations according to the operational equation (Figure 4).

1.3 COMBINED IN VITRO RECEPTOR AUTORADIOGRAPHY AND IN VIVO [^{14}C]-2-DEOXYGLUCOSE AUTORADIOGRAPHY

An *a priori* requirement for the combination of [^{14}C]-2-deoxyglucose autoradiography and in vitro receptor autoradiography is the elution of [^{14}C] from slide-mounted tissue sections which may then be used in ligand binding studies. Figure 5A (overleaf) illustrates the elution of [^{14}C] from slide-mounted tissue sections taken from a brain labelled with [^{14}C]-2-deoxyglucose. After washing, sections were wiped into scintillation vials using Whatman GB/F filter papers and counted on a scintillation counter. Division of resulting dpm/section values by 2220 (dpm/nCi) and subsequent division of this calculated value by wet section weight allowed determination of nCi/g values. Figure 5B illustrates the effect of longer wash times on the elution of [^{14}C] from slide-mounted tissue sections. Routinely, in combined

autoradiographic studies, tissue sections received two 30-second washes in an appropriate buffer prior to preincubation for ligand binding autoradiography. Any small residual amounts of [^{14}C] which may have remained in tissue sections would have been accounted for in the non-specific signal determined in the receptor binding studies.

LEGEND TO FIGURE 5

The elution of [^{14}C] from slide mounted tissue sections was determined in coronal sections taken from a brain labelled with [^{14}C]-2-deoxyglucose.

Figure 5A - the rapid elution of [^{14}C] using wash times of 2 - 12 seconds.

Figure 5B - elution of [^{14}C] using increased number of 30 second washes.

After washing, sections were wiped into scintillation vials using Whatman GB/F filter papers and the level of radioactivity determined using LSA. For each wash time, the number of nCi/g was determined from three individual sections and is presented as the mean ± SEM.

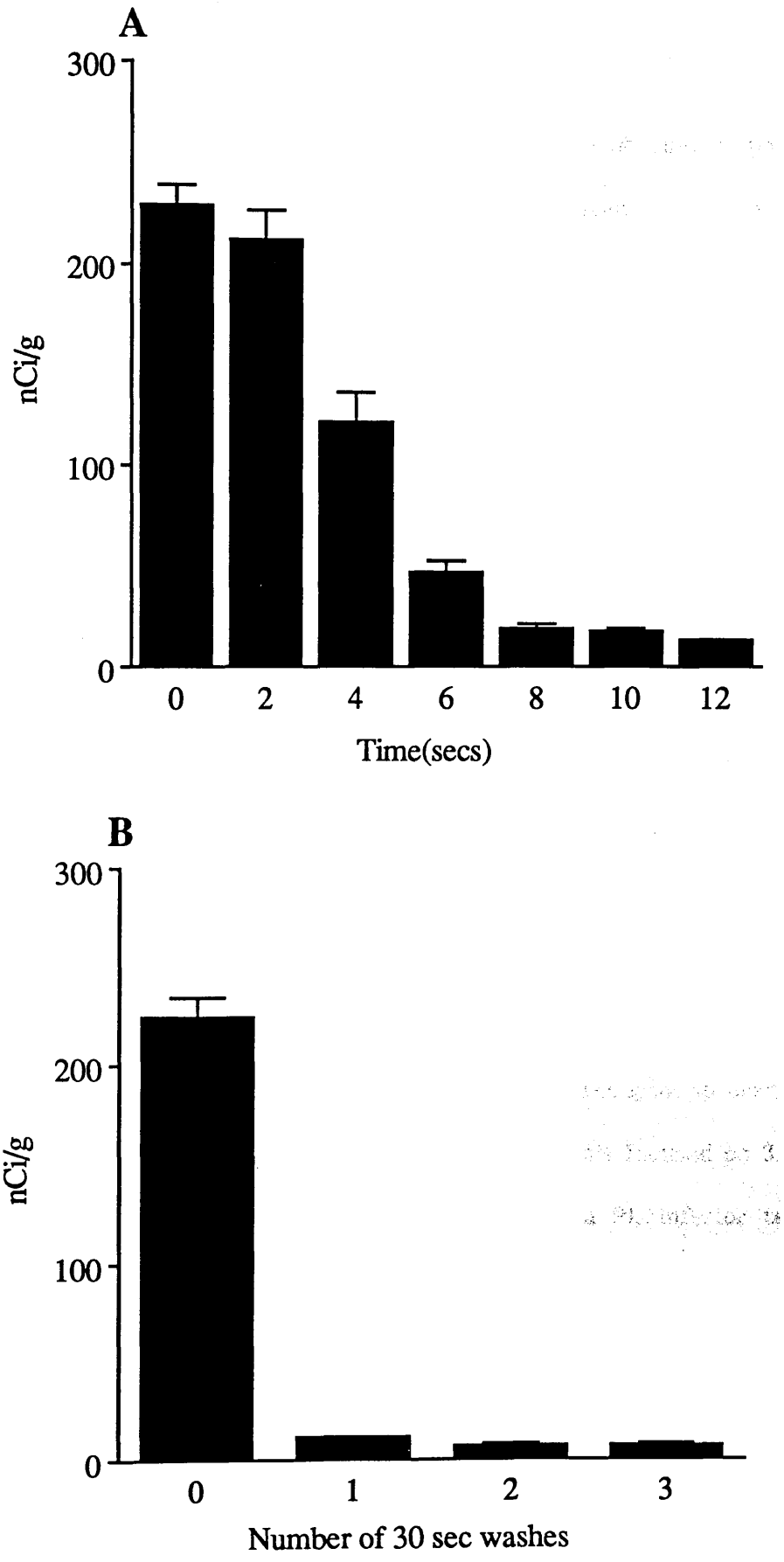

FIGURE 5 ELUTION OF [^{14}C] FROM SLIDE-MOUNTED TISSUE SECTIONS

2. HUMAN POSTMORTEM STUDIES

2.1 CLINICAL INFORMATION

Both control and Alzheimer brains were obtained at postmortem from local psychiatric and geriatric hospitals. As part of an ongoing longitudinal study of Alzheimer's Disease, (A.D.), A.D. patients were diagnosed antemortem according to the criteria set out in CAMDEX (Roth et al. 1986). All Alzheimer patients used in these studies had subsequent postmortem neuropathological confirmation of the clinical diagnosis of A.D. (Khachaturian, 1985). All control patients used had no known neurological or neuropsychiatric disorders. The relevant brain statistics for both control and Alzheimer patient groups are listed in Table 3 (overleaf).

2.2 BRAIN DISSECTION

At autopsy, brains from both control and Alzheimer patients were cut into 1cm thick coronal slabs and discrete anatomical regions of interest were dissected out, frozen in isopentane (-40°C) and stored at -80°C in preparation for subsequent receptor autoradiography. The receptor studies described in this thesis focused on 3 brain regions; middle frontal gyrus (Brodmann area 9), inferior temporal gyrus (Brodmann area 20) and cerebellar cortex. Adjacent undissected tissue was fixed in 10% formalin and processed for senile plaque quantification. Only tissue from the left hemisphere was used in these studies.

TABLE 3 - PATIENT STATISTICS

CONTROL					
CASE	AGE	PM. DELAY HOURS	SEX	CAUSE OF DEATH	MEDICATION
A	88	13.5	M	Peritonitis	-
B	80	23	F	Bronchopneumonia	Thyroxine
C	88	11	F	Bronchopneumonia	Thyroxine
D	87	12	F	Pulmonary Thromboembolism	Temazepam Diamorphine
E	76	17	M	Bronchopneumonia	Metoclopramide Diamorphine
F	86	19	F	Sigmoid Carcinoma	Diamorphine Ampicillin
Mean Age = 84 ± 2		Mean PM. Delay = 15 ± 2			
ALZHEIMER					
CASE	AGE	PM. DELAY HOURS	SEX	CAUSE OF DEATH	MEDICATION
G	83	10	M	Bronchopneumonia	Pethidine
H	89	9.5	M	Intra-abdominal Abscess	Mianserin Metoclopramide
I	89	3.5	F	Bronchopneumonia	Temazepam Diamorphine
J	85	3	F	Bronchopneumonia	Diamorphine
K	92	15	F	Bronchopneumonia	-
L	97	7	F	Bronchopneumonia	-
Mean Age = 89 ± 2		Mean PM. Delay = 8 ± 2		(M = Male, F = Female)	

Brain dissection was carried out in a containment facility and material was only used after clearance for hepatitis B and C-J disease.

2.3 SENILE PLAQUE QUANTIFICATION AND CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY

Senile plaque numbers were determined in sections taken from tissue blocks no more than 1cm caudal to those used for receptor auto-radiography. Cryostat-cut sections (28μm thick) were stained with King's Amyloid and subsequent quantification of senile plaques was performed using a light microscopic image analysis system (Quantimet Q250, Cambridge Instruments). In frontal and temporal cortex, mean senile plaque numbers were calculated from 6 individual readings (3 on gyri, 3 on sulci) in both superficial (cortical layers I-III) and deep (cortical layers IV-VI) cortical laminae. Similarly, in cerebellar cortex, mean senile plaque numbers were calculated from 6 individual readings encompassing both molecular and Purkinje/granule cell layers. Senile plaque quantification was performed independently by an investigator who had no knowledge of the ligand binding studies. In both control and Alzheimer patients, ChAT activity was determined in frontal and temporal cortex using the method of Fonnum (1975).

2.4 IN VITRO LIGAND BINDING AUTORADIOGAPHY

The receptor protocols employed in these studies were outlined in section 1 of this chapter. This section describes aspects of the technique relevant to human brain investigations.

2.4.1 Background

In human postmortem studies, the major advantages of ligand binding autoradiography over homogenate binding techniques are:-

(1) increased anatomical resolution, permitting light microscopic examination of neurotransmitter receptors in single cortical layers

and (2) the ability to perform serial ligand binding studies in adjacent tissue sections, allowing meaningful comparisons of neurotransmitter receptor parameters (pre-/postsynaptic; receptor subtypes) in the same brain region.

In addition, by quantifying senile plaque numbers in adjacent tissue blocks to those used for ligand binding studies, the relationship between local neuropathology and neurotransmitter receptor alterations can be determined in the same region.

As applied to human postmortem material, a general possible limitation of the technique is increased inter-individual variability related to such factors as age of patient, drug treatment history and postmortem delay. This problem was minimised in the studies described in this thesis by matching control and A.D. patient groups as closely as possible (Table 3). A more specific problem in using receptor autoradiography to study postmortem material from A.D.

patients relates to the possible alterations in grey-white matter ratios in different brain regions due to the neurodegenerative process. It has been known for some time that white matter, because of its greater density causes a relatively greater absorption of tritium-emitted β rays than grey matter (Kuhar and Unnerstall, 1985; Geary and Wooten, 1985) and that optical density values for tritium-labelled ligands are consequently underestimated (quenched) in regions of high white matter density. Hence any alterations in grey-white matter ratios in A.D. may influence absolute quantification of ligand binding densities in A.D. tissue. The use of iodonated ligands, whose higher energy emissions are not significantly altered by tissue composition, avoids the problem of white matter auto-absorption. However, as excitatory amino acid receptor ligands are, as yet, unavailable in iodonated form, tritium signal quenching remains a possible complicating factor in the comparison of these tritiated ligand binding densities in control and A.D. tissue.

2.4.2 Practice

Serial sections from each region of interest were used for the determination of [^{3}H]-D-aspartate, [^{3}H]-kainate, [^{3}H]-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H]AMPA) and NMDA-sensitive [^{3}H]-glutamate binding in both A.D. and control brains. As illustrated in Figure 6, by employing these selective ligands in adjacent brain sections it was possible to image and quantify both

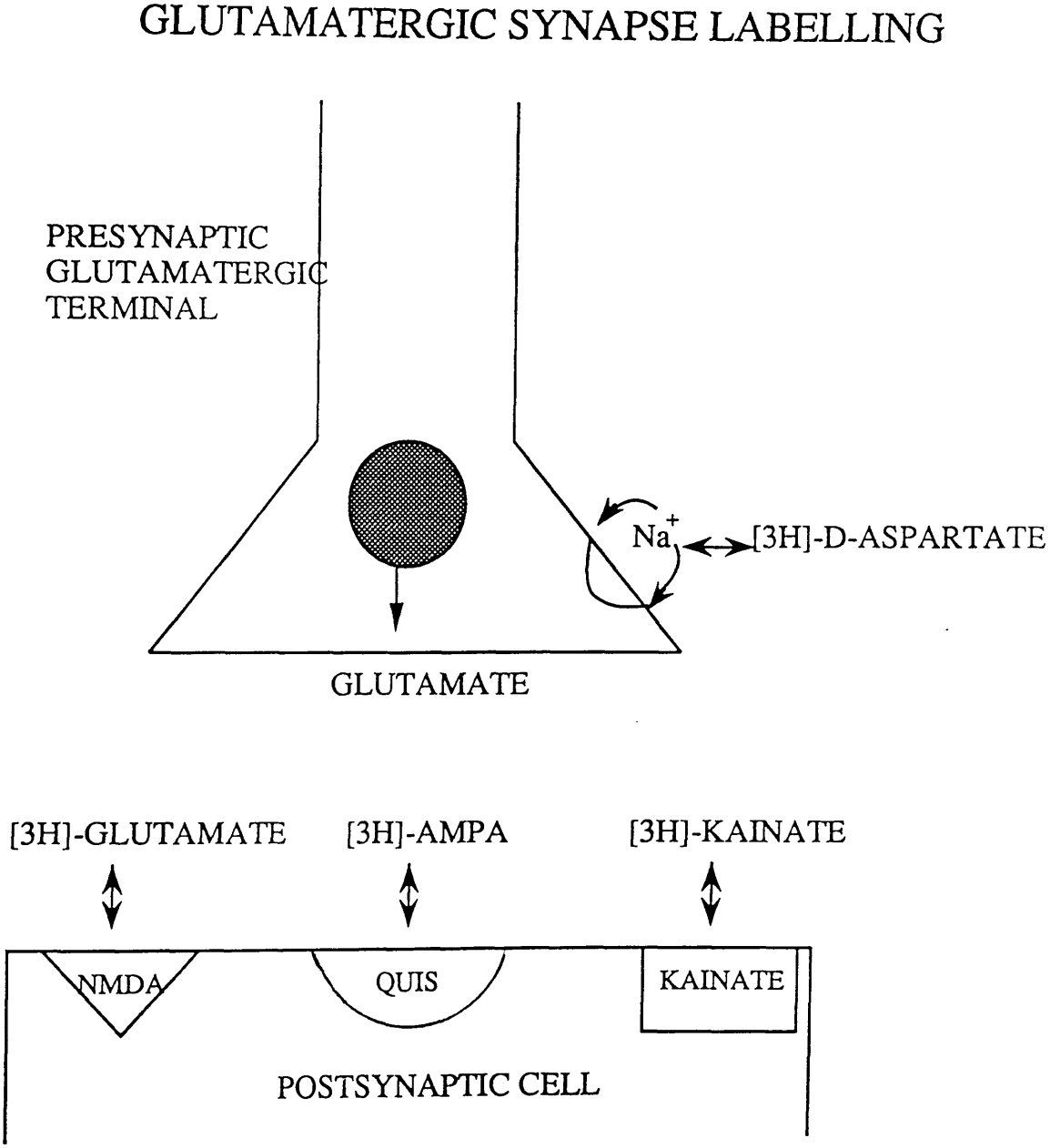

FIGURE 6 DIAGRAMMATIC REPRESENTATION OF GLUTAMATERGIC BINDING SITES

presynaptic glutamatergic input and postsynaptic glutamate receptors in the same brain region. The ligand binding protocols employed are outlined in section 1 of this chapter. For each ligand, control and A.D. sections were incubated and washed simultaneously. Sections were then placed in x-ray cassettes (1 A.D. and 1 control case/x-ray film) with tritium microscales (Amersham) and apposed to tritium hyperfilm (Amersham) for 4 - 6 weeks. Resulting autoradiograms were analysed as described in section 3 of this chapter. Binding to discrete cortical laminae was determined by comparison of autoradiograms to adjacent cresyl violet stained sections. Mean optical density measurements for each laminae were calculated from 6 individual readings (3 on gyri, 3 on sulci) averaged over 3 sections. Optical density values were converted to pmoles of ligand bound/g tissue with reference to precalibrated tritium microscales and the specific activities of the ligands.

2.4.3 Analysis

Statistical differences between control and AD cases were determined using an unpaired, two-tailed Student's t-test. Linear regression analysis was used to determine the relationship between ligand binding and senile plaque data.

3. VISUAL SYSTEM STUDIES

3.1 GENERAL

Studies in the rat visual system utilised two experimental techniques; [^{14}C]-2-deoxyglucose autoradiography and *in vitro* ligand binding autoradiography. Animal surgery employed in these studies and experimental elements common to both techniques are described in this section.

3.2 ANIMALS

All experiments were performed on male adult black-hooded rats of the PVG strain. Animals used weighed between 250g - 400g corresponding to a post-weaning age of 10 to 20 weeks. On delivery from the supplier (Harlan Olac), rats were allowed to re-acclimatise for at least 2 days (3 nights) prior to initiation of any experimental procedures in order to avoid the possibility of starvation-induced physiological changes. In holding, rats were exposed to a natural day/night cycle and room temperature was maintained at approximately 21°C. Animals were fed *ad libitum* until the day of the experiment.

3.3 SURGICAL PREPARATION OF ANIMALS

3.3.1 Unilateral Orbital Enucleation

Prior to surgery, animals were placed in a perspex box into which anaesthetic gas mixture was flowing (70% nitrous oxide: 30% oxygen, containing 5% halothane). During surgery (no more than 3 minutes) anaesthesia was maintained by means of a face mask through which

a 2% halothane mixture was delivered. The surgical approach involved exposing the eyeball by retracting the eyelid muscles with a curved haemostat and clamping the underlying retractor oculi muscle, enclosed blood vessels and optic nerve. With curved scissors the eyeball was then removed by incision between the posterior globe wall and the haemostat. Haemorrhage from the severed artery and vein was prevented by sealing the vessels with a bipolar coagulator.

3.3.2 Cannulation of Femoral Vessels

Anaesthesia was induced and maintained as described in 3.3.1. The femoral vessels were exposed by making small incisions (1cm or less) at the groin in either side of the animal. Both femoral arteries and femoral veins were freed from connective tissue and polythene cannulae (Portex: external diameter 0.96mm; internal diameter 0.58mm, 15cm long and filled with heparinised saline (10 I.U./ml) were inserted a distance of 2cm into both femoral arteries and one femoral vein. Having verified the patency of the cannulae, each cannula was tied in place and the wounds were sutured closed. Sites of incision were smeared with a local anaesthetic gel, covered with gauze pads and the entire pelvic area enveloped in a surgical stocking. A plaster of Paris bandage (Gypsona: 7.5cm wide) was applied around the lower abdomen, pelvis and hindquarters, immobilising the rear legs without impairing normal respiration. The plaster cast and the animal's hindlimbs were taped to a lead support brick to completely immobilise the animal.

Core temperature was monitored by a rectal probe and a pressure transducer (P23 1D Gould Stratham) attached to a chart recorder (Gould Stratham, Model 2202) which was connected to one of the arterial femoral catheters to monitor blood pressure. Anaesthesia was then withdrawn and the animal allowed to recover for at least 2 hours prior to tracer administration.

3.4 EXPERIMENTAL ANALYSIS

3.4.1 Liquid Scintillation Analysis (LSA)

LSA was a necessary component of both [^{14}C]-2-deoxyglucose experiments and ligand binding studies using tritiated ligands. Plasma samples containing [^{14}C] or [^{3}H] were pipetted into 1ml of distilled water in plastic scintillation vials. 10ml of a proprietary scintillation cocktail (Ecoscint A, National Diagnostics) was added to each counting vial and samples were counted for 4 minutes in a refrigerated scintillation counter. Counts per minute were converted to disintegrations per minute using the external channels ratio method (Peng, 1977) and a standard quench correction calibration curve. For each [^{14}C]-2-deoxyglucose experiment the accuracy of the computerised correction was evaluated in 2 vials subjected to the internal standard method (Peng, 1977).

3.4.2 Preparation of [^{14}C]-2-Deoxyglucose Autoradiograms

At the end of the sampling period for the [^{14}C]-2-deoxyglucose technique, animals were sacrificed by decapitation. A midline incision was made at the level of the snout and extended to the level of the forelimb. Skin and the underlying extracranial muscle were then reflected to expose the skull. The dorsal cranium was removed, the underlying dura reflected, and the whole brain removed intact from the skull. All adhering bone fragments were removed and the brain was frozen in isopentane which had been precooled to -42°C. Total dissection and freezing time did not exceed 5 minutes. The brain was mounted to a swivel-headed microtome chuck with a plastic embedding matrix (Lipshaw) over a bed of solid CO_2. Coronal brain sections, 20μm thick, were cut in a cryostat maintained at -22°C. For all studies, brains were cut from the level of the inferior colliculi to the level of the dorsal thalamus. In combined [^{14}C]-2-deoxyglucose and ligand binding studies, 1 section in 7 was picked up onto a thin glass coverslip and rapidly dried on a hot plate (60°C), whilst the intervening 6 sections were picked up onto subbed glass slides and dried at room temperature in preparation for receptor autoradiography. The coverslips were glued onto thin card and apposed to x-ray film (Kodak-GRL-A) in light-tight cassettes, together with a set of [^{14}C]-labelled epoxy resin standards (18-1880μCi/g tissue equivalents). Autoradiograms were obtained after 14 days exposure. Films were processed using a standard Kodak automatic processor.

3.4.3 Preparation of Ligand Binding Autoradiograms

After incubation with any radioligand, tissue sections were washed and dried, then securely mounted to rigid cardboard. Under red safelight, sections were apposed to tritium-sensitive film (Hyperfilm B_{max}, Amersham) and locked in light-tight cassettes for periods ranging from 10 to 28 days. To enable quantification of the resulting autoradiograms, precalibrated tritium standards (Appendix 1) were co-exposed with each film. At the end of the exposure time, films were manually developed in Kodak D-19 developer for 5 minutes at 17°C with intermittent agitation. Development was stopped with a 30 second rinse in deionised water at 20°C. After fixing for 10 minutes at 20°C (Kodak Kodafix), films were washed for 40 minutes in running filtered water, rinsed in deionised water and suspended in a drying cabinet.

3.4.4 Quanitification of Autoradiograms

Analysis of resultant [^{14}C]-2-deoxyglucose and ligand binding autoradiograms was performed using a computer-assisted image analysis system (Quantimet 970, Cambridge Instruments). All optical density measurements were carried out under constant low lighting to minimise the contribution of stray light. Illumination of autoradiograms was provided by reflected white light from four 24W lamps; the light intensity being computer-controlled.

The image of an autoradiogram was captured by a Cholnicon video-camera fitted with a Tamron zoom lens (2.5x) and digitised into an array of image points (pixels) each with a grey level value in the range 0 - 255, (grey level = 255 corresponds to optical density = 0; grey level = 1 corresponds to optical density = 2.41). In order to make reproducible measurements, the image-analyser was calibrated to establish calibration coefficients and the maximum optical density measurable. The scanner was first set up in the autobrightness mode on a blank part of film giving a white level of 1.0 (100% transmission). The output of the video scanner at zero illumination (dark current grey level) was determined by interrupting the light path with an opaque object and measuring the mean grey level produced. Finally, the image-analyser was calibrated against a Kodak neutral density filter (O.D. = 1.0) to convert the grey level values of each pixel into optical density values.

Having calibrated the image analyser, the optical densities of the images produced by precalibrated standards were measured, providing a calibration curve of optical density versus isotope concentration (^{14}C or ^{3}H) and allowing subsequent quantification of tissue isotope concentration for any area of interest. For each anatomical area of interest, the image analyser calculated the mean of three integrated optical density measurements (integrated optical density = sum of all point optical densities within the measurement frame). The size of the measuring frame was variable (9 - 900 pixels/frame) but was

maintained constant for each anatomical region between animals. In visual system studies, optical density values for each area of interest were calculated from 3 to 6 sections. Structures were defined anatomically with reference to a stereotaxic atlas (Paxinos and Watson, 1986).

3.4.5 Statistical Analysis

Asymmetries in glucose utilisation and ligand binding densities were determined using Student's paired t-test. In view of the large number of interhemispheric comparisons made for each parameter, statistical significance was taken at $P<0.01$. The stability of glucose utilisation and ligand binding in anatomical stuctures of the visually-intact hemipshere up to 20 days post-enucleation was assessed using the analysis of variance (Scheffé, 1959) and the multiple comparisons method of Dunnett (1964). Statistically significant intrahemispheric variation for any measured parameters was attained at $P<0.01$ for the appropriate degrees of freedom between and within groups.

4. MATERIALS

[^{14}C]-2-deoxyglucose (50Ci/mmol), [^{3}H]-D-aspartate (25Ci/mmol), [^{3}H]-glutamate (50-59Ci/mmol), [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB) (44Ci/mmol), [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) (75Ci/mmol) and [^{3}H]-muscimol (21.2Ci/mmol) were all supplied by Amersham International. [^{3}H]-Kainate (58-60Ci/mmol), [^{3}H]-α-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H]-AMPA) (27.6 - 29.2Ci/mmol), [^{3}H]-MK-801 (15Ci/mmol), [^{3}H]-5-hydroxytryptamine ([^{3}H]-5HT) (20Ci/mmol) and [^{3}H]-ketanserin (61Ci/mmol) were obtained from New England Nuclear. The Sigma Chemical Company supplied 5-hydroxytryptamine, γ-amino-butyric acid, atropine sulphate, propranolol hydrochloride, D-aspartate, kainate, quisqualate, N-methyl-D-aspartate and 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (S.I.T.S.). MK-801 maleate and methysergide were kindly donated by Merck Sharpe and Dohme, and Sandoz Laboratories, respectively.

CHAPTER III

RESULTS AND COMMENTARY

1. PRE- AND POSTSYNAPTIC EXCITATORY AMINO ACID (E.A.A.) RECEPTOR BINDING SITES IN HUMAN POSTMORTEM TISSUE.

1.1 E.A.A. RECEPTOR BINDING SITES IN CONTROL BRAINS.

[^{3}H]-D Aspartate binding in sections of temporal cortex was entirely dependent on the presence of sodium ions (Figure 7A). No significant specific binding was detectable in the absence of sodium. The mean EC_{50} for sodium stimulation of binding was 25mM. [^{3}H]-D-aspartate binding (1-2000nM) in the presence of 300mM sodium chloride is illustrated in Figure 7B).

[^{3}H]-Kainate binding was saturable in the range 1-65nM (Figure 8A) whilst, in the presence of 100mM potassium thiocyanate, [^{3}H]-AMPA exhibited saturable binding in the range 10-1200nM (Figure 8B). Total [^{3}H]-glutamate binding, in the absence of quisqualate and S.I.T.S., is illustrated in Figure 8C in the range 1-1500nM.

1.2 ANATOMICAL DISTRIBUTION OF E.A.A. RECEPTOR BINDING SITES IN CONTROL BRAINS.

1.2.1 Middle Frontal Gyrus and Inferior Temporal Gyrus

The general distribution of both presynaptic glutmate uptake site binding and postsynaptic glutamate receptor binding was similar in both frontal and temporal cortex. The most prominent feature of

LEGENDS TO FIGURES

FIGURE 7A

The sodium dependency of [^{3}H]-D-aspartate binding was determined in temporal cortex sections from three control brains. [^{3}H]-D-aspartate (500nM) binding was performed in the presence of sodium ions (1-350mM) added as the chloride salt sections were then wiped into scintillation vials and the level of radioactivity determined using LSA. Non-specific binding was determined in the presence of 500μM D-aspartate. Values are presented as the mean specific binding levels calculated from 3 total and 2 non-specific sections/sodium ion concentration/brain.

FIGURE 7B

[^{3}H]-D-Aspartate binding (1 - 2000nM) was performed in the presence of 300mM sodium chloride in temporal cortex sections from three control brains. Radioactivity levels were calculated from wiped sections using LSA. Values are presented as mean binding levels calculated from 9 total and 6 non-specific sections for each ligand concentration. Non-specific binding at each concentration was determined in the presence of D-aspartate (1000 x in excess of ligand concentration).

FIGURE 7 [^{3}H]-D-ASPARTATE BINDING IN HUMAN POSTMORTEM TISSUE

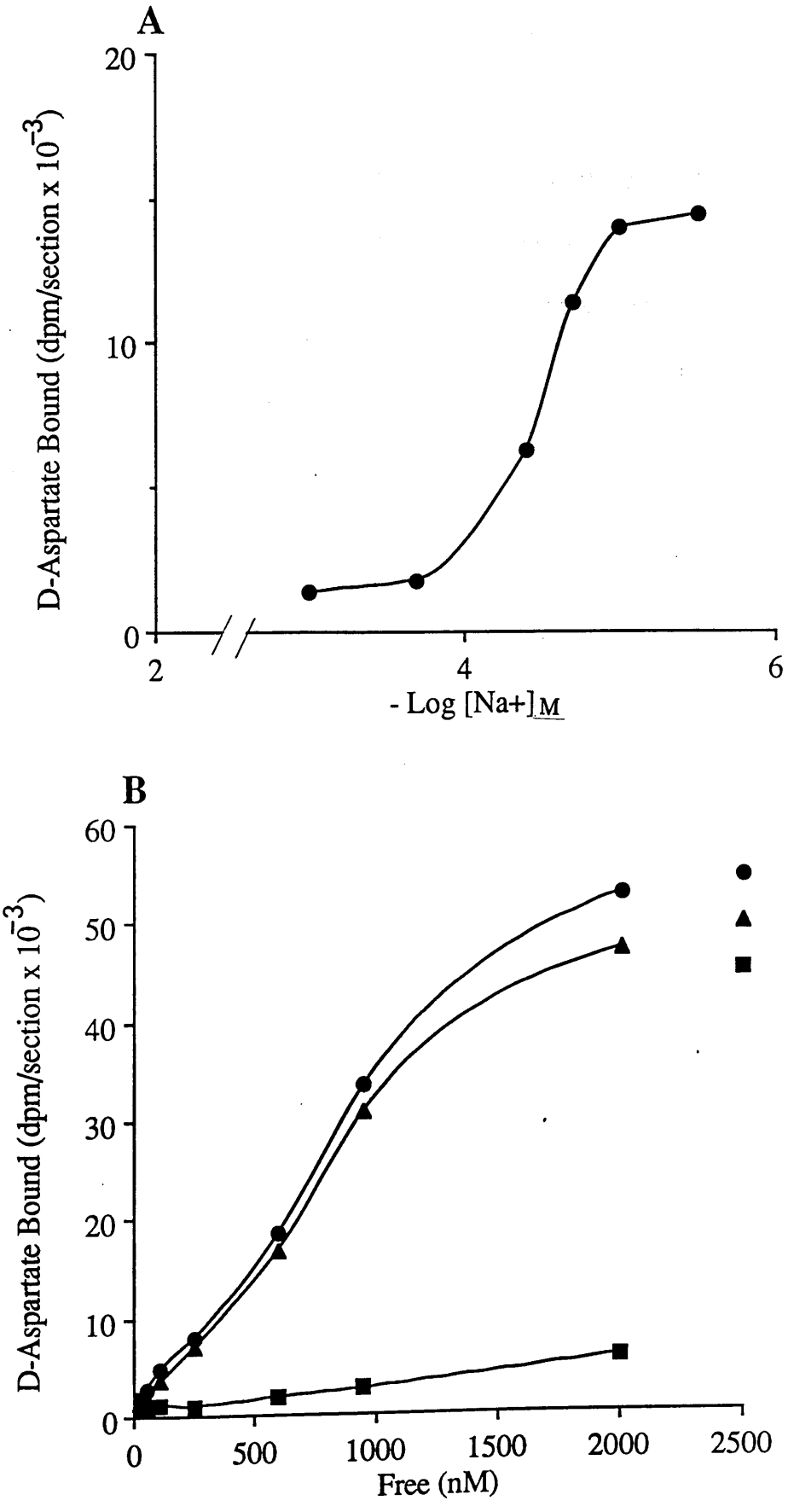

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FIGURE 8

[^{3}H]-Kainate (1-65nM) (A), [^{3}H]-AMPA (10-1200nM) (B) and [^{3}H]-glutamate (1-1500nM) (C) binding were determined in sections of temporal cortex from 3 control brains. Values were presented as mean binding levels calculated from 9 total and 6 non-specific sections for each ligand concentration. For each ligand, non-specific binding was determined in the presence of kainate, quisqualate and L-glutamate.

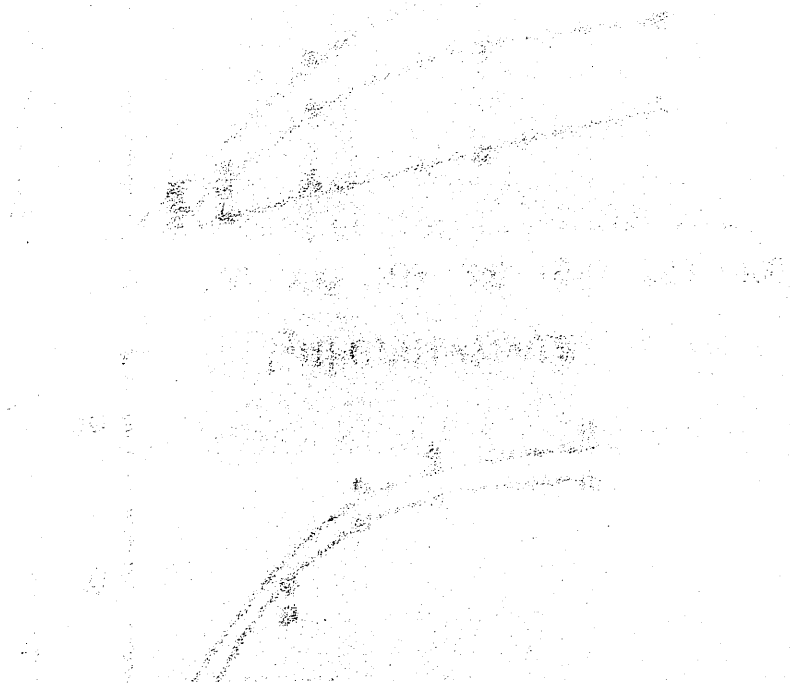
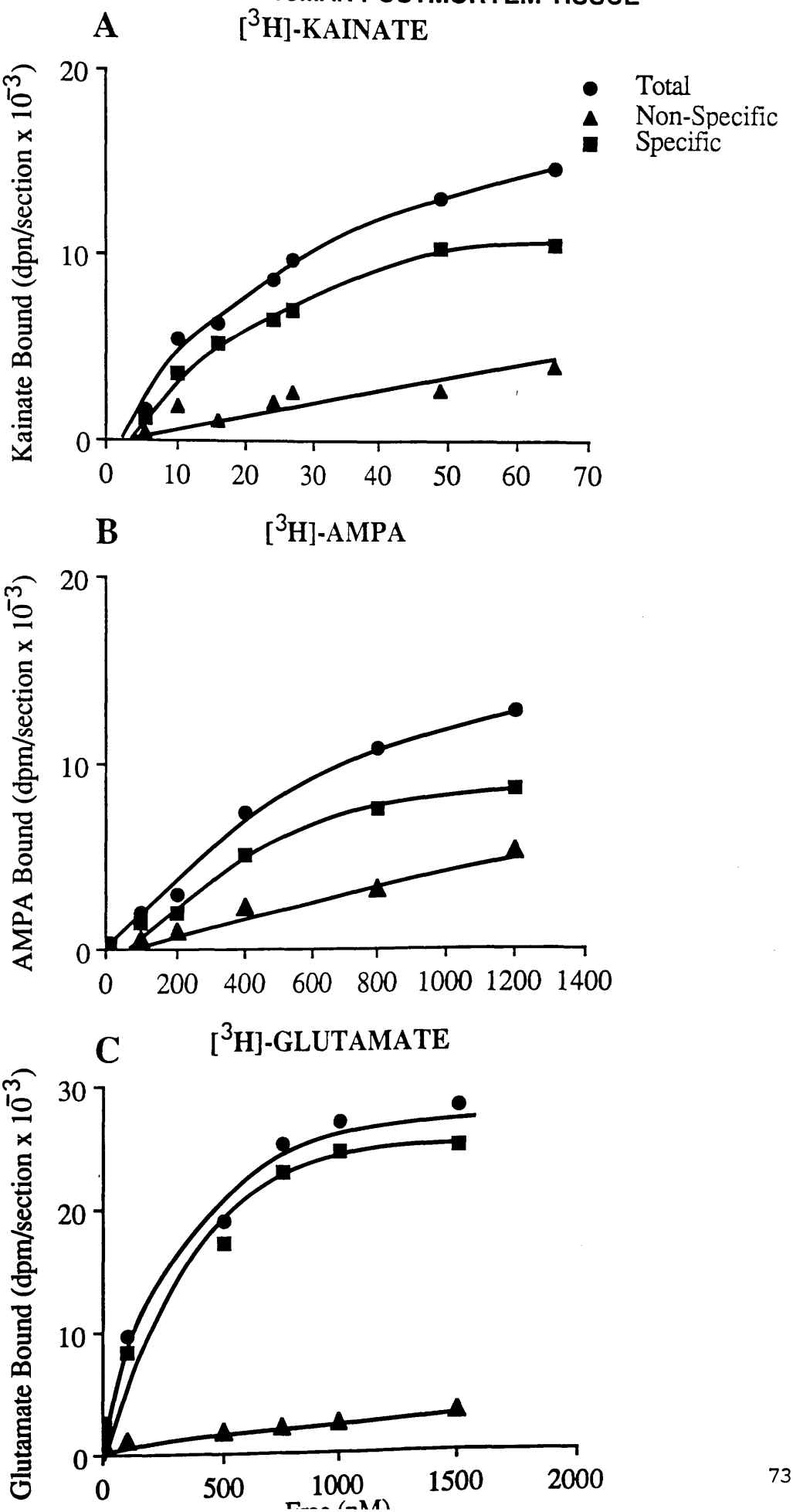


FIGURE 8 [^{3}H]-KAINATE, [^{3}H]-AMPA AND [^{3}H]-GLUTAMATE BINDING IN HUMAN POSTMORTEM TISSUE

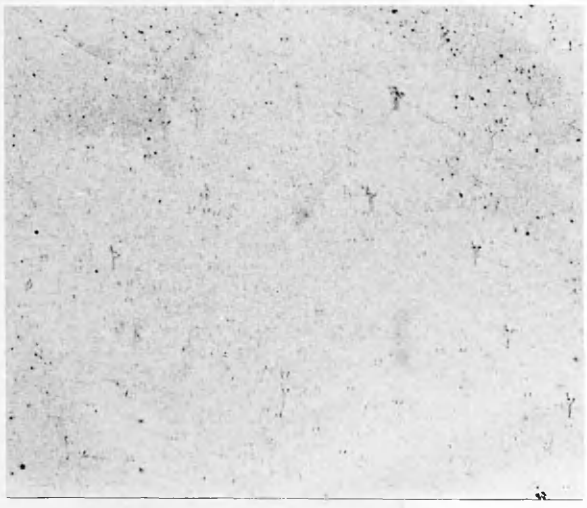


[^{3}H]-D-aspartate autoradiograms was the presence of two bands of high optical density corresponding to cortical layers III and V/VI (Figure 9), indicating a high density of Na^+- dependent glutamate uptake sites in these laminae. [^{3}H]-Kainate autoradiograms exhibited a bilaminar pattern; [^{3}H]-kainate binding levels in cortical layers V/VI being approximately 50% greater than those in cortical layers I-III (Figure 9). By way of contrast, NMDA-sensitive [^{3}H]-glutamate binding levels in cortical layers I-III were approximately 20% greater than those in cortical layers V/VI (Figure 9). Similarly, [^{3}H]-AMPA binding was concentrated in cortical layers I/II, indicating a high density of quisqualate receptors in these laminae (Figure 9).

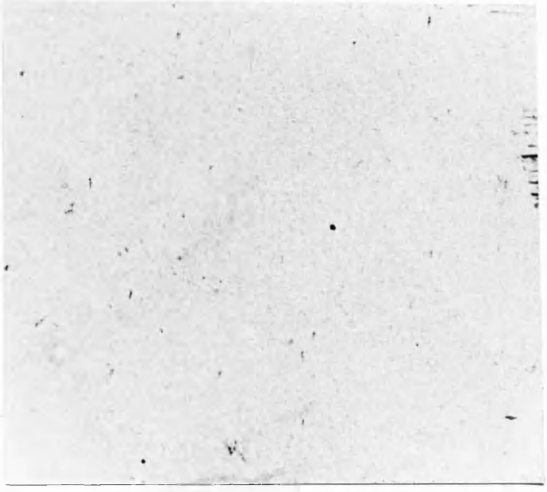
1.2.2 Cerebellar Cortex

[^{3}H]-D-Aspartate binding in the granule cell layer of the cerebellar cortex was approximately 30% greater than that in the molecular layer (Figure 10). Similarly, both [^{3}H]-kainate and NMDA-sensitive [^{3}H]-glutamate autoradiograms were characterised by a high band of optical density corresponding to the granule cell layer (Figure 10). In contrast to the distribution of these ligands, [^{3}H]-AMPA binding in the molecular layer was approximately four times greater than that in the granule cell layer (Figure 10). Thus it would appear that quisqualate receptors are concentrated in the molecular layer of cerebellar cortex, whilst both NMDA and kainate receptor sites are more numerous in the granule cell layer.

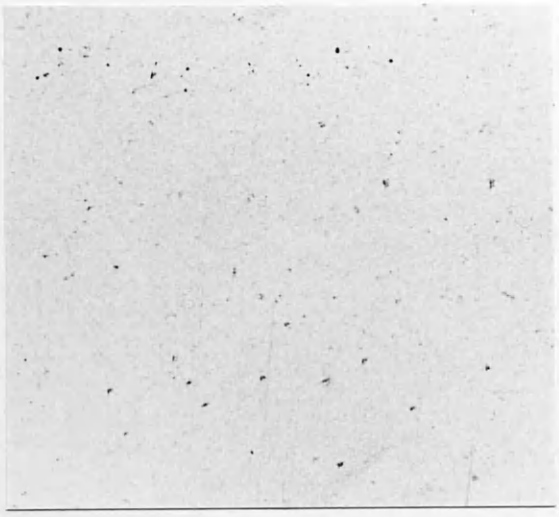
UPTAKE SITES



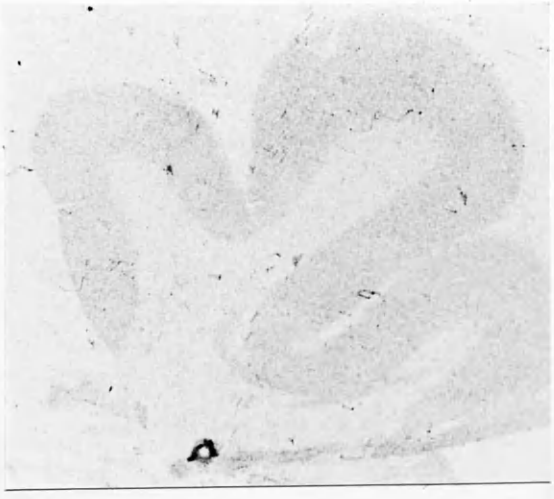
KAINATE



QUISQUALATE



NMDA



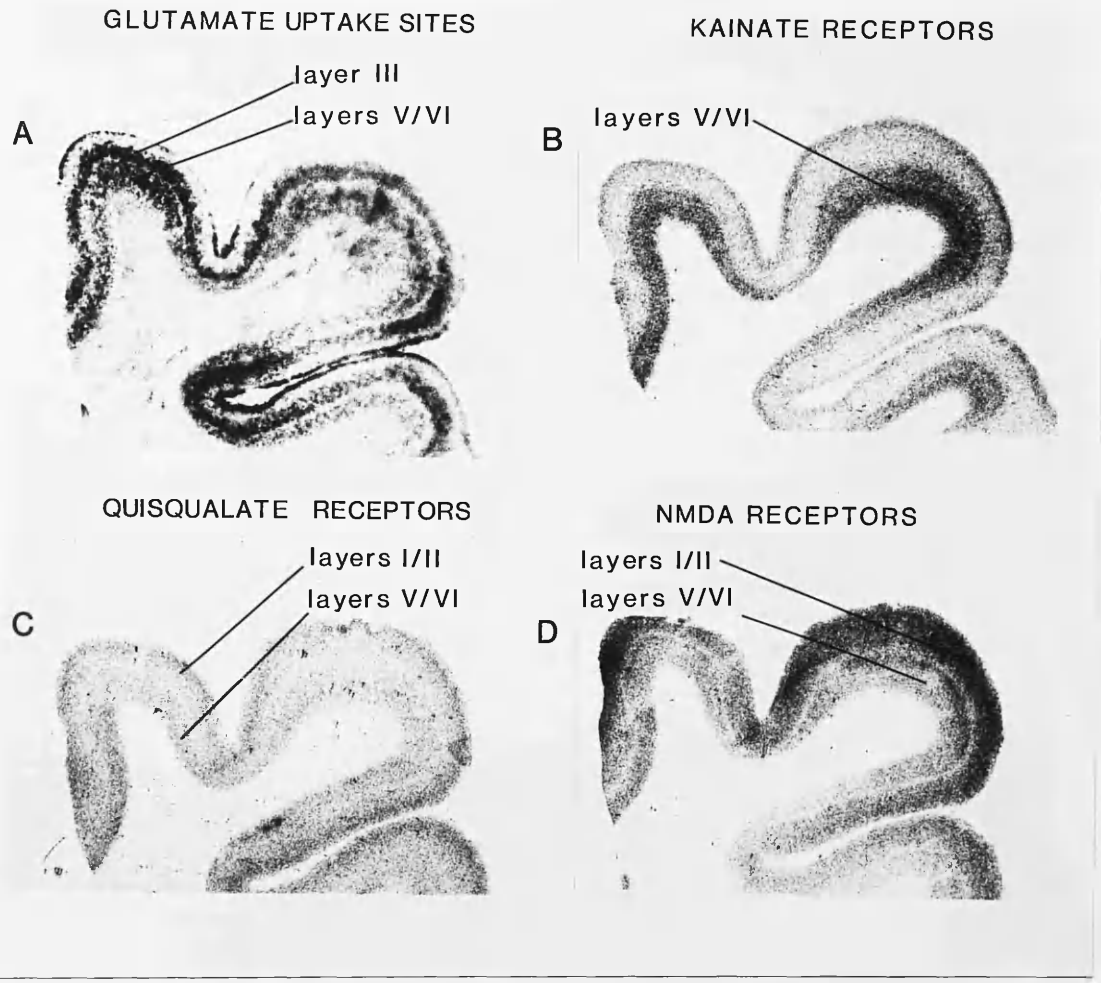
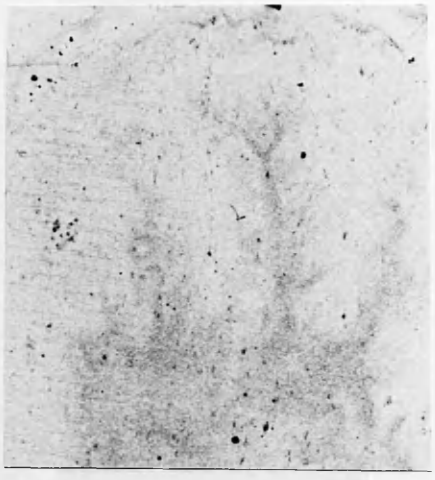


FIGURE 9 GLUTAMATERGIC SITES IN FRONTAL CORTEX.

Representative autoradiograms of (A) glutamate uptake site binding, (B) kainate receptor binding, (C) quisqualate receptor binding and (D) NMDA receptor binding in adjacent sections of frontal cortex.

For respective non-specific images see opposite page.

UPTAKE SITES



KAINATE

QUISQUALATE

NMDA

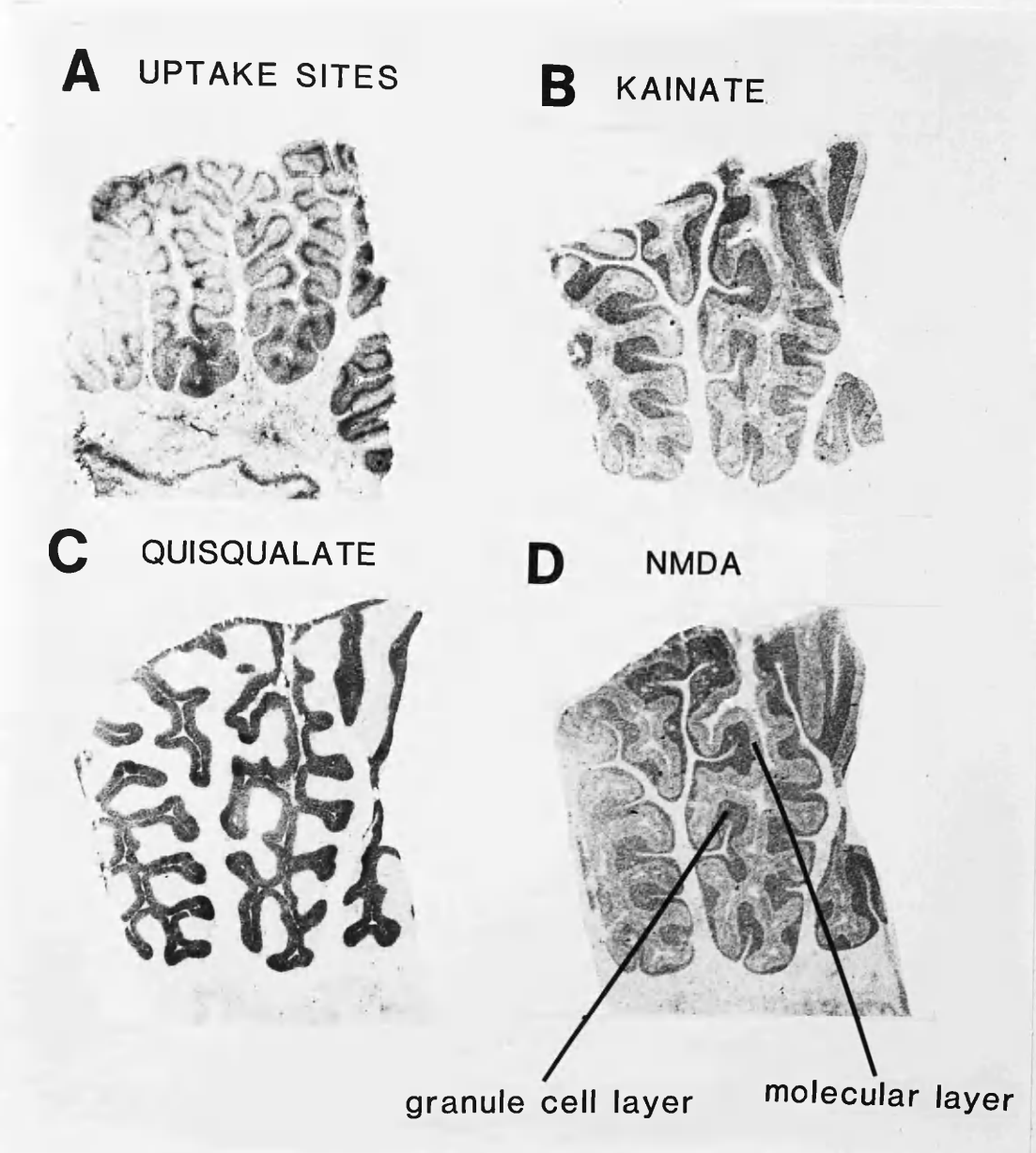


FIGURE 10 GLUTAMATERGIC SITES IN CEREBELLAR CORTEX

Representative autoradiograms of (A) glutamate uptake site binding, (B) kainate receptor binding, (C) quisqualate receptor binding and (D) NMDA receptor binding in adjacent sections of cerebellar cortex.

For respective non-specific images see opposite page.

1.3 E.A.A. RECEPTOR BINDING SITES IN CONTROL AND ALZHEIMER BRAINS

Full quantitative analysis of [^{3}H]-D-aspartate, [^{3}H]-kainate, [^{3}H]-AMPA and NMDA-sensitive [^{3}H]-glutamate binding in control and A.D. brains is presented in Appendix II.

1.3.1 E.A.A. Receptor Binding Sites in Control and A.D. Frontal Cortex

In A.D. brains, there was a marked reduction in [^{3}H]-D-aspartate binding throughout middle frontal gyrus compared to controls (Figure 11C). The magnitude of this alteration was similar in every cortical laminae. Comparison of Figures 11A and B illustrates the clear diminuation in the prominence of the laminar pattern of [^{3}H]-D-aspartate binding in A.D. frontal cortex relative to control.

In contrast to the homogeneous loss of presynaptic glutamatergic binding, there were heterogeneous alterations in glutamate receptor subtype binding in this region of the A.D. brain. [^{3}H]-Kainate binding in layers IV and V/VI of frontal cortex from A.D. patients was significantly greater (70%) than that in control frontal cortex, although [^{3}H]-kainate binding in cortical layers I-III was similar in control and A.D. brains (Figure 12C). The magnitude of the increase in [^{3}H]-kainate binding in A.D. frontal cortex was such that every A.D. brain could be readily distinguished from controls on visual inspection of the autoradiograms by the presence of a band of high optical density in the deep cortical laminae (Figure 12B).

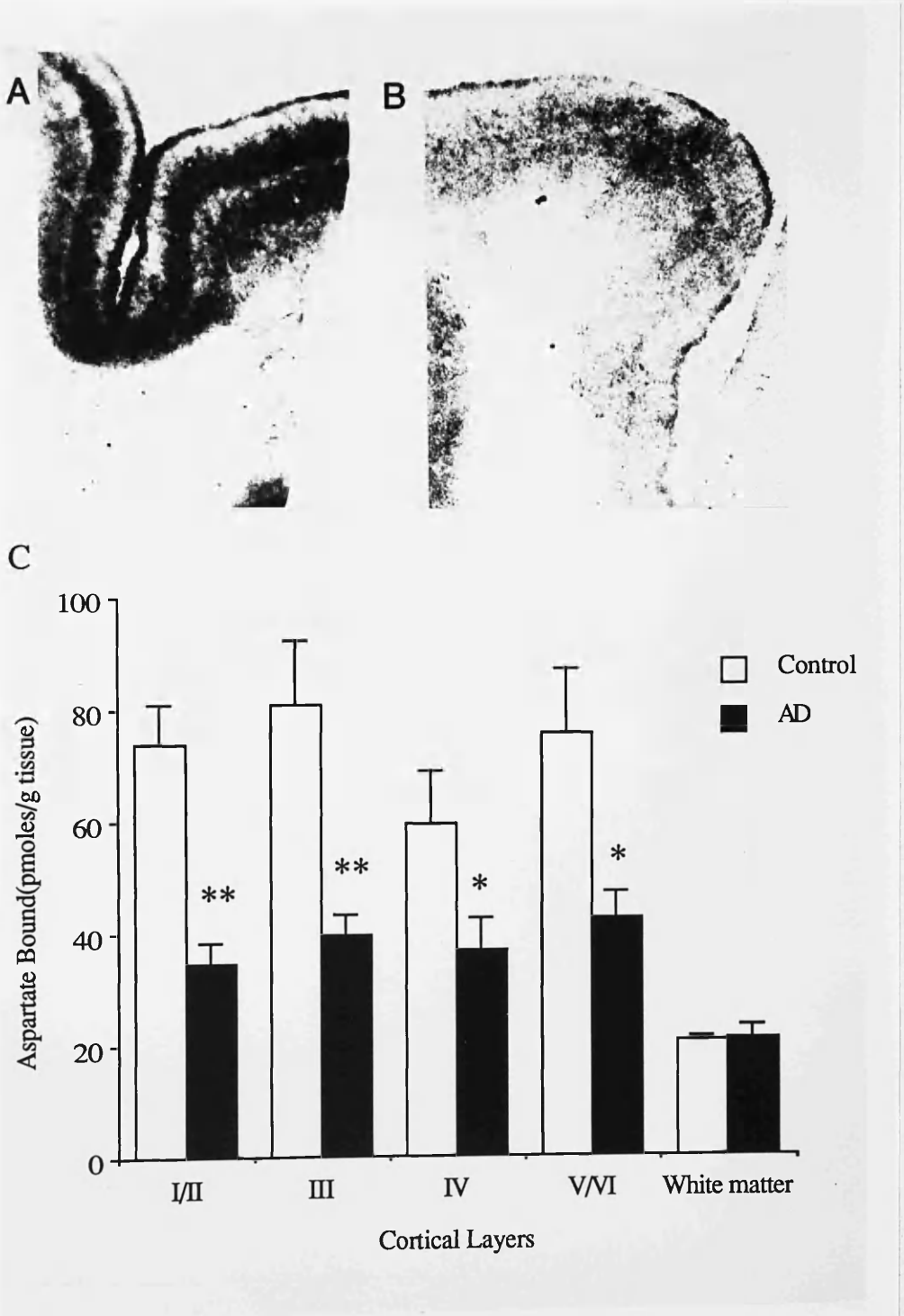


FIGURE 11 [^{3}H]-D-ASPARTATE BINDING IN CONTROL AND A.D. FRONTAL CORTEX

In representative autoradiograms, note the reduction in [^{3}H]-D-aspartate binding throughout A.D. frontal cortex (B) in comparison to control frontal cortex (A). *P<0.05, **P<0.01.

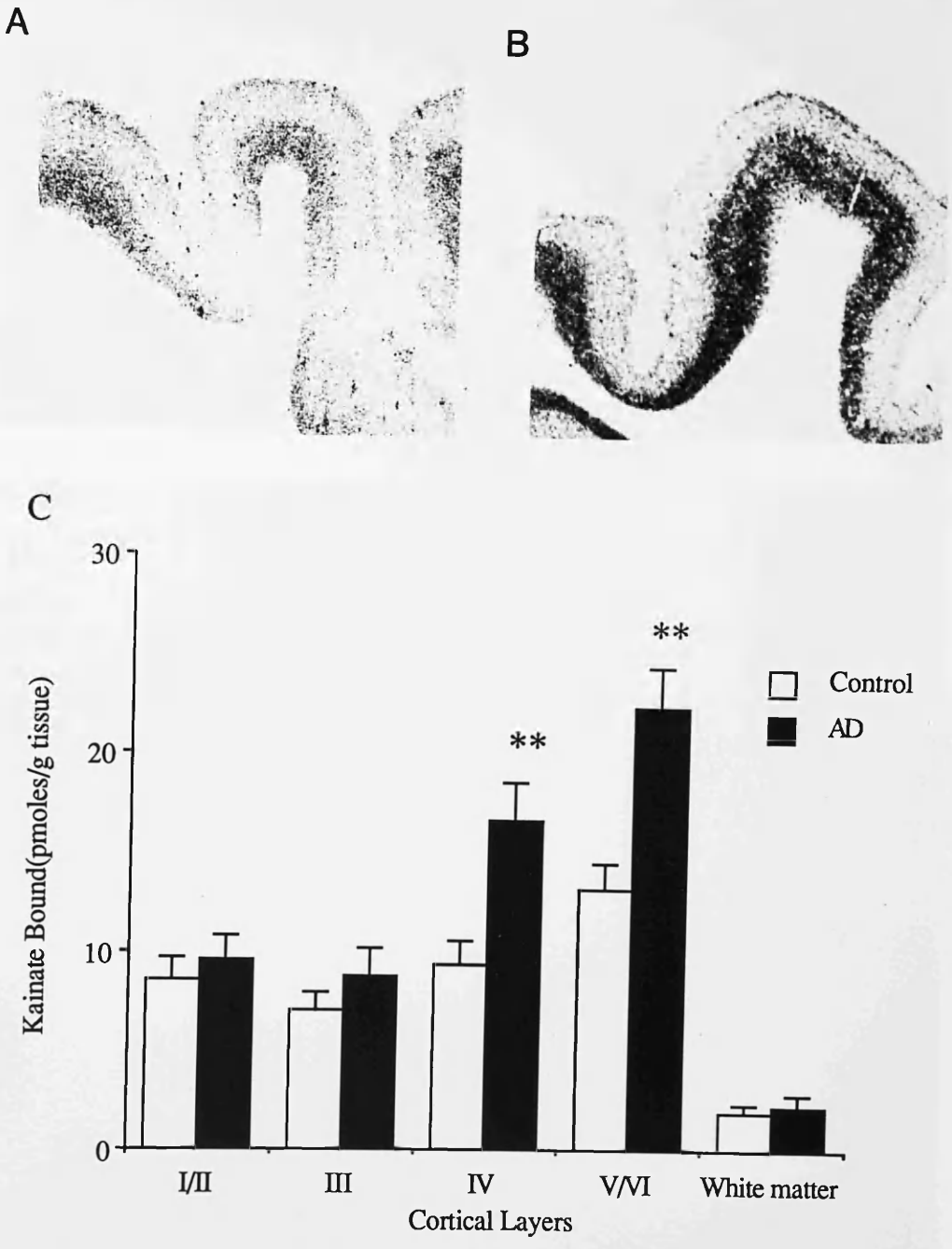


FIGURE 12 [^{3}H]-KAINATE BINDING IN CONTROL AND A.D. FRONTAL CORTEX

In representative autoradiograms, note the selective increase in optical density in the deep layers of A.D. frontal cortex (B) in comparison to control (A). **P<0.01.

In contrast to [^{3}H]-kainate binding, [^{3}H]-AMPA binding remained unaltered in A.D. frontal cortex (Figure 13A), while NMDA-sensitive [^{3}H]-glutamate binding was slightly reduced (25%) only in cortical layers I-II of A.D. frontal cortex compared to control brains (Figure 13B). This difference was significant at the 5% level (P=0.05).

1.3.2 Binding Parameters and Pharmacological Profile of [^{3}H]-Kainate Binding in Control and A.D. Frontal Cortex

Full autoradiographic saturation analysis of [^{3}H]-kainate binding (2.5 - 50nM) was only possible in one control and two A.D. brains in which sufficient frontal cortex tissue remained. Using Scatchard analysis of this data, calculated B_{max} values in cortical layers V/VI of both A.D. brains (61 and 63 pmoles/g tissue) were substantially greater than that in the control case (43 pmoles/g tissue), while apparent K_D values in these cortical layers were similar in all three brains (19 and 21nM in A.D. brains, 16.5nM in control), (Figure 14A). Calculated B_{max} and K_D values in cortical layers I-III were similar in both the control and A.D. brains (Figure 14B); B_{max} values being 26 and 28 pmoles/g tissue in A.D. brains and 27pmoles/g tissue in control, and K_D values being 18.5 and 20nM in A.D. brains and 20nM in control.

Autoradiographic displacement analysis of [^{3}H]-kainate binding was performed in the same brains using a range of excitatory amino acid analogues. Displacer potencies were similar in both the control and A.D. brains in both superficial and deep cortical layers (Figures 15 and

FIGURE 13 [^{3}H]-AMPA AND NMDA-SENSITIVE [^{3}H]-GLUTAMATE BINDING IN CONTROL AND AD FRONTAL CORTEX
*p<0.05

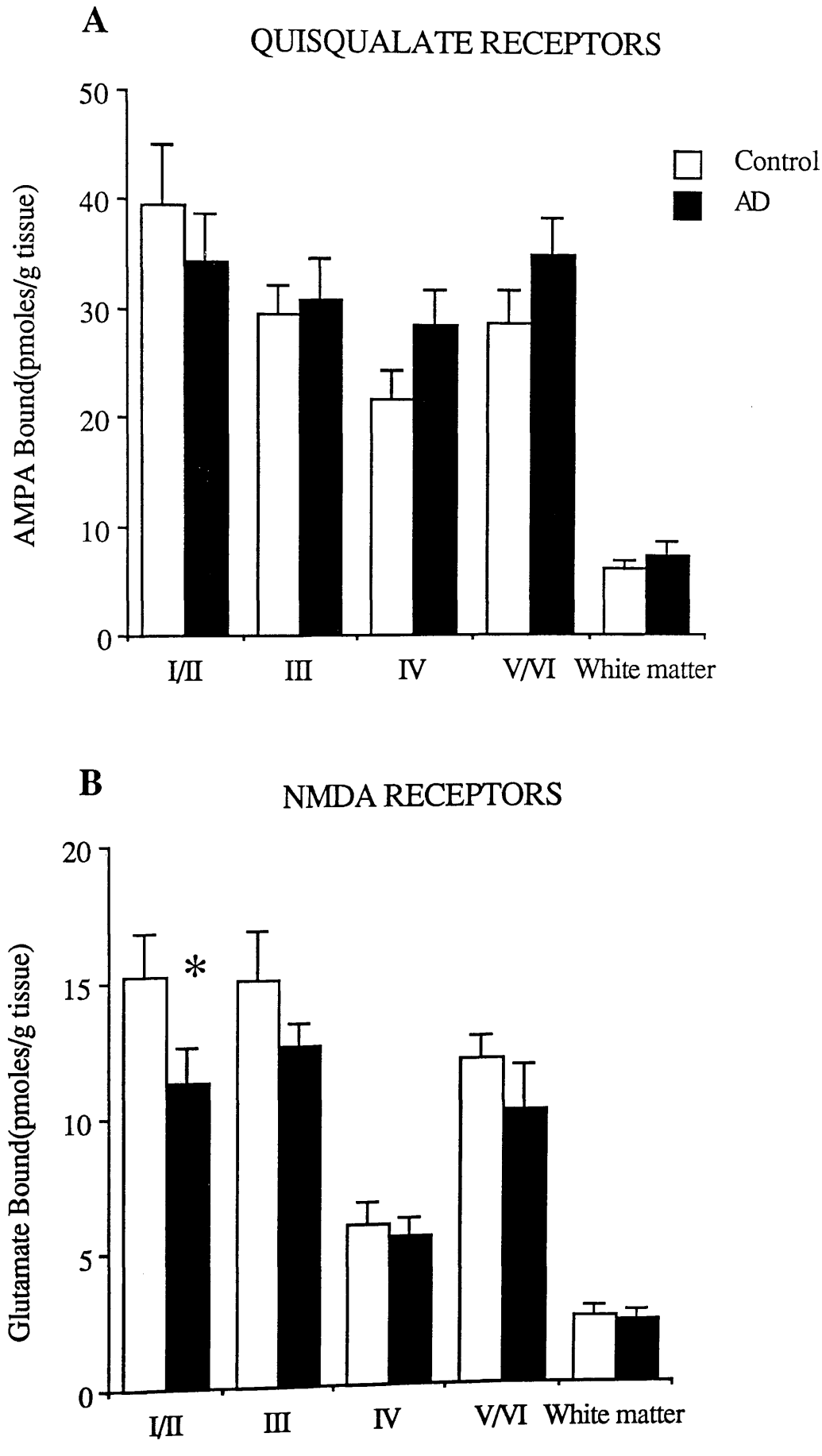


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FIGURE 14.

Scatchard analysis of [^{3}H]-kainate binding (2.5 - 50nM) in one control and two Alzheimer brains in (A) cortical layers IV-VI and (B) cortical layers I-III of frontal cortex. For both the control and Alzheimer brains, calculation of ligand bound at each concentration of [^{3}H]-kainate was determined from four total and two non-specific sections. Calculated K_d and B_{max} values were obtained by linear regression analysis.

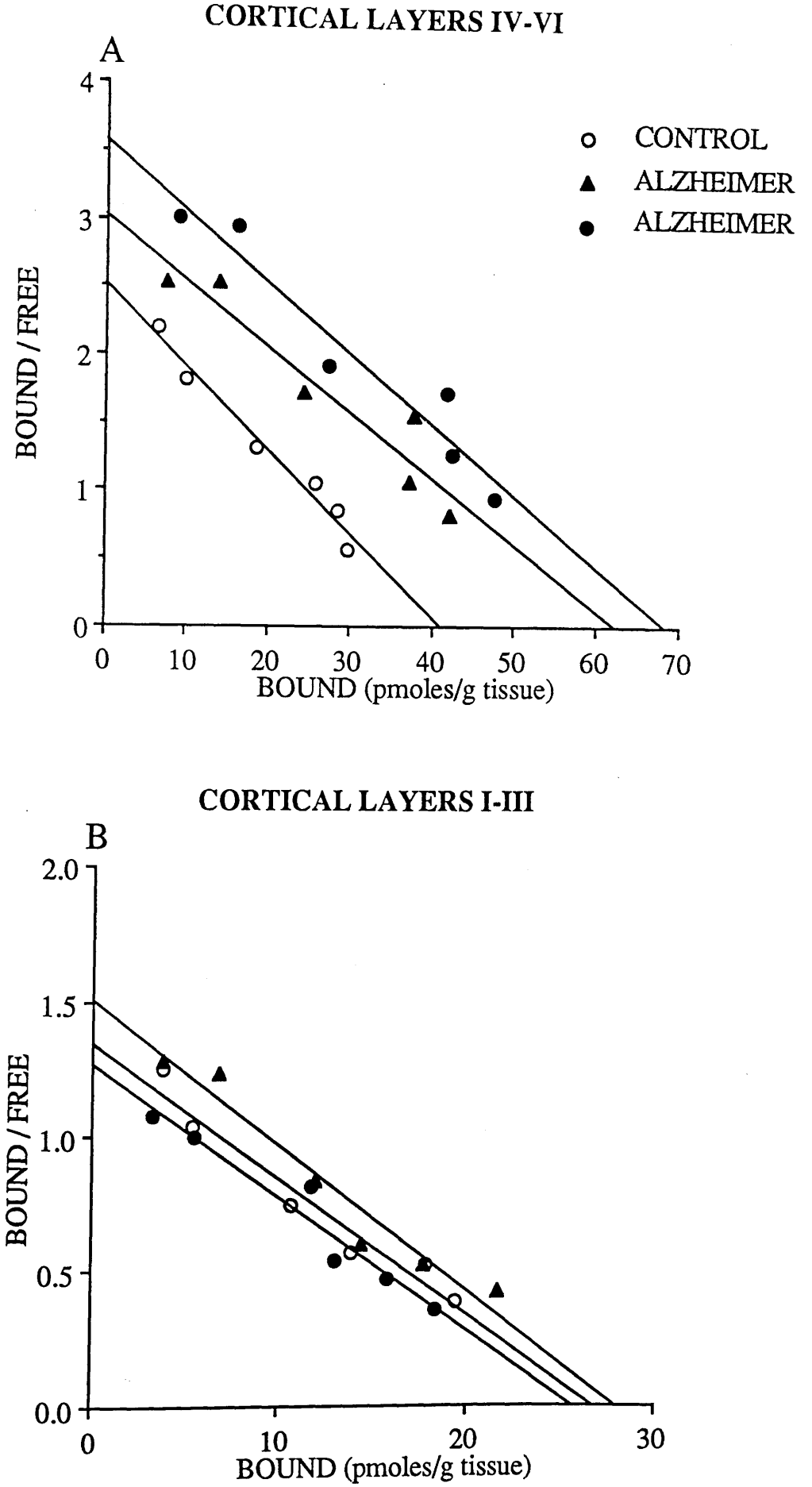


FIGURE 14 SCATCHARD ANALYSIS OF [^{3}H]-KAINATE BINDING IN FRONTAL CORTEX

16); the order of potency being domoate ≃ kainate, >> quisqualate ≃ glutamate, while AMPA and NMDA produced negligible inhibition. This pharmacological profile of [^{3}H]-kainate binding is consistent with results using membrane preparations in both rat (Foster and Fagg, 1984) and human (Cowburn et al. 1989) brain.

Although by no means conclusive, these saturation and displacement data indicate that the increase in [^{3}H]-kainate binding in deep layers of A.D. frontal cortex (at a single ligand concentration) is likely to reflect an increase in kainate receptor numbers rather than an alteration in kainate receptor affinity or recognition properties.

1.3.3 E.A.A. Receptor Binding and Local Neuropathology in A.D. Frontal Cortex

In A.D. frontal cortex, senile plaque numbers (mean ± SEM) were 35 ± 6 per mm^2 in cortical layers I-III and 21 ± 4 per mm^2 in layers IV-VI. The number of senile plaques did not exceed 2 per mm^2 in any control subjects.

Using least squares linear regression analysis, [^{3}H]-kainate binding was positively correlated with senile plaque numbers in cortical layers IV (r=0.901) and V/VI (r=0.914) of A.D. frontal cortex (Figure 17B). [^{3}H]-Kainate binding and senile plaque numbers were, however, unrelated (r=0.089) in cortical layers I-III of A.D. brains (Figure 17A). [^{3}H]-D-aspartate, [^{3}H]-AMPA and NMDA-sensitive [^{3}H]-glutamate binding

LEGENDS TO FIGURES

FIGURES 15 AND 16. Displacement of [^{3}H]-kainate binding by domoate (10^{-5}M - 10^{-11}M), kainate (10^{-4} - 10^{-11}M), glutamate (10^{-4}M - $5x10^{-10}$M) and quisqualate (10^{-4}M - 10^{-10}M) in superficial layers (Figure 15) and deep layers (Figure 16) of frontal cortex from one control and two Alzheimer brains. In both the control and Alzheimer brains total (100%) kainate bound was calculated from 12 sections incubated with 50nM [^{3}H]-kainate. For each concentration of displacer, the level of kainate bound was calculated from three sections incubated simultaneously with 50nM [^{3}H]-kainate and displacer and subsequently converted to a percentage of total (100%) kainate bound. AMPA and NMDA produced negligible inhibition of [^{3}H]-kainate binding up to a concentration of 1mM.

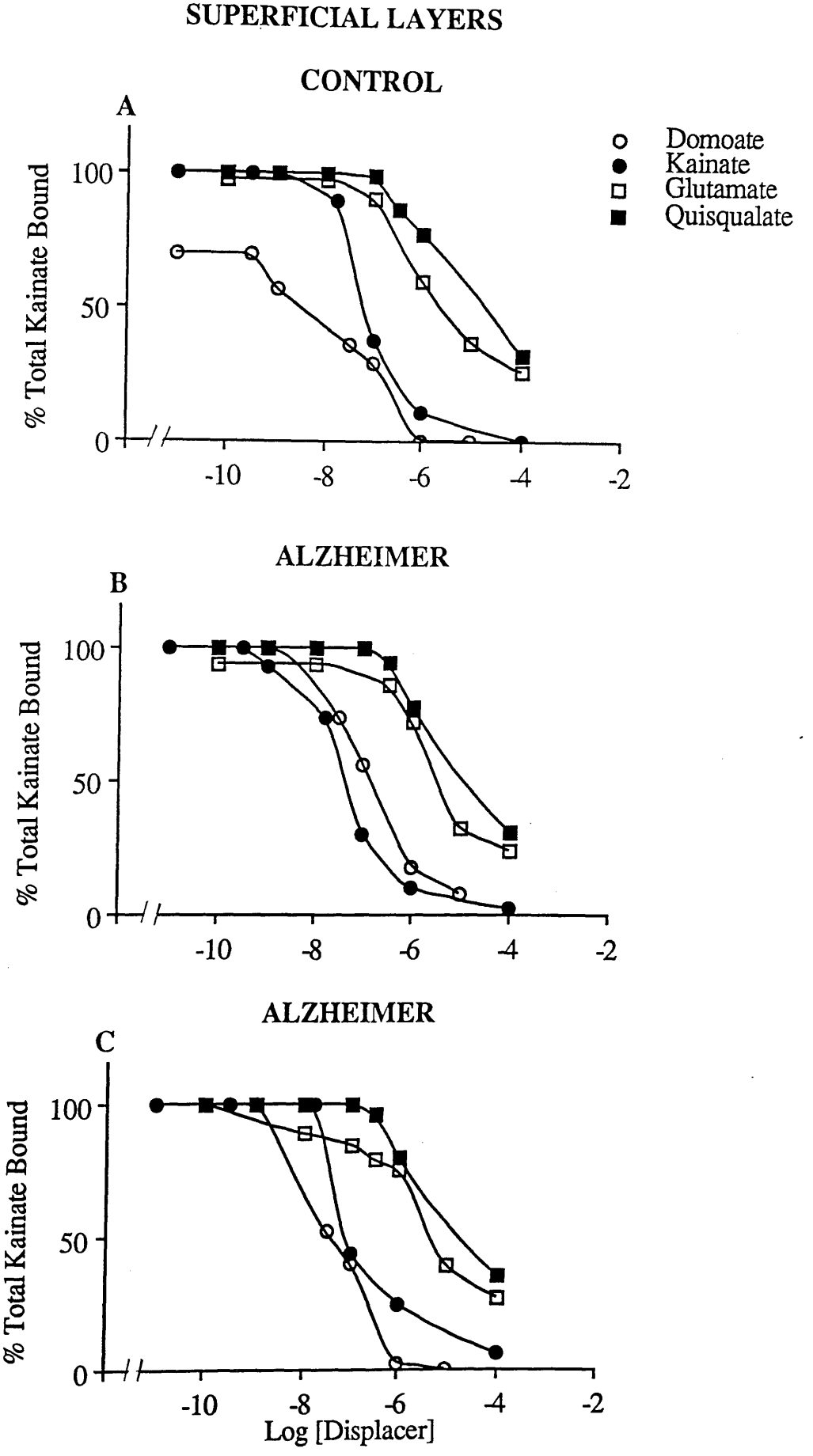


FIGURE 15 DISPLACEMENT ANALYSIS OF [^{3}H]-KAINATE BINDING IN SUPERFICIAL LAYERS OF FRONTAL CORTEX

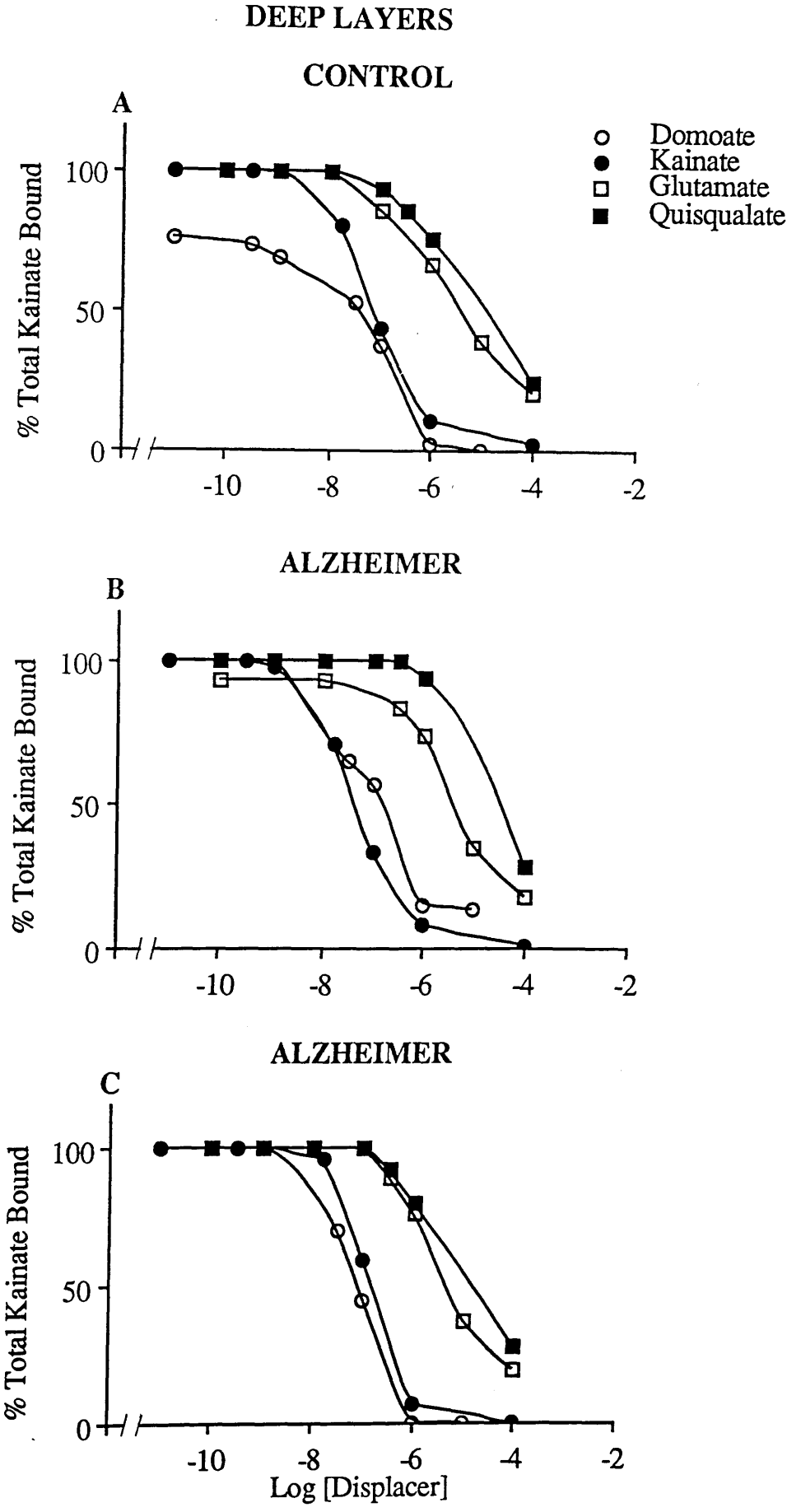


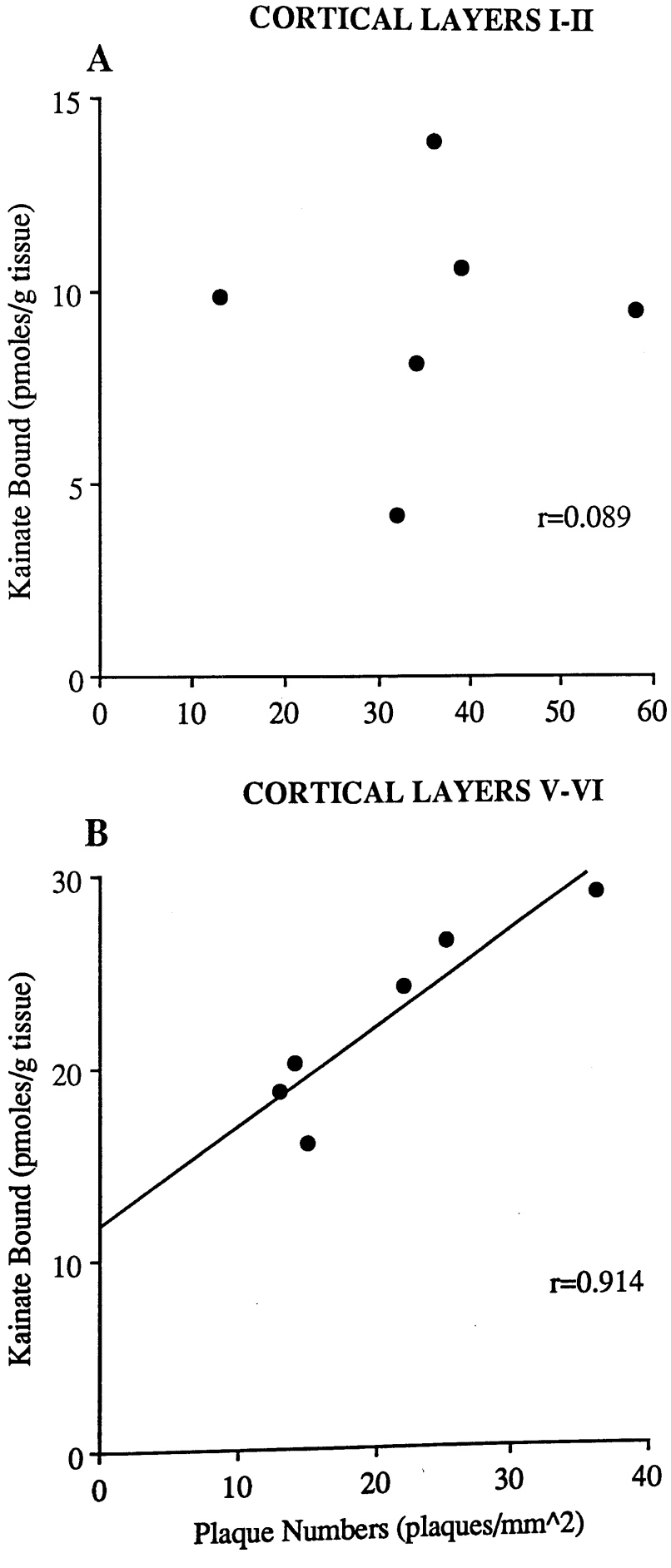
FIGURE 16 DISPLACEMENT ANALYSIS OF [^{3}H]-KAINATE BINDING IN DEEP LAYERS OF FRONTAL CORTEX

LEGEND TO FIGURE

FIGURE 17

Correlation of kainate receptor binding and senile plaque numbers in cortical layers I and II and V-VI of A.D. frontal cortex. Kainate receptor binding and senile plaque numbers were quantified as described in Chapter II. The correlation was significant ($p<0.02$) in deep layers ($r=0.914$) but not in superficial layers ($r=0.089$)

FIGURE 17 RELATIONSHIP BETWEEN [^{3}H]-KAINATE BINDING AND SENILE PLAQUE NUMBERS IN SUPERFICIAL AND DEEP LAYERS OF AD FRONTAL CORTEX



were unrelated to senile plaque numbers in either superficial or deep layers of A.D. frontal cortex (Appendix II).

1.3.4 E.A.A. Receptor Binding Sites in Control and A.D. Temporal Cortex

In A.D. brains, there was no significant alteration in [^{3}H-]-D-aspartate binding throughout inferior temporal gyrus compared to controls (Figure 18A). Similarly, [^{3}H]-kainate, [^{3}H]-AMPA and NMDA-sensitive [^{3}H]-glutamate binding were unaltered in any cortical layer in this region of the A.D. brain (Figures 18B and 19).

1.3.5 E.A.A. Receptor Binding and Local Neuropathology in A.D. Temporal Cortex

In A.D. temporal cortex, senile plaque numbers (mean ± SEM) were 29 ± 8 per mm^2 in cortical layers I-III and 19 ± 4 per mm^2 in layers IV-VI. The number of senile plaques did not exceed 2 per mm^2 in any control subjects.

[^{3}H]-D-Aspartate, [^{3}H]-kainate, [^{3}H]-AMPA and NMDA-sensitive [^{3}H]-glutamate binding were unrelated to senile plaque numbers in either superficial or deep layers of cortex (Appendix II).

1.3.6 E.A.A. Receptor Binding and ChAT Activity in A.D. Cerebral Cortex

Mean ChAT activity in A.D. frontal and temporal cortex (2.03 ± 0.3 and 1.6 ±0.4 nmoles/mg protein/hr) was significantly less ($p<0.05$,t-test) than that in control brains 5.28 ± 0.8 and 3.3 ± 0.1 nmoles/mg

LEGEND TO FIGURE

FIGURE 18

[^{3}H]-D-Aspartate binding (A) and [^{3}H]-kainate binding (B) in control (□) and A.D. (■) temporal cortex. Data are means ± SEM, n=5 and n=6 for control and A.D. groups respectively.

FIGURE 18 [^{3}H]-D-ASPARTATE AND [^{3}H]-KAINATE BINDING IN CONTROL AND AD TEMPORAL CORTEX

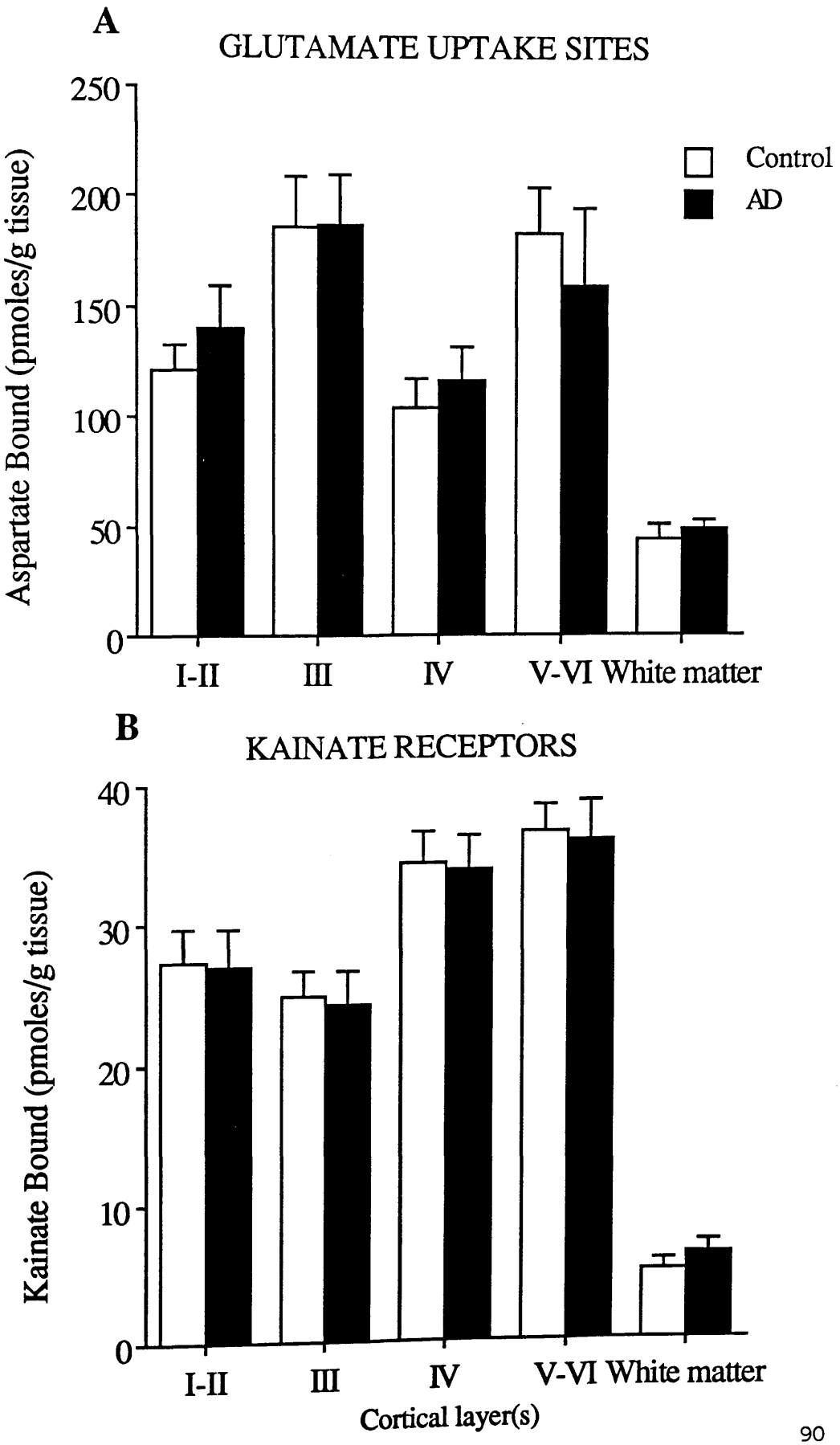


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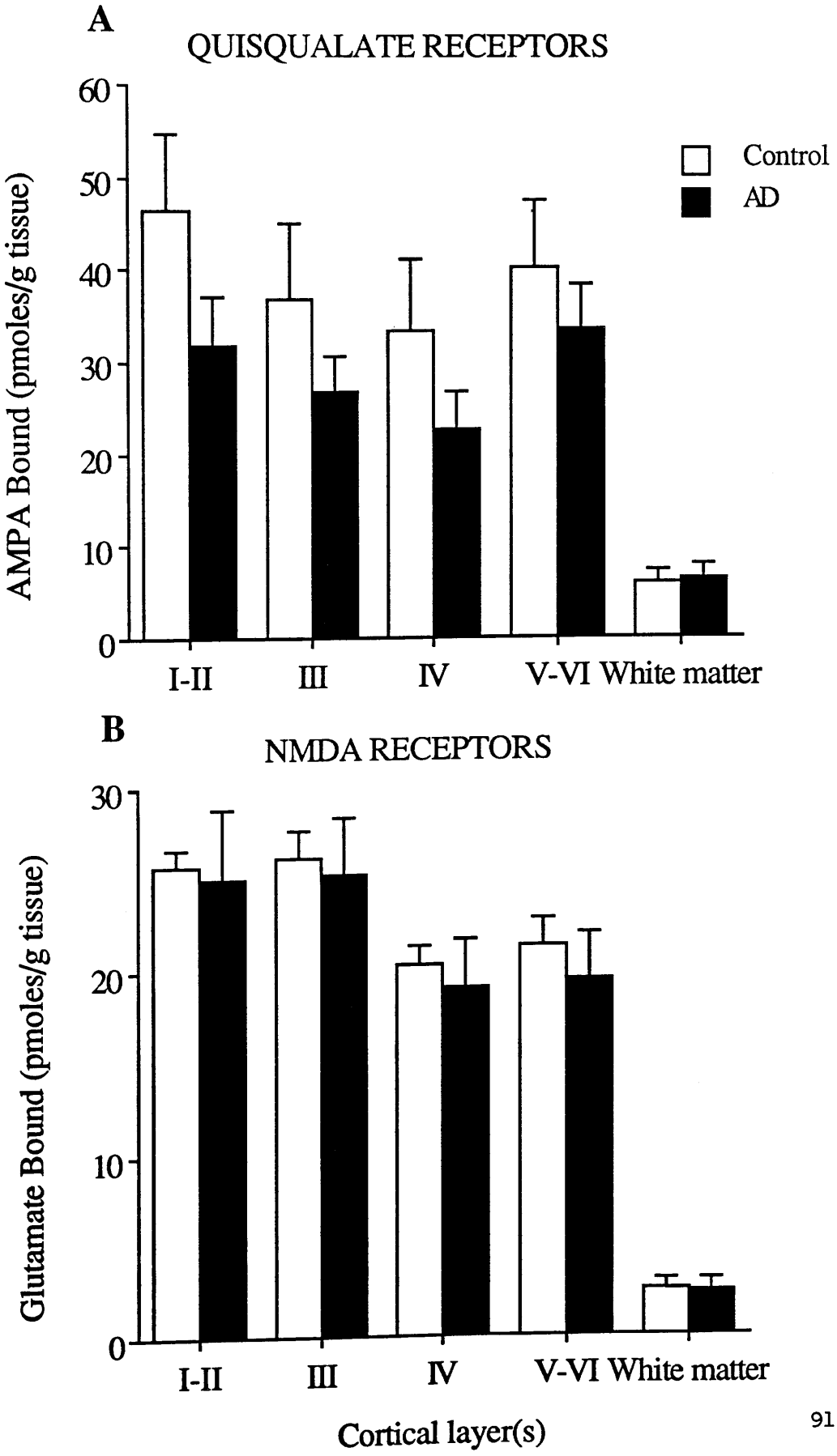
FIGURE 19

[^{3}H]-AMPA (A) and NMDA-sensitive [^{3}H]-glutamate binding (B) in control (□) and A.D. (■) temporal cortex. Data are means ± SEM, n=5 and n=6 for control and A.D. groups respectively.

FIGURE 19 **[^{3}H]-AMPA AND NMDA-SENSITIVE [^{3}H]-GLUTAMATE BINDING IN CONTROL AND AD TEMPORAL CORTEX**



protein/hr in frontal and temporal cortex respectively). There were, however, no statistically significant correlations between E.A.A. ligand binding levels and ChAT activity in either frontal or temporal cortex in A.D. brains (Appendix II).

1.3.7 E.A.A. Receptor Binding Sites in Control and A.D. Cerebellar Cortex

In A.D. brains, there was no significant alteration in [^{3}H]-D-aspartate, [^{3}H]-kainate, or NMDA-sensitive [^{3}H]-glutamate binding in either the molecular or granule cell layers of cerebellar cortex compared to controls (Figure 20). There was, however, a significant reduction in [^{3}H]-AMPA binding in the molecular layer of cerebellar cortex from A.D. subjects (Figure 21C), although [^{3}H]-AMPA binding in the granule cell layer was similar in both control and A.D. brains. The magnitude of the reduction in [^{3}H]-AMPA binding in this region of the A.D. brain is evident from comparison of Figures 21A and B. The obvious bilaminar pattern of [^{3}H]-AMPA binding in the cerebellar cortex from a control subject (Figure 21A) contrasts markedly with the homogeneous distribution of [^{3}H]-AMPA binding in this region in the A.D. brain (Figure 21B).

1.3.8 Binding Parameters for [^{3}H]-AMPA Binding in Control and A.D. Cerebellar Cortex

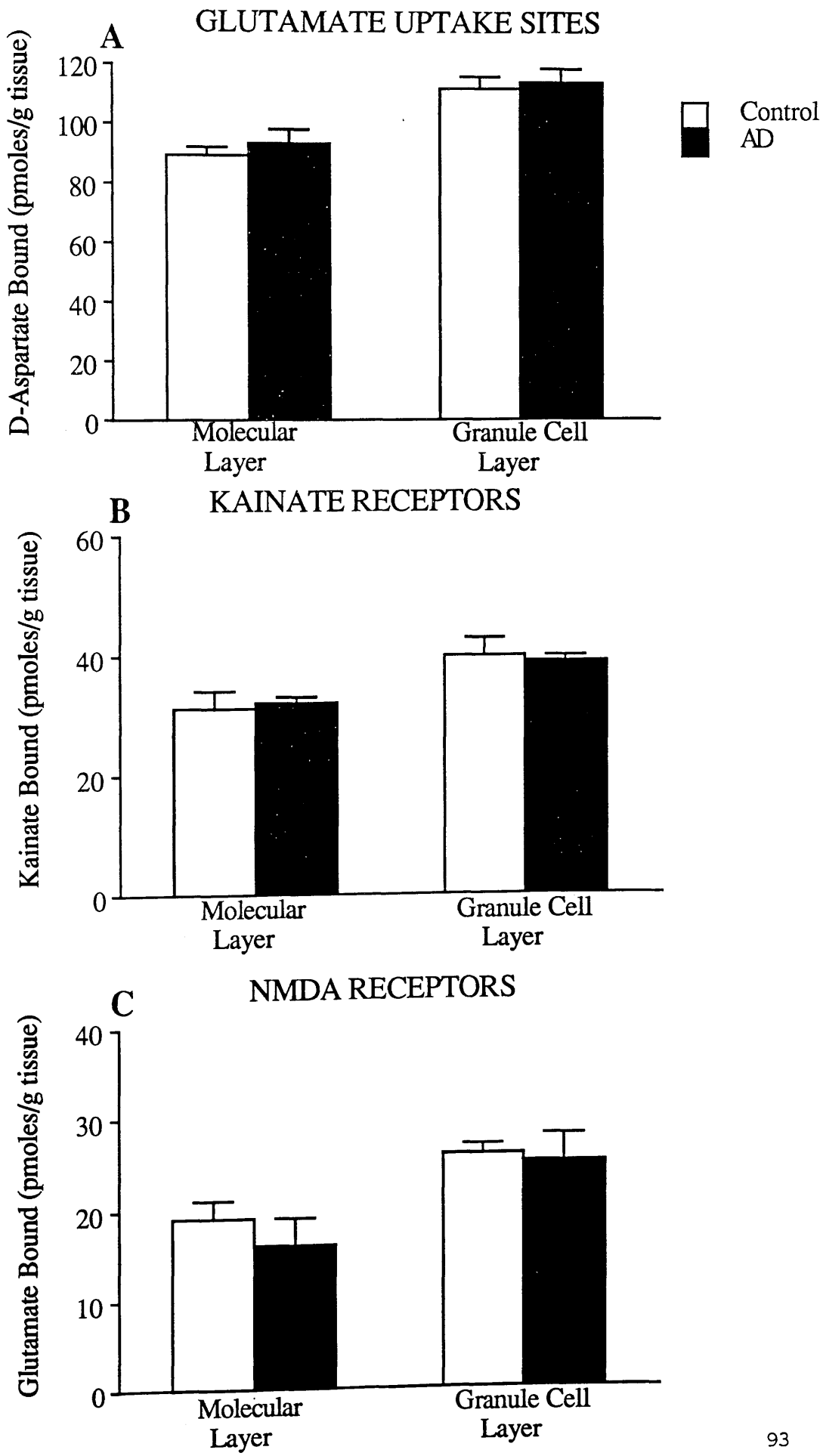
Full autoradiographic analysis of [^{3}H]-AMPA binding (25-800nM) was performed in cerebellar sections from five control and five A.D. brains. In cerebellar molecular layer, calculated B_{max} values in A.D.

LEGEND TO FIGURE

FIGURE 20

[^{3}H]-D-Aspartate (A), [^{3}H]-kainate (B) and NMDA-sensitive [^{3}H]-glutamate binding in control (□) and A.D. (■) cerebellar cortex. Data are means ± SEM, n=6 for each group.

FIGURE 20 GLUTAMATERGIC SITES IN CONTROL AND AD CEREBELLUM



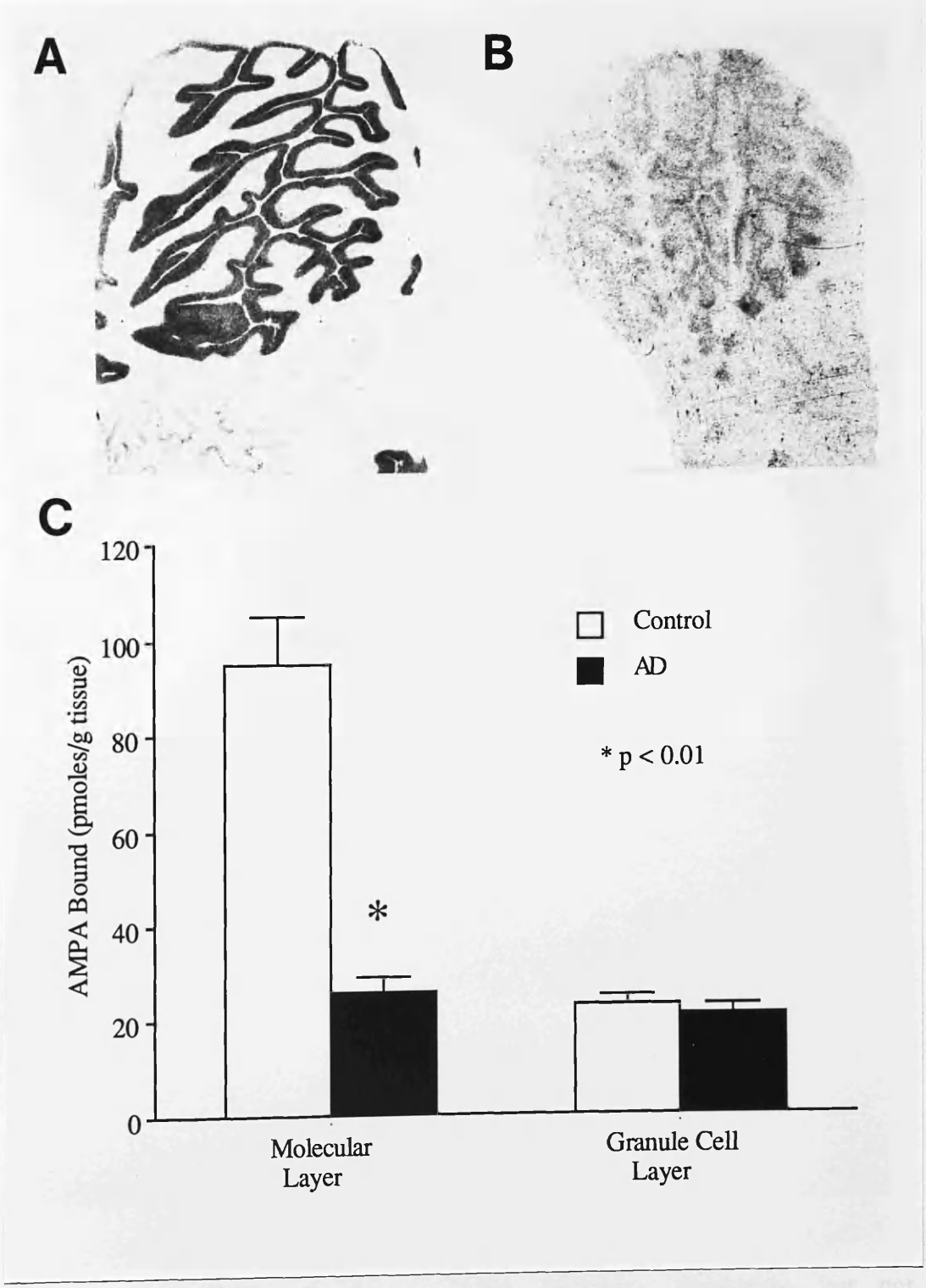


FIGURE 21 [^{3}H]-AMPA BINDING IN CONTROL AND A.D. CEREBELLAR CORTEX

In representative autoradiograms, note the striking loss of optical density in the molecular layer of A.D. cerebellar cortex (B) in comparison to control (A).

brains (167 ± 13 pmoles/g tissue) were significantly lower than those in control brains (280 ±34 pmoles/g tissue) whilst K_D values were similar in both control and A.D. brains (417 ± 53 nM and 450 ± 50nM). B_{max} and K_D values for [^{3}H]-AMPA binding in granule cell layer of A.D. brains were not significantly different from control; B_{max} , 95 ± 11 and 79 ± 8 pmoles/g tissue and K_D, 400 ± 41nM and 367 ± 57nM in control and A.D. brains respectively. Figure 22 illustrates the Scatchard analysis of [^{3}H]-AMPA binding in a typical control and Alzheimer brain.

1.3.9 E.A.A. Receptor Binding and Local Neuropathology in A.D. Cerebellar Cortex

In contrast to the high numbers of senile plaques found in frontal and temporal cortex, senile plaques were absent in cerebellar cortex in all but two A.D. brains. In these cases, plaque counts were 10 and 3 plaques per mm^2. No senile plaques were present in any control brains.

1.3.10 Cortico-Cerebellar Ligand Binding and Neuropathology

Within A.D. brains, [^{3}H]-D-aspartate binding in both superficial and deep layers of frontal cortex correlated negatively, but not significantly with [^{3}H]-AMPA binding in the molecular layer of the cerebellum: r=0.733, P<0.1; and r=0.679, P<0.2 for superficial and deep layers respectively. [^{3}H]-Kainate binding in frontal cortex was unrelated to [^{3}H]-AMPA binding levels in cerebellar molecular layer

LEGEND TO FIGURE

FIGURE 22. Scatchard analysis of [^{3}H-AMPA binding in (A) molecular layer and (B) granule cell layer of cerebellar cortex from a typical control and Alzheimer brain. For both the control and Alzheimer brain, calculation of AMPA bound at each concentration of [^{3}H]-AMPA was determined from four total and two non-specific sections. Calculated K_d and B_{max} values were obtained by linear regression and analysis.

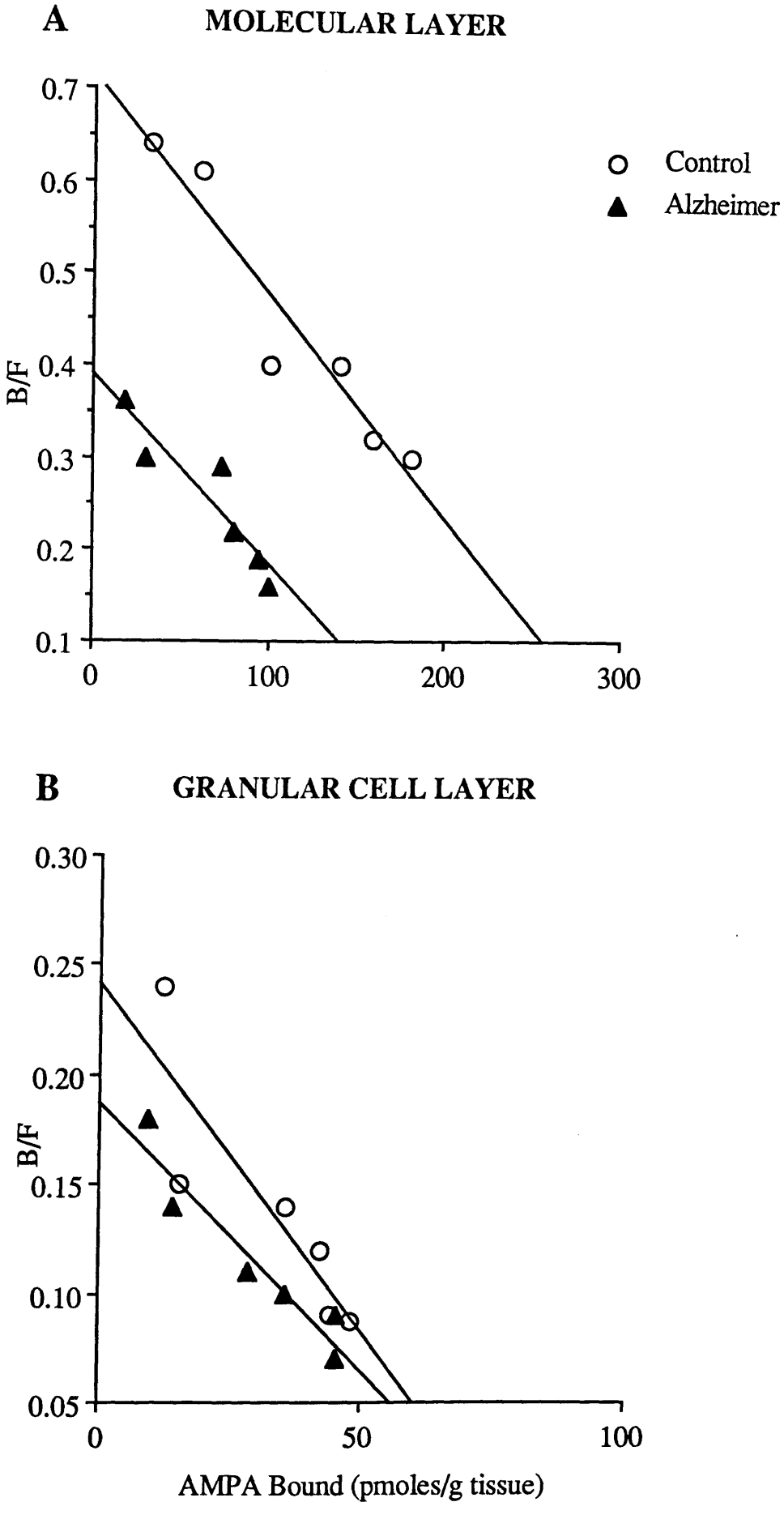


FIGURE 22 SCATCHARD ANALYSIS OF [^{3}H]-AMPA BINDING IN CEREBELLAR CORTEX

(r=0.202 for superficial layers and r=0.109 for deep cortical layers) as was NMDA-sensitive [^{3}H]-glutamate binding (r=0 and r=0.054 for superficial and deep cortical layers).

Senile plaque numbers in both frontal and temporal cortex were unrelated to [^{3}H]-AMPA binding in the cerebellum.

1.4 COMMENTARY: DISCUSSION OF E.A.A. RECEPTOR BINDING IN ALZHEIMER'S DISEASE POSTMORTEM TISSUE

Alzheimer's disease (A.D.) is characterised neuropathologically by neuronal cell loss, the presence of higher than normal densities of senile plaques and the development of neurofibrillary tangles in neocortical and archicortical regions (Perry, 1986; Terry et al. 1981 and Mountjoy et al. 1983). Glutamate is putatively the major excitatory transmitter of cortical pyramidal neurons (Fonnum, 1984; Lund-Karlsen and Fonnum, 1978; Baughman and Gilbert, 1981; Conti et al. 1987), which mediate cortico-cortical and cortico-fugal neurotransmission (Jones, 1981). A loss of cortical pyramidal cells in higher order polymodal association areas, such as the frontal and temporal lobes, is a prominent feature of Alzheimer's pathology (Terry et al. 1981; Mountjoy et al. 1983; Pearson et al. 1985) and has been shown to be directly related to the level of cognitive decline in Alzheimer patients (Neary et al. 1986). Cortical glutamatergic dysfunction may thus be a contributory factor in the patho-physiological progression of the disease. The present study represents a comprehensive postmortem examination of multiple elements of glutamatergic transmission in three regions of the Alzheimer brain: frontal and temporary cortex, where neuropathological alterations and pyramidal cell loss are prominent; and cerebellar cortex, an area exhibiting minimal neuropathological change.

Frontal Cortex. The high density of [^{3}H]-D-aspartate binding in cortical layers III and V in both frontal and temporal cortex supports the concept of excitatory amino acid-mediated intracortical transmission in these cortical regions as cortico-cortical fibres are believed to terminate in these laminae (Jones, 1981). However, although Na^+-dependent high affinity glutamate uptake sites are present on glutamatergic terminals, glial cells also possess high affinity glutamate re-uptake mechanisms (Hertz, 1979) and consequently it should be borne in mind that a proportion of Na^+-dependent [^{3}H]-D-aspartate binding is likely to be non-neuronal.

The marked reduction in [^{3}H]-D-aspartate binding throughout frontal cortex from A.D. subjects indicates a significant loss or de-sensitisation of Na^+-dependent glutamate up-take sites in this region. Previous reports using homogenate preparations of frontal cortex have indicated that [^{3}H]-D-aspartate binding was reduced by 37% (Cross et al. 1987) and 30% (Simpson et al. 1988) of control values, while the maximal velocity of the synaptosomal uptake of [^{3}H]-D-aspartate binding in A.D. frontal cortex was only 35% of control levels (Hardy et al. 1987). In view of the prominent loss of cortical pyramidal cells in A.D., it is likely that all of the above data indicates a loss of glutamatergic cortico-cortical innervation in A.D. frontal cortex.

It has been suggested that degenerating cortical glutamatergic fibres contribute to the formation of plaque neurites within the cerebral cortex (Perry, 1986). Despite the overall (50%) reduction in [^{3}H]-D-aspartate binding in the present study there was no correlation with senile plaque numbers in any cortical layer. However, in view of the probable ceiling effects in relation to presynaptic glutamatergic loss at end-stage of the disease and the contribution of other neurotransmitter systems to senile plaque formation, this finding may not be unexpected.

Ligand binding studies of glutamate receptors in A.D., to date, have concentrated on NMDA receptors with minimal examination of quisqualate or kainate receptors. In this respect, the most striking finding of the present study has been the substantial increase in kainate receptor binding in deep layers of frontal cortex in Alzheimer patients. Autoradiographic saturation and displacement analysis indicates that the increase in kainate receptor binding reflects an increase in kainate receptor numbers, rather than a change in receptor recognition properties.

An increase in kainate receptor numbers in A.D. contrasts with the great majority of reports in which reductions of many neurotransmitters and their receptors including glutamate, have been described (Coyle et al. 1983; Reynolds et al. 1984; Beal et al. 1985; Chu et al. 1987; Greenamyre et al. 1987; Maragos et al. 1987b). A

rare exception, however, reports an expansion of the kainate receptor field in the dentate gyrus of the hippocampus in A.D. patients (Geddes et al. 1985). Using quantitative receptor autoradiography, Geddes and colleagues identified that kainate receptors, which usually occupy only the inner one-third of the molecular layer in the dentate gyrus of control patients, had expanded to cover the inner one-half of the layer in A.D. patients. Furthermore, a similar expansion in this portion of the molecular layer was observed in rats which had received lesions of the entorhinal cortex, effectively removing the perforant path input into the outer two-thirds of the dentate molecular layer (Geddes et al. 1985). On the basis of these experiments, the authors concluded that the expansion of the kainate receptor field in the dentate gyrus of A.D. patients reflected compensatory sprouting of both commissural and associational fibres into that space vacated as a consequence of reduced perforant path input from the entorhinal cortex. This important study indicates that, even in the A.D. brain, neurons are capable of plasticity and growth reactions to reduced excitatory input. The possibility that increased kainate receptor binding in cortical layers IV-VI of frontal cortex in A.D. brains also reflects regenerative changes in response to a loss of intracortical glutamatergic fibres remains to be investigated.

The loss of a presynaptic glutamatergic marker ([^{3}H]-D-aspartate) together with an increase in the postsynaptic kainate receptor may,

at a simplistic level, be considered as evidence for denervation supersensitivity in A.D. frontal cortex. Such a response is not unprecedented in human neurodegenerative disorders, as increased numbers of neurotransmitter receptors have been reported, for example, in both Parkinsons's disease (Guttman and Seeman, 1985) and Huntington's Chorea (Trifiletti et al. 1987). However, in the present study, the laminar specific increase in kainate receptors contrasts with the widespread loss of presynaptic glutamatergic terminals from all cortical layers. The neuroanatomical basis for the laminar specificity of the kainate receptor response is unclear. One possibility is that kainate receptor up-regulation in response to denervation only occurs in those cortical layers in which kainate receptors are physiologically important in mediating fast excitatory transmission, i.e. deep cortical layers. The high density of kainate receptors in deep layers of human frontal cortex may be interpreted as evidence for their relatively greater importance in these laminae. If glutamatergic denervation is the sole stimulus for kainate receptor up-regulation, however, we may have expected a direct correlation of presynaptic deficit ([^{3}H]-D-aspartate) and postsynaptic response ([^{3}H]-kainate) in deep layers of frontal cortex from Alzheimer patients, which was not the case (r=0.439). Again, a possible explanation for the lack of correlation may be ceiling effects in relation to presynaptic glutamatergic deficit.

While [^{3}H]-kainate binding in deep layers of A.D. frontal cortex was unrelated to [^{3}H]-D-aspartate binding, this response was directly related to senile plaque numbers in both cortical layers IV (r=0.901) and V/VI (r=0.914). [^{3}H]-Kainate binding in superficial cortical layers, which was unaltered in comparison to control brains, was unrelated to senile plaque numbers (r=0.089). This suggests that the increase in kainate receptors in cortical layers IV-VI of A.D. frontal cortex is intimately associated with the level of local neuropathology in these laminae. It is noteworthy that in these same patients [^{3}H]-kainate binding in deep layers of frontal cortex was unrelated to senile plaque numbers quantified in the opposite hemisphere (r=0.148). Comparison of neuropathological and neurochemical measurements determined in opposite hemispheres has been a common experimental procedure in studies of A.D. to date (e.g. Cross et al. 1988; Shimohama et al. 1988), the implicit assumption being interhemispheric plaque symmetry. The lack of interhemispheric correlation between [^{3}H]-kainate binding and senile plaque numbers in the present study highlights the importance of experimental design in studies of A.D. Indeed, recent investigations from this laboratory indicate that hemispheric asymmetry of plaque numbers in frontal cortex in A.D. may be more than exceptional (Dewar et al. unpublished observations).

The distribution of senile plaques in A.D. cerebral cortex appears to have an anatomical basis, possibly associated with zones of association

fibre termination (Pearson et al. 1985). In addition, the density of senile plaques in certain areas of the neocortex, including frontal cortex, correlates with the degree of cellular degeneration in the related parts of the cholinergic basal nucleus (Arendt et al. 1985). Thus the degree of kainate receptor up-regulation in A.D. frontal cortex may reflect the level of intracortical dysfunction which in turn relates to the degree of cholinergic subcortical degeneration. The ability of excitatory amino acid-induced neurotoxicity in the cerebral cortex of rats to produce retrograde degeneration of cholinergic neurons in the nucleus basalis (Sofroniew and Pearson, 1985), further suggests that the primary "insult" in A.D. is cortical.

In addition to the termination of cortico-cortical fibres in cortical layer V (Jones, 1981), pyramidal cells in cortical layers V/VI receive interlaminar excitatory amino acid connections from cortical layers II, III and IV (Baughman and Gilbert, 1981; Kisvarday et al. 1989). These neurons are in turn the source of cortical efferent projections to the caudate putamen, thalamus and brainstem (Jones, 1981). Preservation of excitatory input to deep layers of A.D. frontal cortex via kainate receptor up-regulation may, at a mechanistic level, act to maintain cortico-fugal information processing, in the presence of intracortical excitatory dysfunction. An alternative, non-functional, interpretation of the correlation of kainate receptor binding and senile plaque numbers stems from the observation that the density of muscarinic receptors in the neuropil of the senile plaque is comparable

to that in the surrounding neuropil (Palacios, 1982). If this were true for kainate receptors, the association of kainate receptor binding and senile plaque density may simply reflect an increasing accumulation of receptors as senile plaque numbers increase. This explanation seems, however, untenable as within the same area of frontal cortex (from the same Alzheimer patients) senile plaque density was greatest in outer cortical layers (35 ± 6 per mm^2 in layers I-III; 21 ± 4 per mm^2 in layers IV-VI) yet kainate receptor binding was unaltered in these laminae in comparison to control subjects. In addition, kainate receptor binding was unaltered in the deep layers of temporal cortex from the same Alzheimer patients, despite the presence of a comparable number of senile plaques to that in frontal cortex (21 ± 4 in frontal cortex; 19 ± 4 in temporal cortex).

The NMDA receptor has been the most extensively studied glutamate receptor in A.D. However, despite the availability of ligands for the NMDA receptor and its related ion channel, no consistent alterations of this receptor complex have emerged in A.D. brain, even in the hippocampus which has been the subject of the majority of studies. In this region, reductions in NMDA receptor binding, using [^{3}H]-glutamate and the non-competitive antagonist, [^{3}H]-TCP, as ligands, have been reported by Greenamyre et al. (1987) and Maragos et al. (1987b) respectively. However, Geddes and colleagues (1986) found no consistent change in hippocampal NMDA-sensitive [^{3}H]-glutamate binding in the A.D. brain and [^{3}H]-TCP binding in this region was also

found to be unaltered in two independent investigations (Monaghan et al. 1987; Simpson et al. 1988). The differences in these studies may relate to the severity of Alzheimer cases studied and/or experimental protocols employed. The original study by Greenamyre et al. (1987) can certainly be criticised on methodological grounds as these investigators defined NMDA receptors in terms of chloride-dependent, quisqualate-insensitive [^{3}H]-glutamate binding. However, a large proportion (50%) of [^{3}H]-glutamate binding under these conditions is also insensitive to NMDA (Bridges et al. 1988), and probably reflects binding to chloride-dependent glutamate uptake sites present on glia (Bridges et al. 1987). A point of more general interest relates to the influence of the degree of morphological change in Alzheimer patients on ligand binding results. Thus, while the average density of NMDA receptors in the hippocampus of A.D. brains was no different to that in age-matched controls, NMDA receptor binding was markedly reduced in one A.D. case in which neuronal cell loss was particularly severe (Geddes et al. 1986). This indicates that NMDA receptor density in A.D. hippocampus can be maintained at normal levels as long as a minimal level of cell survival is maintained. The existence of such "threshold" phenomena in the A.D. brain suggests that receptor plasticity may be important in sustaining functional activity, even at later stages of the disease process.

The small reduction in NMDA receptor binding in the outer layers of A.D. frontal cortex, in the present study, is consistent with the

reported loss of non-competitive NMDA receptor antagonist binding in this region (Simpson et al. 1988). This may be interpreted as a loss of postsynaptic receptors on degenerating cells receiving a glutamatergic input, providing some evidence for the involvement of excitotoxic mechanisms in cortical damage. If such mechanisms are relevant in the outer layers of A.D. frontal cortex, however, the stability of both quisqualate and kainate receptor binding in these laminae in the same patients must indicate a differential distribution of NMDA and non-NMDA receptors on postsynaptic membranes. This seems an unlikely possibility, as NMDA receptor activation requires prior membrane depolarisation initiated by quisqualate and/or kainate receptors (Cotman et al. 1987). The functional importance of this small NMDA receptor loss is, thus, unclear but may be gauged in relation to the large alterations in both presynaptic glutamatergic sites and kainate receptors in the same region. Interesting recent results, however, indicate that although both the agonist site and cation channel of the NMDA receptor complex are not significantly altered in A.D. cerebral cortex, their coupling to the glycine regulatory site is selectively impaired in frontal cortex (Procter et al. 1989). In vivo inhibition of facilitatory NMDA receptor regulation may act to diminish the efficacy of NMDA receptor function in A.D. frontal cortex. The possibility that this represents an intrinsic neuroprotective mechanism merits further investigation.

Although the quisqualate receptor, like the kainate receptor, is involved in fast excitatory postsynaptic transmission (MacDermott and Dale, 1987), quisqualate receptor binding in A.D. frontal cortex, as assessed by [^{3}H]-AMPA, did not differ from control values. The absence of changes in quisqualate receptors in A.D. frontal cortex contrasts with the reported marked reduction (50%) of these sites, as labelled with [^{3}H]-glutamate in A.D. hippocampus (Greenamyre et al. 1987). On the other hand, using [^{3}H]-AMPA as a receptor ligand, no significant differences in quisqualate receptors were found in the hippocampal region of brains (Geddes et al. 1987) except in one A.D. case which exhibited severe cell loss. These results, again, underline the importance of methodological and morphological influences on ligand binding studies in A.D.

Temporal Cortex. In the same A.D. brains in which frontal cortex was examined, there were no significant alterations in [^{3}H]-D-aspartate, [^{3}H]-kainate, [^{3}H]-AMPA or NMDA-sensitive [^{3}H]-glutamate binding in temporal cortex in comparison to control subjects. The lack of alteration in [^{3}H]-D-aspartate binding in the temporal cortex is somewhat surprising in view of previous homogenate binding results which have indicated deficits in temporal cortex of between 40-60% in comparison to control values (Procter et al. 1986; Palmer et al. 1986; Cowburn et al. 1988). However, in contrast to the present autoradiographic study which concentrated on inferior temporal gyrus, all of these investigations relied on "pooled" tissue from the temporal

lobe, suggesting that the differing results may have a neuroanatomical basis.

Previous studies of glutamate receptors in A.D. temporal cortex have focused, almost entirely, on the NMDA receptor. Greenamyre et al. (1984b) found a 57% decrease in low affinity quisqualate-sensitive [^{3}H]-glutamate binding in the most superficial layers of temporal cortex from A.D. patients compared to controls. However, although these authors equate these sites to the NMDA receptor, for reasons outlined above, it is likely that a large proportion of these binding sites are non-receptor sites. Subsequent investigations using both [^{3}H]-glutamate and [^{3}H]-MK-801 (Cowburn et al. 1988; Mouradian et al. 1988), and the results of the present autoradiographic study, all indicate stability of NMDA receptors in A.D. temporal cortex.

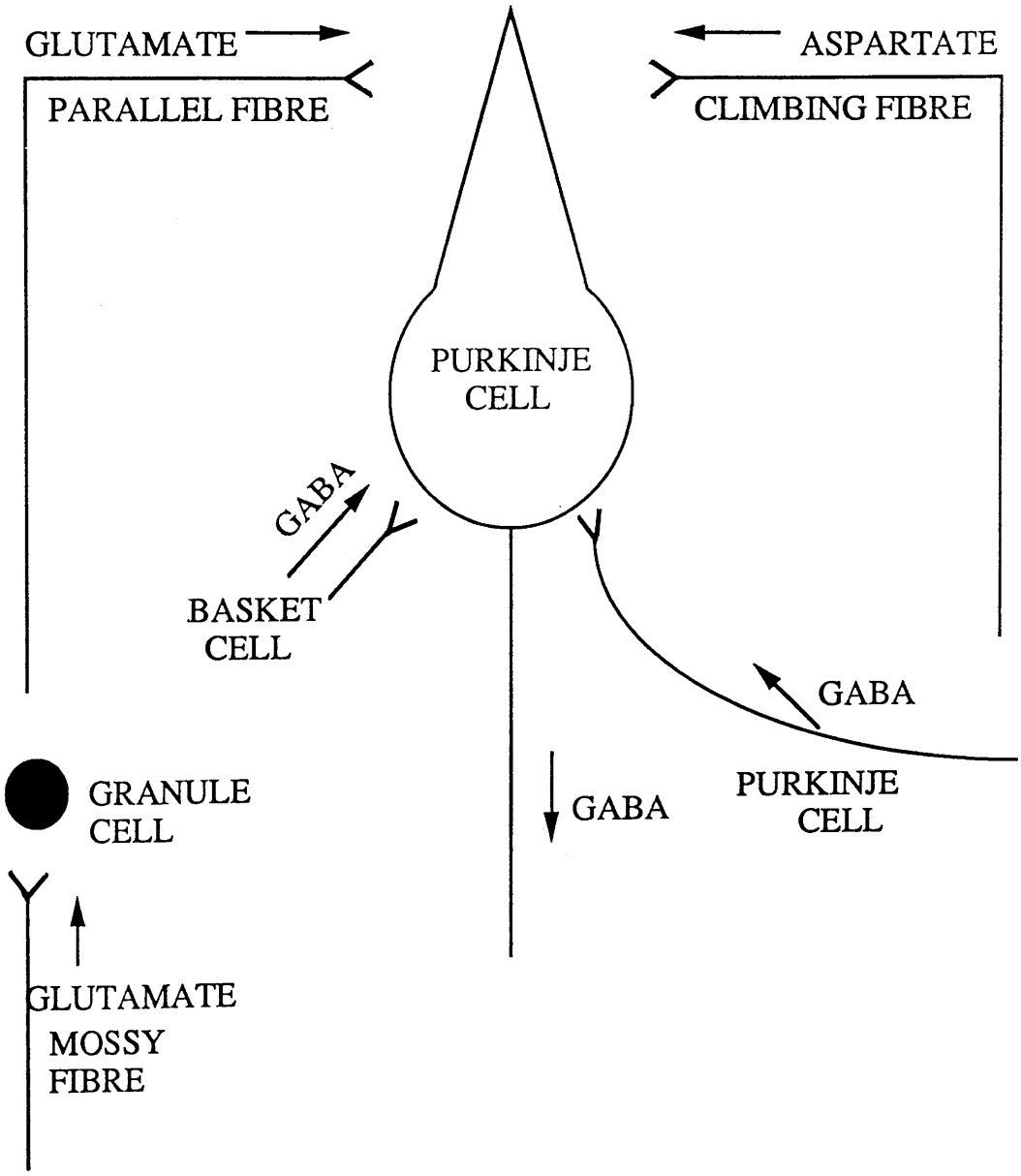
The stability of glutamatergic parameters in temporal but not frontal cortex does not appear to be related to the level of local neuropathology. Quantification of senile plaques in adjacent tissue sections to those used for receptor autoradiography revealed that both temporal and frontal cortex possessed similar numbers of these lesions. Simpson and colleagues (1988) have proposed that differences in ligand binding results between the frontal and temporal cortices in the A.D. brain may be explained in terms of tissue atrophy. These investigators identified a reduction in NMDA receptor binding in frontal but not temporal cortex from A.D. patients, and suggested

that NMDA receptor loss in the temporal cortex was "masked" by greater tissue shrinkage in this region than in frontal cortex (Hubbard and Anderson, 1981). The appropriateness of such an explanation is unclear as the authors do not indicate whether allowance for reduced cerebral cortical tissue in the A.D. brain would have altered their results. Nevertheless, a "collapse" of cortical grey matter due to pyramidal cell loss (Pearson et al. 1985; Rogers and Morrison, 1985) would act to concentrate glutamate receptor sites in temporal cortex and may provide an explanation for their stability in this region. Future investigations which allow simultaneous assessment of neuronal numbers and glutamate recognition sites are obviously desirable.

Cerebellar Cortex. The present study represents the first simultaneous quantitative autoradiographic study of Na^+-dependent glutamate uptake sites and glutamate receptors in the human cerebellum and the first systematic investigation of these sites in A.D.

In contrast to the cerebral cortex, the cerebellar cortex possesses a relatively simple laminar cytoarchitecture and a neuronal circuitry which is well defined anatomically and physiologically (Figure 23). Within this system it has been proposed that excitatory amino acids are the transmitters of the afferent climbing fibres (Wiklund et al. 1982; Foster and Roberts, 1983) mossy fibres (Freeman et al. 1983; Beitz et al. 1986) and intrinsic parallel fibres (Hudson et al. 1976;

FIGURE 23



EXCITATORY AND INHIBITORY AMINO ACID PATHWAYS IN CEREBELLAR CORTEX.

Sandoval and Cotman, 1978). The high concentration of [^{3}H]-AMPA binding in the molecular layer of the human cerebellum suggests that quisqualate receptors are principally located on the dendrites of Purkinje cells where they probably mediate the parallel fibre-Purkinje cell synapse (Olson et al. 1987). The high densities of both [^{3}H]-kainate and NMDA-sensitive [^{3}H]-glutamate binding in the granule cell layer indicates that kainate and NMDA receptors are associated with granule cells and perhaps Golgi and Basket cells. This general distribution of glutamate receptor subtypes in the human cerebellar cortex is consistent with results from both rat (Greenamyre et al. 1985) and mouse brain (Olson et al. 1987).

Senile plaques and neurofibrillary tanges, the characteristic neuropathological lesions in A.D., are found in great numbers in certain areas of the neocortex and hippocampus but rarely in cerebellar cortex (Tomlinson and Corselis, 1984). Consequently, neurochemical investigations in the A.D. brain have focused on those areas most severely affected neuropathologically, such as the hippocampus, with minimal examination of the cerebellar hemisphere. However, perhaps the most striking result from the present investigations has been the substantial loss of quisqualate receptors from the molecular layer of cerebellar cortex in A.D. brains in comparison to control subjects. Concomitant assessment of Na^+-dependent glutamate uptake sites and NMDA and kainate receptors in the same patients indicated no change in these parameters in either

molecular or granule cell layer. The loss of glutamate receptors in A.D. cerebellum is thus selective for the quisqualate subtype and occurs in the absence of a loss of glutamatergic terminals.

Long-term modification of transmission efficacy at synapses is proposed to be the cellular basis of memory and learning, and in this respect, the NMDA receptor has received much attention because of its role in the induction of long-term potentiation (LTP) at central synapses (see Collingridge and Bliss, 1987). However, within the cerebellum it would appear that quisqualate receptors, and not NMDA receptors, are specifically involved in a form of cerebellar synaptic plasticity, long-term depression (LTD) (Ito et al. 1982). When the dual inputs to the cerebellar Purkinje cells from parallel fibres and climbing fibres (Figure 23) are conjunctively activated, parallel fibre-Purkinje cell transmission undergoes LTD. This alteration in synaptic efficacy is related to a desensitisation of quisqualate receptors on Purkinje cell dendrites and may play a role in the motor learning processes of the cerebellum (Kano and Kato, 1987). A loss of quisqualate receptors at this synapse in the A.D. brain may indicate an enhancement of LTD in this region. Whether this receptor loss is epiphenomenal or an hitherto undiscovered extension of the patho-physiological process in the A.D. brain, is open to question. However, it is highly significant that this glutamate receptor alteration occurs in the absence of any gross neuropathological insults, with only two A.D. cases in this study exhibiting very low numbers of senile plaques

(10 and 4) in cerebellar cortex. The possibility of cerebellar circuitry damage prior to neuropathological change is further suggested by the work of Shimohama and colleagues (1988). These investigators, using membrane preparations, identified a 20% reduction in benzodiazepine receptor binding in the cerebellar hemisphere of A.D. cases. Although this response failed to achieve statistical significance, it suggests that inhibitory amino acid systems may also be affected in this region and indicates the need for an autoradiographic investigation of these sites. Quisqualate receptor numbers are reduced in the A.D. cerebellum in the absence of significant numbers of senile plaques, however, it is possible that neuropathological and neurochemical changes in the cerebral cortex of the same patients could be related to this receptor alteration. The concept of "diaschisis" was developed by von Monakow in 1914 to explain temporary impairment of function in an undamaged area of brain following permanent injury to a remote but anatomically related site (for review see Feeney and Baron, 1986). One example of this effect is crossed cerebellar dischisis, in which damage to one cerebral hemisphere can produce functional depression in the contralateral cerebellar hemisphere. Such a phenomenon has recently been described in vivo in a group of severely demented Alzheimer patients using positron emission tomography (PET) (Akiyama et al. 1989). This effect appears to be mediated by cortico-fugal fibres from various regions of the cerebral cortex to the pontine nuclei and, from there, in a crossed tract to the cerebellum. Interestingly, in the patients

examined by Akiyama and colleagues, frontal cortex showed a very high correlation for crossed cerebellar diaschisis which they attributed to the substantial cortico-pontine projections from this region. If the loss of quisqualate receptors in the left cerebellar hemisphere, described in the present investigations, are related to dysfunction in the cerebral cortex, however, this must originate in the right hemisphere. Although substantial glutamatergic dysfunction was evident in the deep layers of left frontal cortex from A.D. patients, in view of the non-symmetrical inter-hemispheric distribution of senile plaques in these cases, assumptions of symmetrical neurochemical changes may be invalid. However, the possibility that cerebral-cerebellar alterations in A.D. have a glutamatergic basis merits further investigation.

The above results indicate anatomically-selective alterations in glutamatergic systems in the A.D. Plastic alterations in glutamate receptors may be associated with local neuropathology or occur in the absence of detectable neuropathological changes, suggesting that the mechanisms of glutamatergic dysfunction in A.D. are heterogeneous with respect to anatomical locus.

2. E.A.A. AND ADENOSINE A_1 RECEPTOR BINDING SITES IN THE RAT VISUAL SYSTEM

2.1 ANATOMICAL DISTRIBUTION OF E.A.A. AND ADENOSINE A_1 RECEPTOR BINDING SITES IN THE RAT VISUAL SYSTEM

Within the rat visual system, the highest levels of all E.A.A. receptor binding sites were found in visual cortex. The laminar distribution of E.A.A. receptor ligands was, however, heterogeneous: [^{3}H]-kainate binding in cortical layers V/VI was approximately double that in superficial cortical layers, while the level of both NMDA-sensitive [^{3}H]-glutamate and [^{3}H]-MK-801 binding was highest in cortical layers II/III. In contrast to the laminar pattern of binding obtained with these ligands, [^{3}H]-AMPA binding was relatively homogenous throughout visual cortex.

In subcortical visual structures, the highest levels of [^{3}H]-AMPA binding were evident in the superficial layer of the superior colliculus. In contrast, the dorsal lateral geniculate and lateral posterior nucleus expressed the highest densities of NMDA-sensitive [^{3}H]-glutamate and [^{3}H]-MK-801 binding.

2.2 E.A.A. AND ADENOSINE A_1 RECEPTOR BINDING SITES AFTER UNILATERAL ORBITAL ENUCLEATION

Full quantitative analysis of [^{3}H]-kainate, [^{3}H]-AMPA, NMDA-sensitive [^{3}H]-glutamate and [^{3}H]-CHA binding after unilateral orbital enucleation is presented in Appendix III.

2.2.1 [^{3}H]-Kainate Binding After Unilateral Orbital Enucleation

At 1, 5 and 10 days post-enucleation [^{3}H]-kainate binding was unaltered in both primary and secondary retinal projection areas in the visually-deprived hemisphere. However, at 20 days post-lesion, a 15% increase in [^{3}H]-kainate binding was evident in the superior colliculus (Figure 24A), although [^{3}H]-kainate binding was unaltered in other visual structures at this time point. There was no significant alteration in [^{3}H]-kainate binding in visual structures of the visually-intact hemisphere post-enucleation (ANOVA). [^{3}H]-Kainate binding was also unaltered in any of the non-visual structures examined at any time point post-enucleation (Appendix III (a)).

2.2.2 [^{3}H]-AMPA Binding After Unilateral Orbital Enucleation

There were no significant alterations in [^{3}H]-AMPA binding at 1 and 5 days post-enucleation in either primary or secondary retinal projection areas in the visually-deprived hemisphere. At 10 and 20 days post-enucleation, however, [^{3}H]-AMPA binding was significantly reduced in the superior colliculus (Figure 24B), but unaltered in other visually-deprived areas. [^{3}H]-AMPA binding was unaltered in visual structures of the visually-intact hemisphere post-enucleation (ANOVA) and unaltered in non-visual structures examined post-enucleation (Appendix III(b)).

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FIGURE 24. Superior colliculus (superficial layer). Alterations in (A) kainate receptors, (B) quisqualate receptors, (C) NMDA receptors, (D) NMDA receptor channel and (E) Adenosine A_1 receptors after unilateral orbital enucleation. Results are presented as the mean percentage change in the visually-deprived left hemisphere (L) as a function of the visually-intact right hemisphere (R) + S.E.M. In (A) - (D), values are given for control and 1, 5, 10 and 20 days survival post-enucleation. In (E), values are given for control and 1, 5, 10 and 20 days survival post-enucleation. *P<0.01, paired t-test.

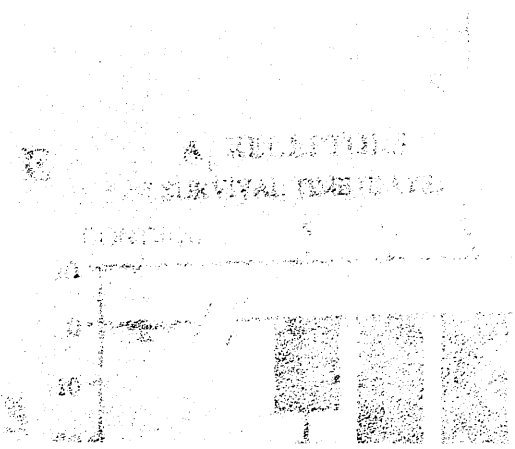
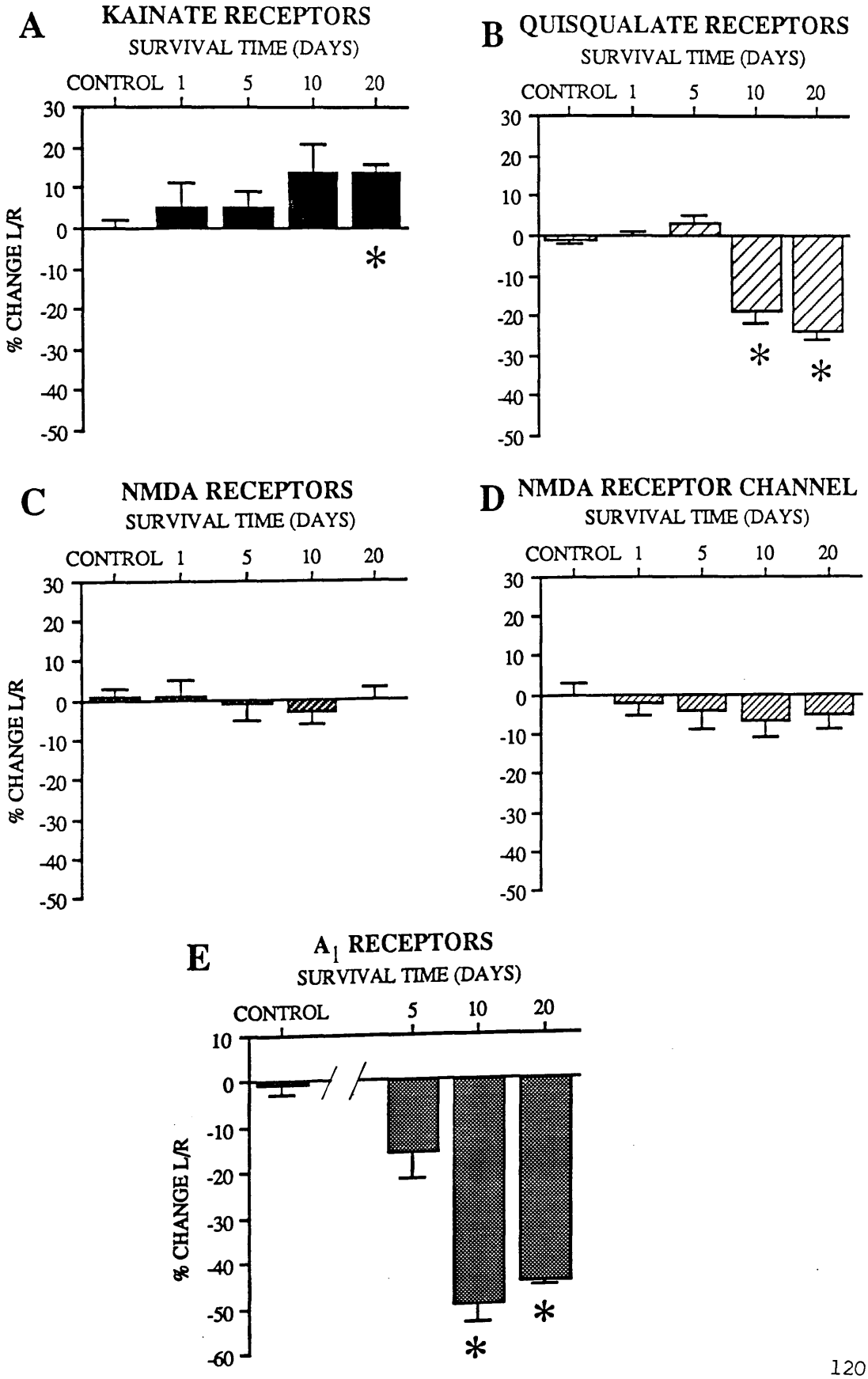


FIGURE 24 E.A.A. AND ADENOSINE A_1 RECEPTORS IN VISUALLY-DEPRIVED SUPERIOR COLLICULUS POST-ENUCLEATION



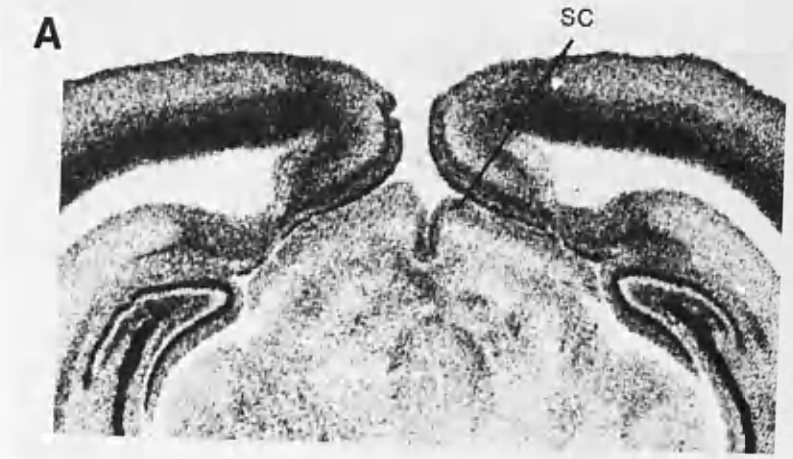
2.2.3. NMDA-Sensitive [^{3}H]-Glutamate and [^{3}H]-MK-801 Binding After Unilateral Orbital Enucleation

There were no significant alterations in NMDA-sensitive [^{3}H]-glutamate of [^{3}H]-MK-801 binding in visual structures in the visually-deprived hemisphere post-enucleation. NMDA-sensitive [^{3}H]-glutamate and [^{3}H]-MK-801 binding were unaltered in visual structures of the visually-intact hemisphere post-enucleation (ANOVA) and unaltered in all non-visual structures examined post-enucleation (Appendix IV (c) and (d)).

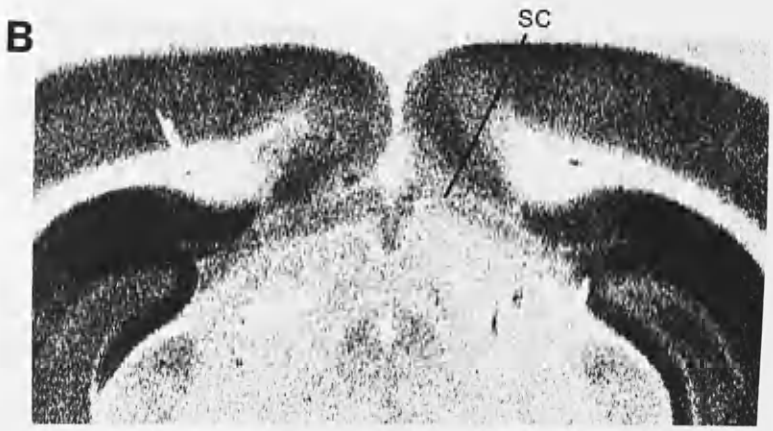
2.2.4 [^{3}H]-CHA Binding After Unilateral Orbital Enucleation

[^{3}H]-CHA binding was not significantly altered in visual structures of the visually-deprived hemisphere up to 5 days post-enucleation. There was, however, a significant reduction (~50%) in [^{3}H]-CHA binding in the superior colliculus at 10 and 20 days post-enucleation (Figure 24E). The [^{3}H]-CHA autoradiograms in Figure 26 illustrate the striking loss of optical density in the visually-deprived superior colliculus at 10 and 20 days post-enucleation.

There were no other significant ($P<0.01$) alterations in [^{3}H]-CHA binding in visual structures of the visually-deprived hemisphere, although a 12% reduction in [^{3}H]-CHA binding in the dorsal lateral geniculate nucleus at 20 days post-enucleation was significant at the 5% level.



KAINATE



QUISQUALATE

NMDA

FIGURE 25 REPRESENTATIVE AUTORADIOGRAMS OF KAINATE, QUISQUALATE AND NMDA RECEPTOR BINDING AT 20 DAYS POST-ENUCLEATION

In the above autoradiograms the right hand hemisphere is visually-deprived. In (A) and (B), note the simultaneous increase and decrease in kainate and quisqualate receptor binding respectively in the visually-deprived superior colliculus (SC). NMDA receptor binding is unaltered (C).

Section co-ordinates are approximately 2.96mm interaural, -6.04mm bregma.

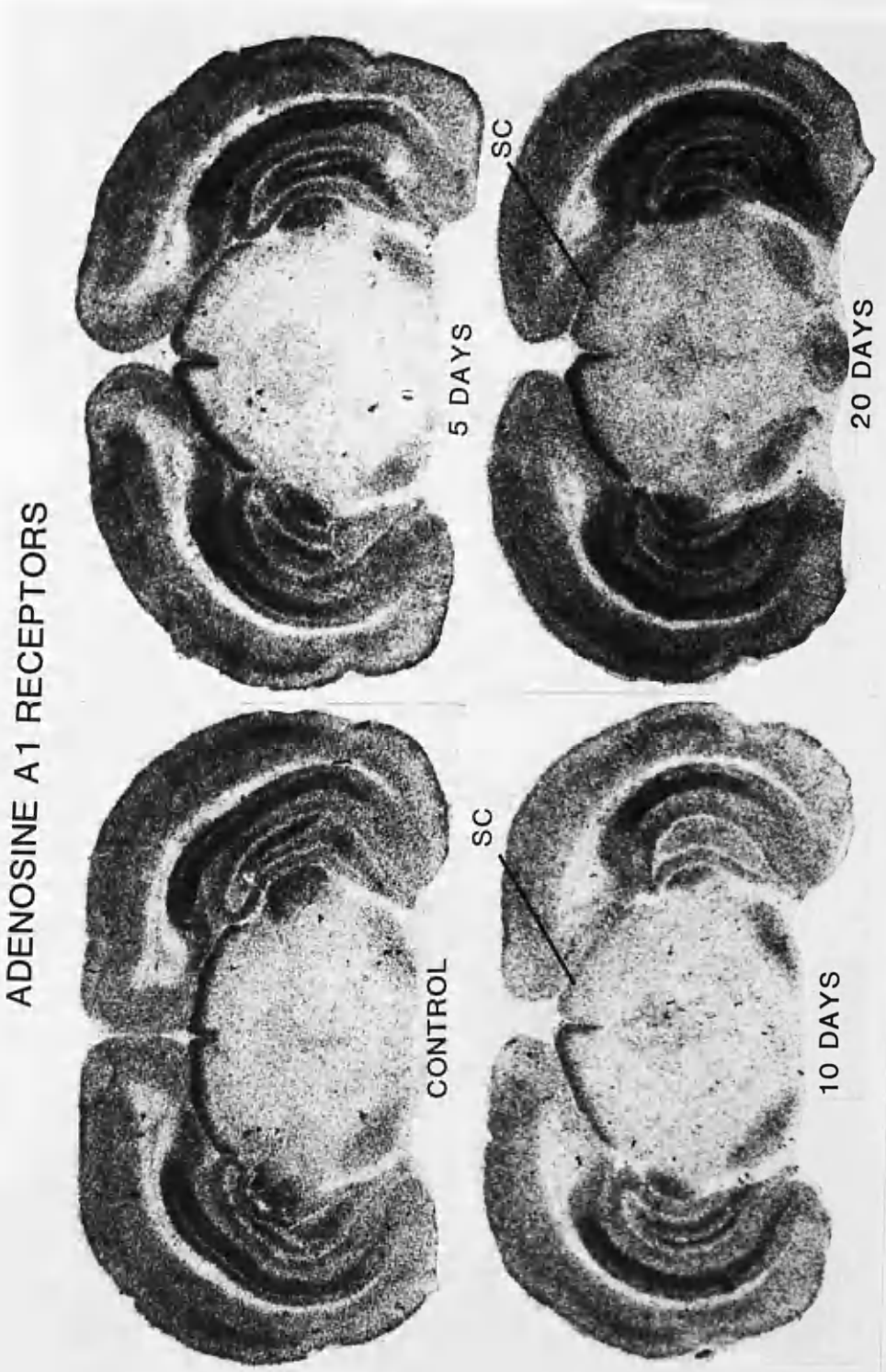


FIGURE 26 ADENOSINE A_1 RECEPTOR BINDING POST-ENUCLEATION

In the above autoradiograms the right hand hemisphere is visually-deprived. In representative autoradiograms, note the reduction in optical density in the visually-deprived superior colliculus at 10 and 20 days post-enucleation.

2.2.5 Binding Parameters for [^{3}H]-Kainate at 20 Days Post-Enucleation

Saturation analysis of [^{3}H]-kainate binding (2.5 - 75nM) was performed in five animals at 20 days post-enucleation. Scatchard analysis of this data in the superior colliculus gave curvilinear plots, ($P<0.05$, F test as compared to linear fit), mean values are illustrated in Figure 27. Assuming that curvilinearity reflects multiple binding sites, these curves could be resolved into high and low affinity sites in the visually-intact superior colliculus with K_D values of 2.5 ± 0.5nM and 35 ± 6nM, and B_{max} values of 21 ± 0.5pmoles/g tissue and 76 ± 7pmoles/g tissue for high and low affinity sites respectively. In the visually-deprived superior colliculus there was a significant increase in the B_{max} value for the high affinity kainate site, 25 ± 1pmoles/g tissue **($p<0.01$)**, with no alteration in the K_D value, 2.5 ± 0.5nM. The K_D and B_{max} values for low affinity kainate sites, 21 ± 5nM and 72 ± 8pmoles/g tissue respectively, were not significantly different from those in the visually-intact hemisphere.

2.2.6 Binding Parameters for [^{3}H]-AMPA at 20 Days Post-Enucleation

Saturation analysis of [^{3}H]-AMPA binding (10-1000nM) in five animals at 20 days post-enucleation revealed curvilinear Scatchard plots ($P<0.05$, F test compared to linear fit). In the visually-intact superior colliculus, these curves could be resolved into high and low affinity sites with K_D values of 42 ± 3nM and 500 ± 53nM, and B_{max} values of

40 ± 2pmoles/g tissue and 240 ± 21pmoles/g tissue respectively. In visually-deprived superior colliculus there was a significant reduction in the B_{max} value for the high affinity AMPA site in comparison to the visually-intact hemisphere, 30 ± 1pmoles/g tissue ($P<0.01$), with no change in K_D, 40 ±2nM. There was no statistically significant alterations in low affinity AMPA binding parameters in the visually-deprived superior colliculus although there was a 20% reduction in the B_{max} value (183 ± 17pmoles/g tissue) in comparison to the intact hemisphere (240 ± 21pmoles/g tissue).

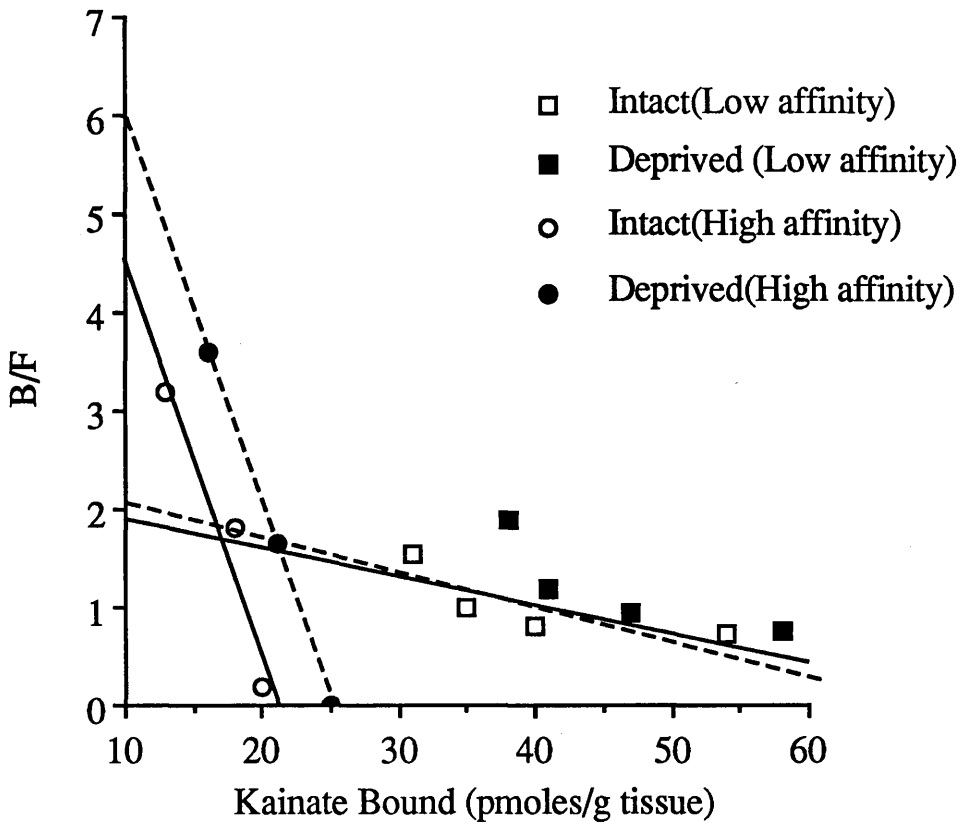


FIGURE 27 SCATCHARD ANALYSIS OF [^{3}H]-KAINATE BINDING IN SUPERIOR COLLICULUS

Scatchard plots of [3H]-kainate binding in the visually-intact(□,○) and visually-deprived(■,●) superior colliculus at 20 days post-enucleation. [3H]-kainate binding was determined using concentrations from 2.5-75nM as described in chaper II. Data are representative of five animals.

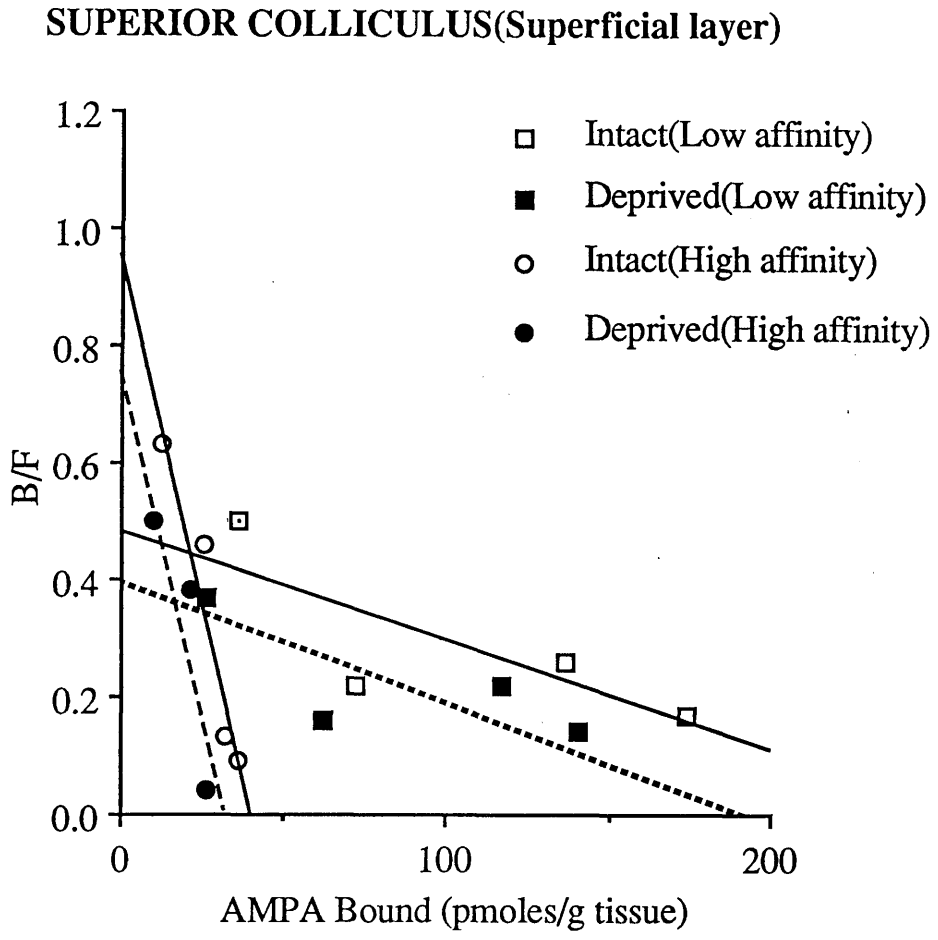


FIGURE 28 SCATCHARD ANALYSIS OF [^{3}H]-AMPA BINDING IN SUPERIOR COLLICULUS

Scatchard analysis of [^{3}H]-AMPA binding in the visually-intact(□,○) and visually-deprived(■,●) superior colliculus at 20 days post-enucleation. [^{3}H]-AMPA binding was determined using concentrations from 10-1000nM as described in chapter II. Data are representative of five animals.

2.3 COMMENTARY: DISCUSSION OF E.A.A. AND ADENOSINE A_1 RECEPTOR BINDING IN THE RAT VISUAL SYSTEM AFTER UNILATERAL ORBITAL ENUCLEATION.

Examination of the electrophysiological and pharmacological properties of the excitatory postsynaptic potentials evoked by electrical stimulation of the optic tract, indicates that an excitatory amino acid acts as transmitter in retinal projection fibres (Crunelli et al. 1987). The competitive antagonism by γ-D-glutamylglycine of both the excitatory postsynaptic potential and the depolarisation produced by exogenous quisqualate and glutamate, indicates that the synaptic receptors of the optic nerve are of the quisqualate/kainate type in the lateral geniculate nucleus. Similarly, glutamate-induced ionic currents in cultured rat superior colliculus neurons, also involve quisqualate and kainate receptor-mediated conductance changes (Grantyn et al. 1987; Perouansky & Grantyn, 1989). In addition to mediating retino-fugal neurotransmission, there is good evidence supporting a transmitter role for glutamate and/or aspartate in the projection fibres from visual cortex to subcortical visual structures (Lund-Karlsen and Fonnum, 1978; Fosse and Fonnum, 1987). Glutamatergic cortico-geniculate cells in layer VI (Baughman and Gilbert, 1981) and cortico-collicular cells in layer V (Huettner and Baughman, 1988) also send collaterals throughout visual cortex, indicating an interlaminar transmitter role for glutamate. Finally, in addition to mediating retino-fugal, cortico-fugal and intracortical transmission, there is some evidence to suggest that cortical

postsynaptic responses to dorsal lateral geniculate input are mediated by excitatory amino acid receptors (Tsumoto et al. 1986; Hagihara et al. 1988).

All of the above evidence indicates the extensive involvement of glutamatergic transmission in visual processing and, in view of the high density of glutamate receptors within visual structures (Greenamyre et al. 1984) allows the rat visual system to act as an appropriate polysynaptic model in which to study post-denervation regulation of glutamate receptors.

Kainate Receptors. Based on cell fractionation studies, kainate binding sites have been shown to be synaptically localised (Foster et al. 1981). The anatomical heterogeneity of [^{3}H]-kainate binding within the rat visual system suggests that kainate receptors mediate excitatory amino acid neurotransmission within specific visual pathways. In this respect, the high density of [^{3}H]-kainate binding in deep layers of visual cortex is in agreement with previous autoradiographic data (Monaghan and Cotman, 1982) and supportive of electrophysiological evidence implicating kainate receptors as mediators of excitatory amino acid transmission in cortical layers IV-VI of visual cortex (Fox et al. 1989). In addition to mediating postsynaptic excitatory transmission, there is evidence to indicate that a population of kainate receptors may exist on the terminals of certain glutamatergic afferent neurones, where they act to modulate

glutamate release. Kainate-induced neurodegeneration in the striatum depends on an intact glutamatergic afferent input (cortico-striatal) (Biziere and Coyle, 1979; McGeer et al. 1978) and results from in vitro and in vivo preparations suggest that in this area, kainate increases extracellular glutamate concentrations by acting on presynaptic positive-feedback autoreceptors on glutamatergic terminals (Young et al. 1988). It therefore seems plausible that kainate receptors may be located pre- and/or postsynaptically within visual pathways.

The stability of [^{3}H]-kainate binding in retinorecipient nuclei (superior colliculus, dorsal lateral geniculate) up to 10 days post-enucleation, suggests that kainate receptors are not located presynaptically on the terminals of retinal projection fibres. At this time point, [^{3}H]-CHA binding to adenosine A_1 receptors was reduced by approximately 50% in the visually-deprived superior colliculus, indicative of the degeneration of retinal fibres in this structure (Goodman et al. 1983). This time course for retinal projection fibre loss is consistent with the ultrastructure study of Lund (1969), showing that the number of degenerating endings reaches a maximum 7 days after eye ablation. It would thus seem safe to assume that, in the superior colliculus at least, kainate receptors are located postsynaptically.

[^{3}H]-Kainate binding was significantly increased (15%) in the visually-deprived superior colliculus at 20 days post-enucleation. Saturation analysis of this response revealed that the increase was due to an increased number of high affinity kainate receptor sites with no significant alteration in receptor affinity. There was also no significant alteration in low affinity kainate receptor numbers or affinity. The existence of high and low affinity kainate receptor sites in rat brain has previously been demonstrated using membrane preparations (Foster et al. 1981; Honoré et al, 1986), and the affinity constants obtained in this autoradiographic study are in good agreement with these investigations. The functional relationship between high affinity and low affinity kainate receptors remains unclear. However, if is of interest that Ca^{2+} ions selectively inhibit high affinity [^{3}H]-kainate binding (Honoré et al. 1986), in view of the evidence that kainate receptor activation is associated with Ca^{2+} channel gating (Hori et al. 1985; Wroblewski et al. 1985). This effect is due to a reduction in receptor affinity (increased K_D) with no significant alteration in the number of sites (Braitman and Coyle, 1987), suggesting an allosteric interaction beween the kainate receptors and Ca^{2+} channels. The high affinity site may therefore represent an "uncoupled" form of the receptor, whereas the low affinity site is associated with Ca^{2+} channel occupancy and represents a "coupled" form of the receptor. On the other hand, on the basis of high-energy radiation inactivation analysis, the Ca^2-insensitive low affinity kainate site has been proposed to be an entirely different

macromolecule from the high affinity site (Honoré et al. 1986) possessing a molecular weight similar to that of quisqualate receptors.

Increased kainate receptor numbers in the visually-deprived superior colliculus may represent a denervation supersensitivity reaction in reponse to reduced glutamatergic input of retinal and/or cortical orgin; although there is some evidence to suggest that cortico-tectal activity may in fact "recover" at longer survival times post-enucleation (see 3.3). A lack of kainate receptor up-regulation in the visually-deprived dorsal lateral geniculate nucleus or pretectal area suggests that the level of normal retinal input may be a determinant of postsynaptic receptor response. Alternatively, kainate receptor up-regulation post-enucleation may be confined to those retino-recipient nuclei in which kainate receptor-mediated responses are of greatest physiological importance. In this respect, the recent electrophysiological study of Perouansky and Grantyn (1989) is of considerable relevance. These investigators used cultured neurons from rat superior colliculus to examine the receptors mediating the compound response to exogenous glutamate. Although all three glutamate receptor subtypes were expressed in the colliculus, kainate-activated currents were larger than those for quisqualate or NMDA and kainate receptors were found to be responsible for 49%-82% of the compound current elicited by glutamate. Thus, from an electrophysiological standpoint, an increase in kainate receptor numbers may represent the most efficacious postsynaptic response to

reduced glutamatergic input.

Intrinsic GABAergic neurons predominate in the superficial layer of the superior colliculus (Ottersen and Storm-Mathisen, 1984). However, the excitotoxic action of stereotaxically-injected kainic acid in this region is most apparent in intrinsic glutamatergic neurons, as reflected in reduced [^{3}H]-D-asparate uptake (Fosse and Fonnum, 1986), suggesting that glutamatergic afferents impinge predominantly on these cells. Interestingly, visual cortex ablation provided an almost complete protection against kainate-induced neurotoxicity, while enucleation was ineffective. This further supports the proposed lack of presynaptic kainate receptors on retinal afferents, as suggested by the results of the present study. However, it raises the intriguing possibility that kainate receptor up-regulation in the colliculus post-enucleation may be localised partially or wholly to cortico-tectal terminals. Such a response may act to amplify cortico-tectal actvity via enhanced positive-feedback mechanisms.

Quisqualate Receptors. Quisqualate receptors, like kainate receptors, mediate fast excitatory transmission and are implicated in visual processing (Crunelli et al. 1987; Kelly and Crunelli, 1988). These receptors were unaltered in the visually-deprived hemisphere up to 10 days post-enucleation. However, at 10 and 20 days post-lesion [^{3}H]-AMPA binding was significantly reduced in visually-deprived superior colliculus, but unaltered in other retinorecipient nuclei or visual

cortex. Saturation analysis of [^{3}H]-AMPA binding at 20 days post-enucleation revealed both high and low affinity sites with K_D values in agreement with published autoradiographic experiments (Westerberg et al. 1989). In the visually-deprived superior colliculus at this time point, there were no significant changes in either high or low affinity K_D values for [^{3}H]-AMPA binding. However the B_{max} values for both binding sites were reduced, although the reduction in low affinity sites failed to achieve statistical significance. From homogenate binding experiments, it has been proposed that the curvilinear Scatchard plots obtained with [^{3}H]-AMPA in the presence of potassium thiocyanate reflect two inter- convertible states of the quisqualate receptor rather than two separate binding sites (Honoré and Drejer, 1988). The equilibirum between the two states being shifted towards the conformation with high affinity for [^{3}H]-AMPA by potassium thiocyanate. In agreement with this kinetic model, absolute levels of [^{3}H]-AMPA binding are reduced in autoradiographic assays in the absence of potassium thiocyanate (personal observations; Westerberg et al. 1989). The loss of [^{3}H]-AMPA binding at 10 days post-enucleation coincides with a significant 50% reduction in A_1 receptor binding in the same region. This suggests that a reduction in [^{3}H]-AMPA binding may reflect the loss of a population of presynaptic quisqualate receptors on degenerating retinal projection fibres. Consistent with this hypothesis, presynaptic quisqualate receptors have been demonstrated on glutamatergic cortico-striatal fibres (Errami and Nieoullon, 1988) where they may act to modulate cortico-striatal

neurotransmission.The proportion of presynaptic quisqualate receptors at the cortico-striatal synapse (30%) is also similar to the percentage reduction in [^{3}H]-AMPA binding in the visually-deprived superior colliculus. On the other hand, the stability of [^{3}H]-AMPA binding in other visually-deprived retinorecipient nuclei (dorsal lateral geniculate nucleus and pretectal nuclei), may argue in favour of selective postsynaptic quisqualate receptor response in the visually-deprived superior colliculus. It is, however, conceivable that the proportionately lower degree of retinal input into these structures in comparison to the superior colliculus precludes detection of small presynaptic binding deficits. In addition, the possibility that quisqualate receptor down-regulation and kainate receptor up-regulation represent a co-ordinated compensatory postsynaptic response seems unlikely as these divergent receptor responses were statistically unrelated (r=0.010) at 20 days post-enucleation.

Degeneration of retinal projection fibres in the visually-deprived colliculus will be associated with a degree of reactive gliosis. Such an alteration in cellular composition could conceivably act to increase the self-absorption of soft β-particles in this region with a resultant increase in the quenching of the [^{3}H]-ligand signal. Asymmetrical tritium quenching would result in subsequent asymmetries in ligand binding and false positive results. It is, however, evident that differential tritium quenching in the superior colliculus is not influential in the present study, in view of the non-homogeneous alterations in ligand binding in this region post-enucleation.

NMDA Receptors.

Both NMDA-sensitive [^{3}H]-glutamate binding and [^{3}H]-MK-801 binding were unaltered in the visually-deprived hemisphere up to 20 days post-enucleation. Thus, within this polysynaptic system, the NMDA recognition site and related ionophore appear to be insensitive to functional changes in their microenvironment. The lack of alterations in NMDA receptor binding in retinorecipient nuclei indicates that these receptor sites are not located presynaptically on retinal afferents. Although NMDA receptors are involved in developmental changes and induction of LTP within visual cortex (Kleinschmidt et al. 1987; Artola and Singer 1987), the stability of these sites post-enucleation may relate to a minor physiological role in mediating visual processing. On the other hand, the robustness of the NMDA receptor complex has been a feature of glutamate receptor studies in both cerebral ischaemia (Bowery et al. 1988; Westerberg et al. 1989; Dewar et al. 1989) and hypoglycaemia (Westerberg and Wieloch, 1989) where not only functional alterations but also neuronal necrosis is significant. The maintenance of NMDA receptors post-enucleation may therefore reflect a general homeostatic mechanism in relation to these sites.

Adenosine A_1 Receptors. Adenosine acts as a neuromodulator within the CNS by altering neuronal excitability or the release of neurotransmitters (Phillis and Wu, 1981). CNS adenosine receptors have been designated A_1 and A_2 according to their respective inhibitory and stimulatory action on cyclic adenosine monophosphate synthesis (Van Calker et al. 1979). The presence of adenosine A_1 receptors on the terminals of retinal fibres (Goodman et al. 1983) has allowed these sites to act as suitable markers of retinal projection fibre degeneration post-enucleation. However, it is evident that A_1 receptors are not present on all excitatory terminals (Goodman et al. 1983) and, consequently, may not act as markers for excitatory cortico-fugal and intracortical fibres within the visual pathway, although, within the time frame of the experiments, significant transynaptic degeneration of these fibres is unlikely.

The stability of cortical glutamate receptor subtype binding in the present study contrasts with a previous investigation of [^{3}H]-glutamate binding after acute orbital enucleation (Chalmers and McCulloch, 1989). At 1 day post-enucleation total [^{3}H]-glutamate binding was found to be reduced throughout visually-deprived visual cortex but unaltered in retinorecipient nuclei, such as the superior colliculus. The present investigations suggests that this alteration in total [^{3}H]-glutamate binding reflects changes in non-receptor sites. In this respect, it is likely that the assay conditions employed for total [^{3}H]-glutamate binding (use of Tris/HCl buffer) would have allowed the

binding of chloride dependent glutamate uptake sites (Bridges et al. 1987) in addition to glutamate receptors. It thus seems probable that chloride-dependent glutamate uptake sites are reduced or desensitised in visual cortex post-enucleation. This transynaptic alteration, at a time when no gross morphological changes would occur, may be a physiologically relevant compensatory mechanism in respect to reduced geniculate input, possibly acting to increase synaptic glutamate concentrations.

The present investigation has demonstrated the presence of two concurrent alterations in glutamate receptor subtypes post-enculeation. The increase in kainate receptors is likely to reflect a postsynaptic up-regulation in response to reduced glutamatergic input while the loss of quisqualate receptor binding is likely to be presynaptic on degenerating retinal fibres. The stability of NMDA receptor binding post-enucleation indicates that these sites are not located on retinal afferents and are not susceptible to lesion-induced modulation in this system.

3. GLUCOSE UTILISATION AND $5HT_1$, $5HT_2$, β-ADRENERGIC, $GABA_A$ AND CHOLINERGIC MUSCARINIC RECEPTOR BINDING IN THE RAT VISUAL SYSTEM

3.1 ANATOMICAL DISTRIBUTION OF SEROTONERGIC, β-ADRENERGIC, $GABA_A$ AND CHOLINERGIC MUSCARINIC RECEPTORS IN THE RAT VISUAL SYSTEM

Each of the ligands employed in these studies exhibited distinctive distributions of binding within the visual system. However, there were some similarities between ligands in relation to the hierachy of binding within anatomical components of the visual pathway and with respect to the laminar distribution of binding within visual cortex.

In subcortical visual structures, high levels of [^{3}H]-5HT (5-hydroxy-tryptamine), [^{3}H]-ketanserin and [^{3}H]-DHA (dihydroalprenolol) binding were found in the superior colliculus and relatively low levels in the dorsal lateral geniculate nucleus, indicating a somewhat similar distribution of $5HT_1$, $5HT_2$ and β-adrenergic receptors in these anatomical structures. Similarly, the density of [^{3}H]-QNB (quinuclidinyl-benzilate) binding to muscarinic receptors in the superior colliculus was double that in the dorsal lateral geniculate nucleus although for this ligand the lowest levels of binding were found in pretectal nuclei. In contrast to these ligands, the level of [^{3}H]-muscimol binding to $GABA_A$ receptors in the dorsal lateral geniculate nucleus was approximately double that in the superior

colliculus, the lowest level of binding being present in pretectal nuclei.

Unlike subcortical visual structures, within visual cortex, the distribution of [^{3}H]-5HT, [^{3}H]-ketanserin and [^{3}H]-DHA binding were very different: [^{3}H]-5HT binding was concentrated in deep cortical layers, while [^{3}H]-DHA binding was highest in cortical layers II-III. In contrast, [^{3}H]-ketanserin binding was low in cortical layers II/III and V/VI and high in layer IV. Dense labelling of layer IV of visual cortex was also a feature of [^{3}H]-muscimol autoradiograms, while [^{3}H]-QNB exhibited a tri-laminar pattern of binding; binding in cortical layer IV being lower than that in cortical layers II/III and V/VI.

3.2 GLUCOSE UTILISATION AND [^{3}H]-5HT, [^{3}H]-KETANSERIN, [^{3}H]-DHA, [^{3}H]-MUSCIMOL AND [^{3}H]-QNB BINDING AFTER UNILATERAL ORBITAL ENUCLEATION

Full quantitative analysis of glucose use, [^{3}H]-5HT, [^{3}H]-ketanserin, [^{3}H]-DHA, [^{3}H]-muscimol and [^{3}H]-QNB binding following unilateral enucleation is presented in Appendix IV.

3.2.1 [^{3}H]-5HT and [^{3}H]-Ketanserin Binding After Unilateral Orbital Enucleation

At 1 day post-enucleation [^{3}H]-5HT binding was unaltered in both primary and secondary retinal projection areas in the visually-deprived hemisphere. However, at 5, 10 and 20 days post-lesion, reductions

in [^{3}H]-5HT binding were evident in both the superior colliculus (Figure 29) and dorsal lateral geniculate nucleus (Figure 30). In both structures the magnitude of the reduction was greatest after 10 days post-enucleation (-27% and -44% in superior colliculus and dorsal lateral geniculate nucleus respectively). There was no significant alteration in [^{3}H]-5HT binding in visual structures of the visually-intact hemisphere post-enucleation (ANOVA). [^{3}H]-5HT binding was unaltered in any of the non-visual structures examined at any time point post-enucleation (Appendix IV (b)).

Unilateral orbital enucleation induced no significant alterations in [^{3}H]-ketanserin binding to $5HT_2$ receptors in any visual structure at any time point examined. [^{3}H]-Ketanserin binding was also unchanged in any non-visual structures examined post-enucleation (Appendix V (c)).

3.2.2 [^{3}H]-DHA Binding After Unilateral Orbital Enucleation

There was no change in [^{3}H]-DHA binding in any visual structures in the visually-deprived hemisphere at 1 day post-enucleation. However, in a similar fashion to [^{3}H]-5HT binding, [^{3}H]-DHA binding was significantly reduced in both the superior colliculus and dorsal lateral geniculate nucleus at 5, 10 and 20 days post-enucleation (Figures 29 and 30).

LEGEND TO FIGURE

FIGURE 29. Superior colliculus (superficial layer). Alterations in (A) glucose use, (B) β-adrenergic receptors, (C) $5HT_1$ receptors, (D) $5HT_2$ receptors, (E) $GABA_A$ receptors and (F) cholinergic muscarinic receptors after unilateral orbital enucleation. Results are presented as the mean percentage change in the visually-deprived left hemisphere (L) as a function of the visually-intact right hemisphere (R) + S.E.M. Values are given for control and 1, 5, 10 and 20 days survival post-enucleation. *P<0.01, paired t-test.

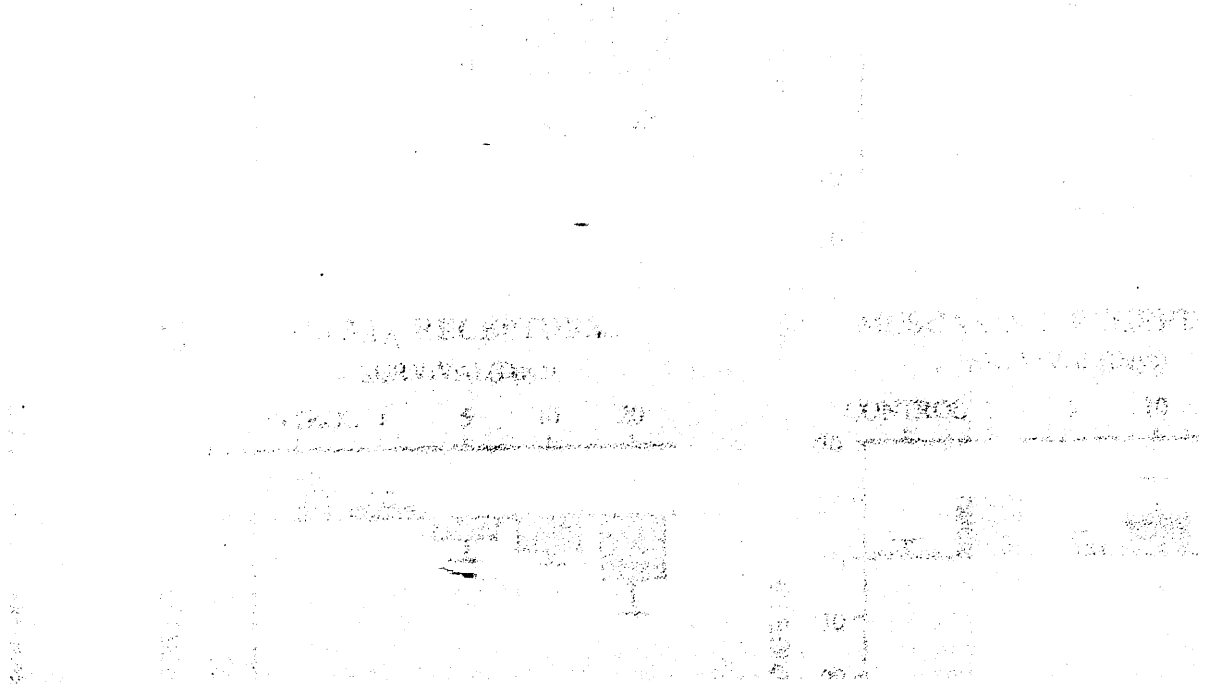
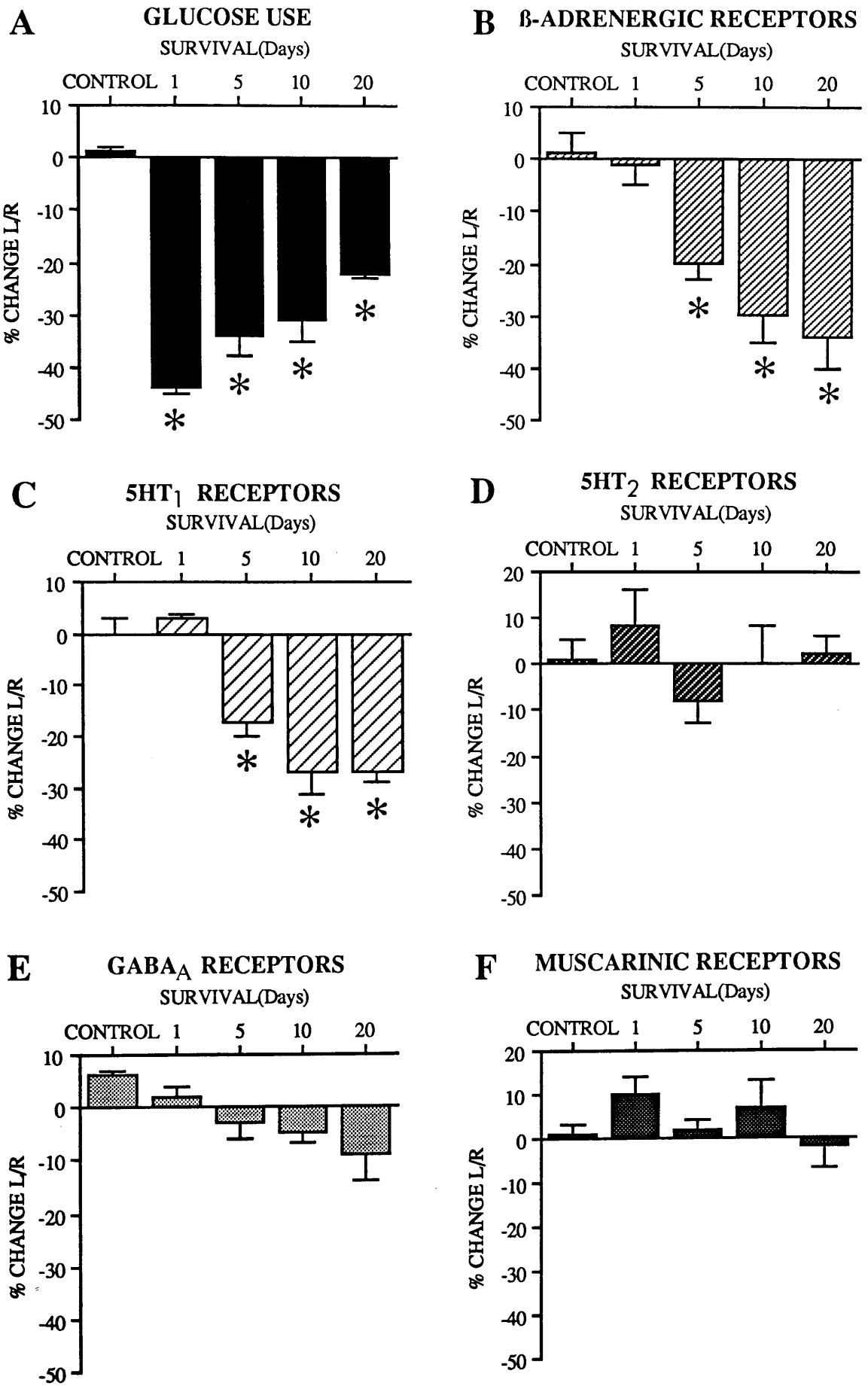


FIGURE 29 GLUCOSE USE AND LIGAND BINDING IN THE VISUALLY-DEPRIVED SUPERIOR COLLICULUS POST-ENUCLEATION



LEGENDS TO FIGURES

FIGURE 30. Dorsal lateral geniculate nucleus. Alterations in (A) glucose use, (B) β-adrenergic receptors, (C) $5HT_1$ receptors, (D) $5HT_2$ receptors, (E) $GABA_A$ receptors and (F) cholinergic muscarinic receptors after unilateral orbital enucleation. Results are presented as the mean percentage change in the visually-deprived left hemisphere (L) as a function of the visually-intact right hemisphere (R) + S.E.M. Values are given for control and 1, 5, 10 and 20 days survival post-enucleation. *P<0.01, paired t-test.

FIGURE 31. Visual cortex, layer IV. Alterations in (A) glucose use, (B) β-adrenergic receptors, (C) $5HT_1$ receptors, (D) $5HT_2$ receptors, (E) $GABA_A$ receptors and (F) cholinergic muscarinic receptors after unilateral orbital enucleation. Results are presented as the mean percentage change in the visually-deprived left hemisphere (L) as a function of the visually-intact right hemisphere (R) + S.E.M. Values are given for control and 1, 5, 10 and 20 days survival post-enucleation. *P<0.01, paired t-test.

FIGURE 30 GLUCOSE USE AND LIGAND BINDING IN THE VISUALLY-DEPRIVED DORSAL LATERAL GENICULATE NUCLEUS POST-ENUCLEATION

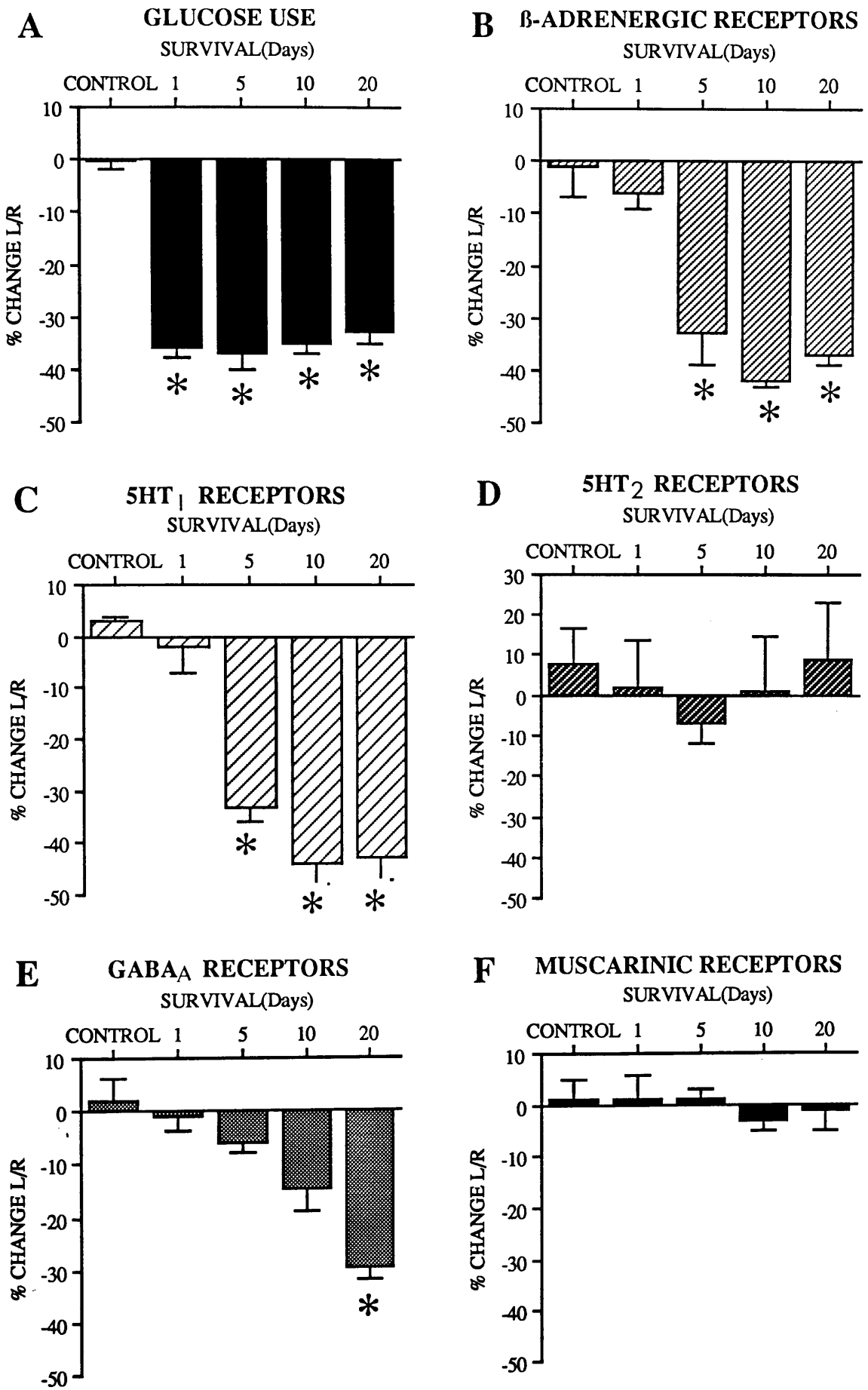
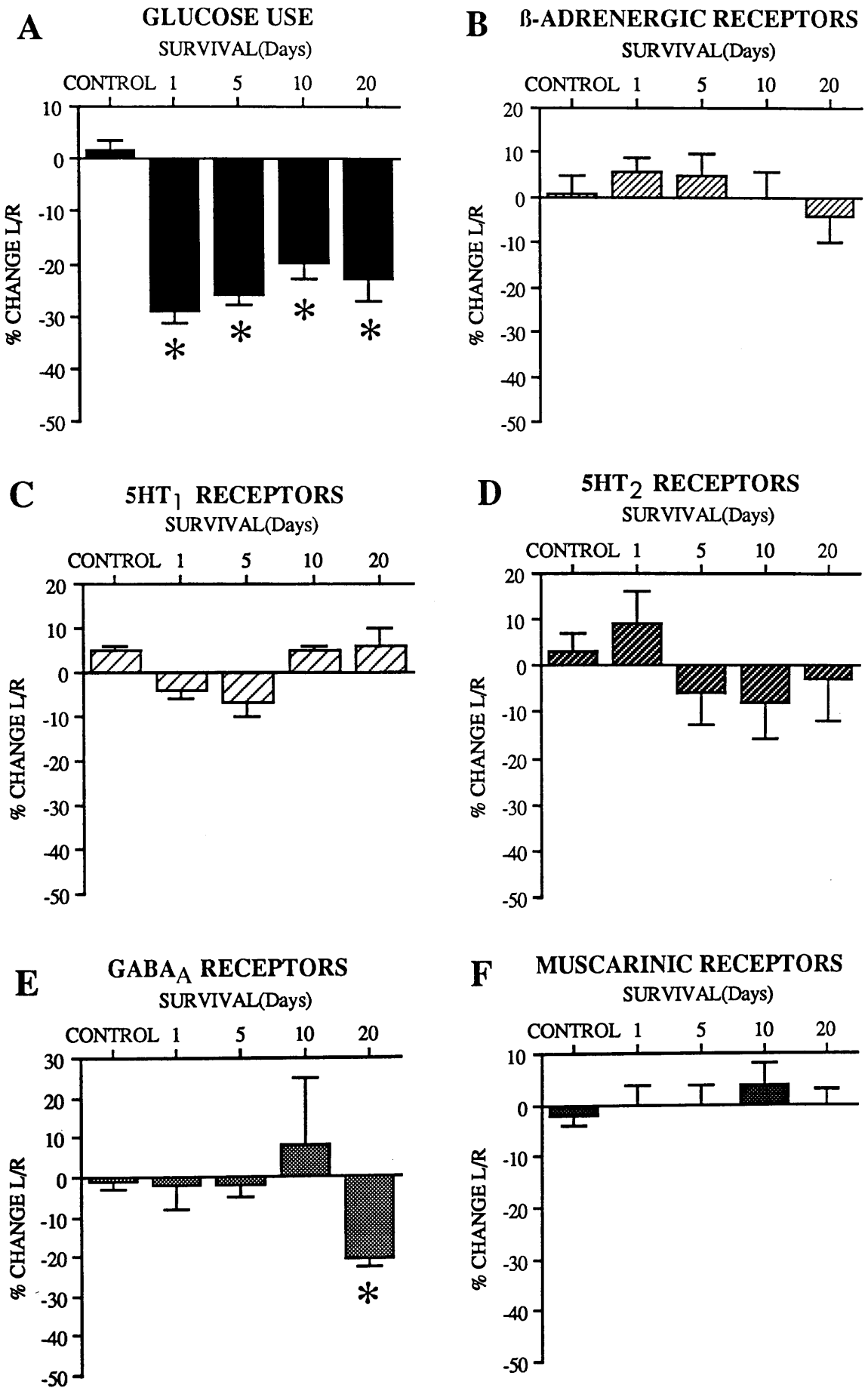


FIGURE 31 GLUCOSE USE AND LIGAND BINDING IN THE VISUALLY-DEPRIVED VISUAL CORTEX POST-ENUCLEATION



[^{3}H]-DHA binding in pretectal nuclei exhibited a statistically significant bilateral reduction 10 and 20 days post-enucleation (F=5.17 and F=4.63 for intact and deprived hemispheres respectively; degrees of freedom between groups 4, within groups 17), although no asymmetry in binding was evident in this region at any time point. There were no significant alterations in [^{3}H]-DHA binding in any other visual structures in the visually-deprived hemisphere post-enucleation. [^{3}H]-DHA binding was not altered in any non-visual structures examined at any time point post-enucleation (Appendix IV(d)).

3.2.3 [^{3}H]-Muscimol Binding After Unilateral Orbital Enucleation

[^{3}H]-Muscimol binding was not significantly altered in either primary or secondary retinal projection areas in the visually-deprived hemisphere at 1, 5 or 10 days post-enucleation. Only at 20 days post-enucleation were reduced levels of [^{3}H]-muscimol binding evident in both dorsal lateral geniculate nucleus (-30%) (Figure 30) and deep layers (IV/VI) (-20%) of visual cortex (Figure 31). There were no significant alterations in [^{3}H]-muscimol binding in visual structures of the visually-intact hemisphere post-enucleation (ANOVA) or in any non-visual structures examined at any time point post-enucleation (Appendix IV (e)).

3.2.4 [^{3}H]-QNB Binding After Unilateral Orbital Enucleation

Unilateral orbital enucleation produced no significant alterations in [^{3}H]-QNB binding in primary visual structures of either hemisphere at 1, 5, 10 or 20 days post-lesion. Similarly, [^{3}H]-QNB binding was unaltered in all non-visual structures examined at any time point post-enucleation (Appendix IV (f)).

3.2.5 Glucose Utilisation after Unilateral Orbital Enucleation

At 1 day post-enucleation there were immediate and maximal deficits in glucose use in all visual structures in the visually-deprived hemisphere. The most pronounced reductions in glucose use were found in the superficial layer of the superior colliculus (-45%) (Figure 29) and in the dorsal lateral geniculate nucleus (-36%) (Figure 30). Glucose use was reduced to a lesser extent in visually-deprived visual cortex (-25%) (Figure 31), pretectal nuclei (-25%) and lateral posterior nucleus (-21%). The degree of metabolic depression within visual structures was thus directly related to the level of retinal input: retinal projection areas (superior colliculus, dorsal lateral geniculate nucleus) exhibited more prominent deficits in glucose use than, for example, visual cortex, which receives no direct retinal input.

The degree of metabolic depression in the visually-deprived superior colliculus varied as a function of post-enucleation survival time (Figure 29). Thus, by 20 days post-enucleation, glucose use in the visually-deprived superior colliculus was significantly greater (23%;

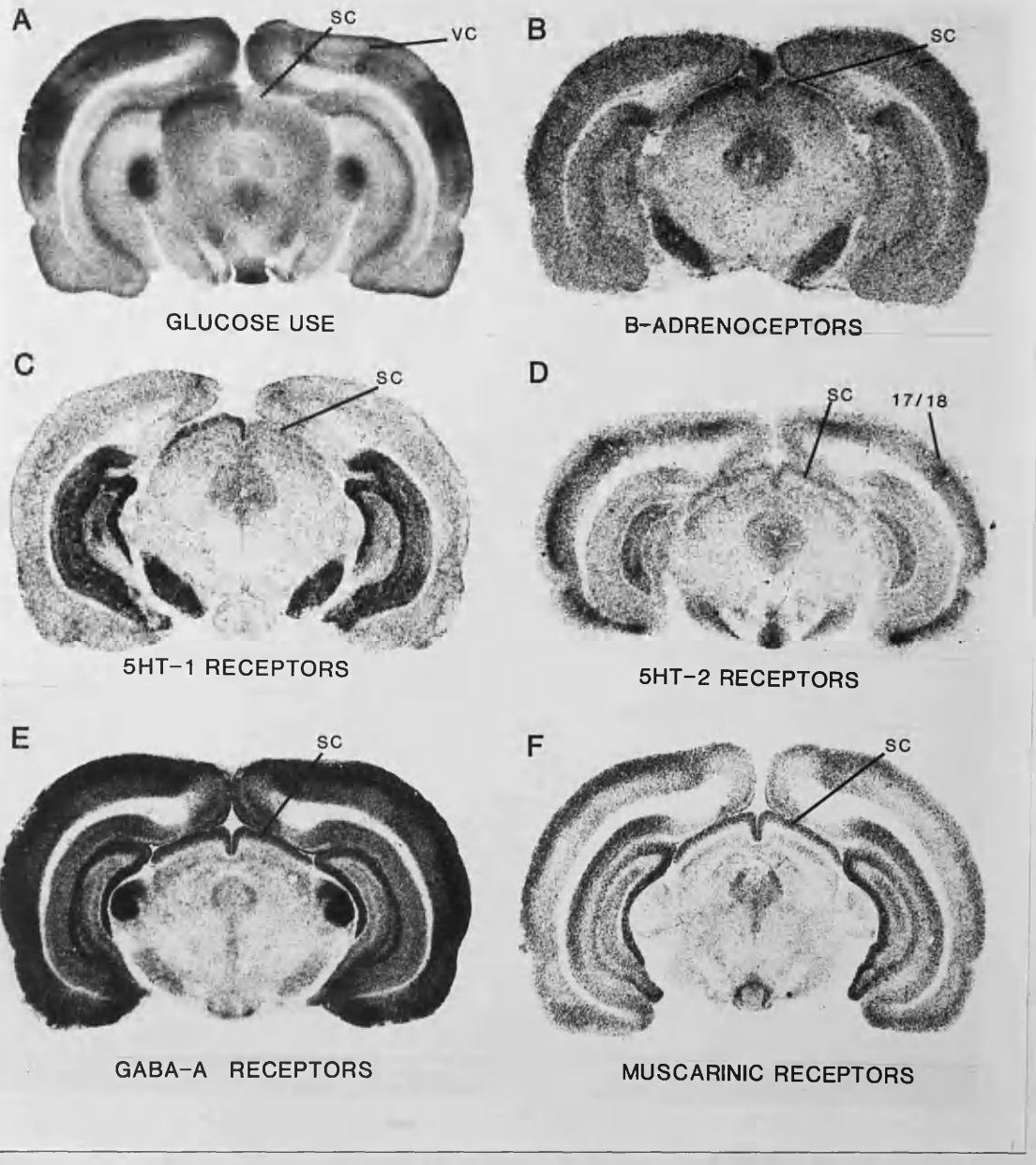


FIGURE 32 REPRESENTATIVE AUTORADIOGRAMS OF GLUCOSE USE AND LIGAND BINDING AT 10 DAYS POST-ENUCLEATION

In the above autoradiograms the right hand hemisphere is visually-deprived. In (A) glucose use is reduced in visually-deprived superior colliculus (SC) and visual cortex (VC). β-Adrenoceptor (B) and $5HT_1$ receptor (C) binding are selectively reduced in SC but unaltered in VC. $5HT_2$ receptor (C), $GABA_A$ receptor (E) and cholinergic muscarinic receptor (F) binding are unaltered in SC or VC. In (D) note the sharp reduction in the density of $5HT_2$ receptor binding in layer IV of primary visual cortex.

Section co-ordinates are approximately 2.96mm interaural, -6.04mm bregma.

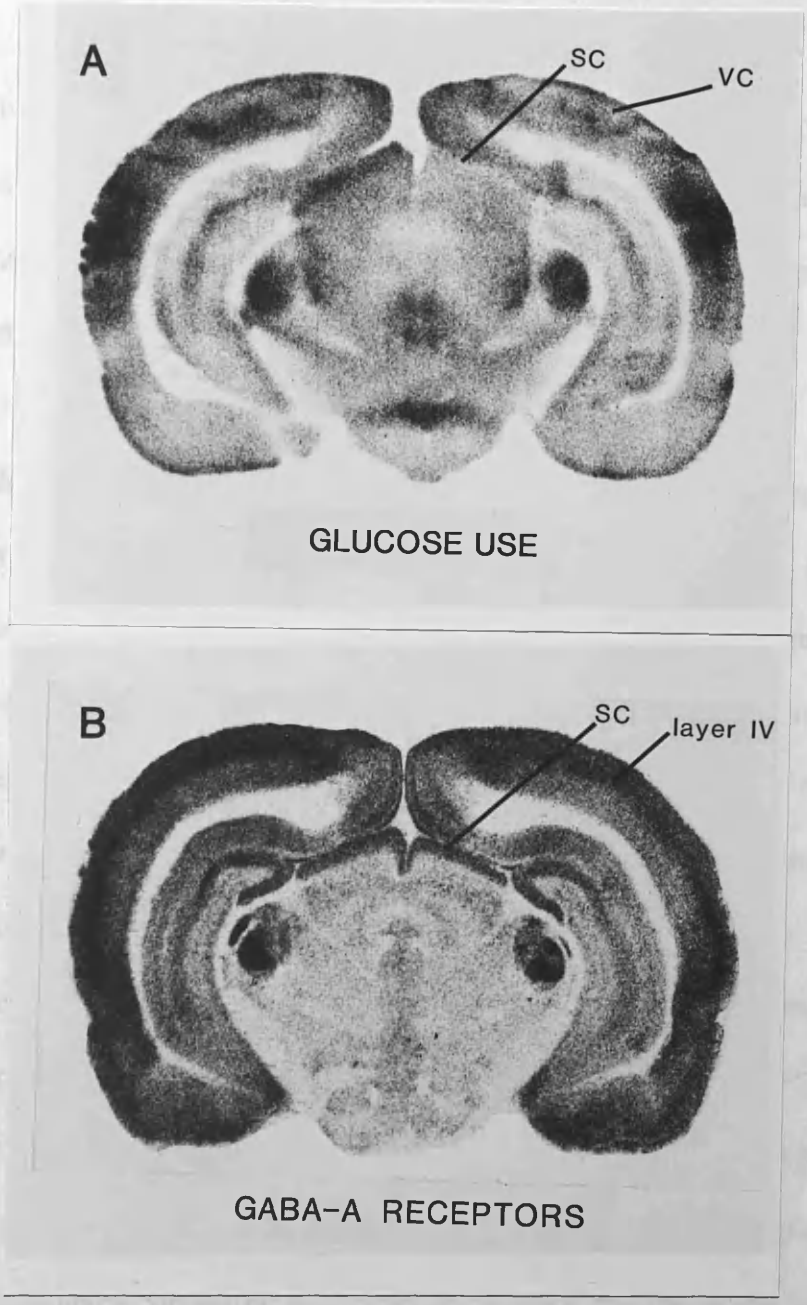


FIGURE 33 REPRESENTATIVE AUTORADIOGRAMS OF GLUCOSE USE AND $GABA_A$ RECEPTOR BINDING AT 20 DAYS POST-ENUCLEATION

In the above autoradiograms the right hand hemisphere is visually-deprived. In (A), note the reduction in glucose use in visually-deprived superior colliculus (SC) and visual cortex (VC). In (B), $GABA_A$ receptor binding is reduced in layers IV-VI of VC in comparison to the contralateral hemisphere but unchanged in SC.

P<0.01 ANOVA) than that at 1 day post-enucleation. There was no statistically significant recovery in metabolic activity in any other visual structures in the visually-deprived hemisphere.

Glucose use was not significantly altered in visual structures of the visually-intact hemisphere post-enucleation (ANOVA). Glucose use was unaltered in any of the non-visual structures examined at any survival time post-enucleation (Appendix IV(a)).

3.2.6 Glucose Use and Ligand Binding After Unilateral Orbital Enucleation

Examination of Figures 29, 30 and 31 clearly reveals that the time courses of functional and receptor binding responses post-enucleation are different. However, at 10 and 20 days post-enucleation, the degree of both [^{3}H]-5HT and [^{3}H]-DHA binding deficits in visual structures in the visually-deprived hemisphere correlated significantly with the level of reduction in function-related glucose use in these structures (Figures 34 and 35). For both ligands this relationship was most significant at 10 days post-enucleation (R=0.700 and r=0.757 for [^{3}H]-5HT and [^{3}H]-DHA respectively). At 20 days post-enucleation, reductions in [^{3}H]-muscimol binding in the visually-deprived hemisphere correlated significantly with the degree of asymmetry in glucose use in visual structures at this time point (r=0.543, P<0.01).

LEGENDS TO FIGURES

FIGURE 34.

(A) Correlation of percentage change in [^{3}H]-5HT binding against percentage change in glucose use in the visually-deprived hemisphere (L) as a function of the visually-intact hemisphere (R) in 7 primary visual structures at 10 days post-enucleation (3 rats).

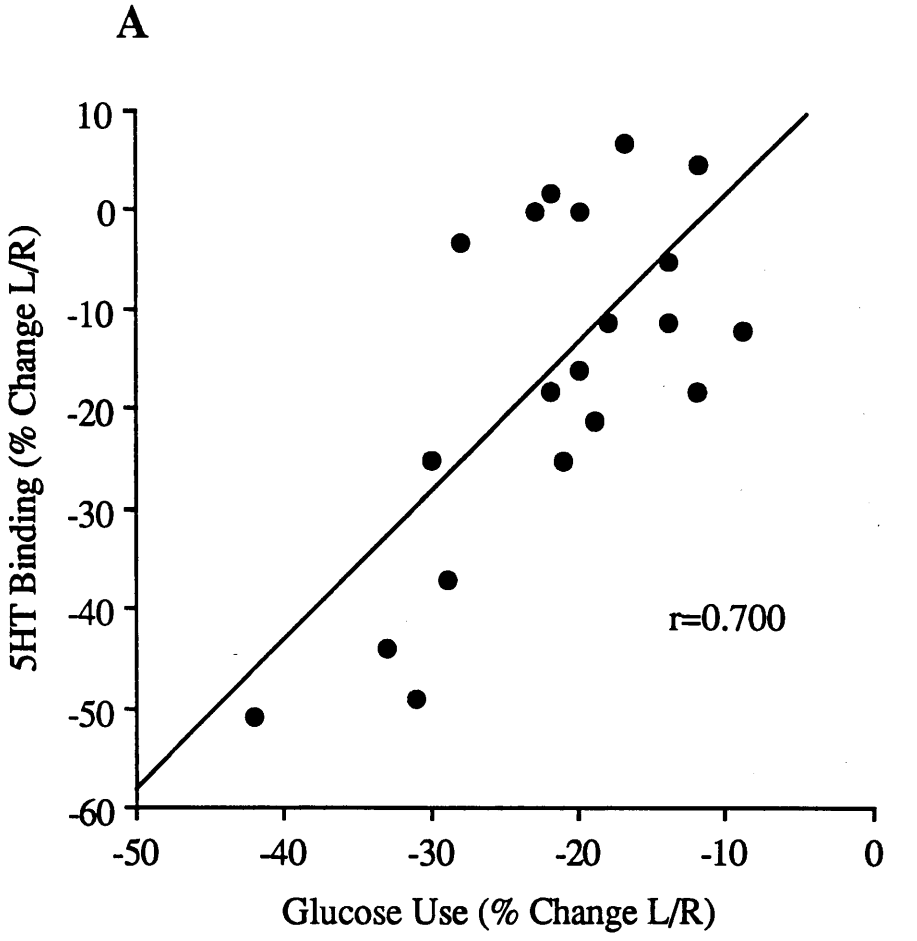
(B) Plot of correlation coefficient for the relationship $y = mx+c$, where y = percentage change in [^{3}H]-5HT binding in the visually-deprived hemisphere as a function of the visually-intact hemisphere and x = percentage change in glucose use in the visually-deprived hemisphere as a function of the visually-intact hemisphere. *$p<0.01$.

FIGURE 35.

(A) Correlation of percentage change in [^{3}H]-DHA binding against percentage change in glucose use in the visually-deprived hemisphere (L) as a function of the visually-intact hemisphere (R) in 7 primary visual structures at 10 days post-enucleation (4 rats).

(B) Plot of correlation coefficient for the relationship $y = mx+c$, where y = percentage change in [^{3}H]-DHA binding in the visually-deprived hemisphere as a function of the visually-intact hemisphere and x = percentage change in glucose use in the visually-deprived hemisphere as a function of the visually-intact hemisphere. *$p<0.01$.

FIGURE 34 RELATIONSHIP BETWEEN 5HT BINDING AND GLUCOSE USE POST-ENUCLEATION



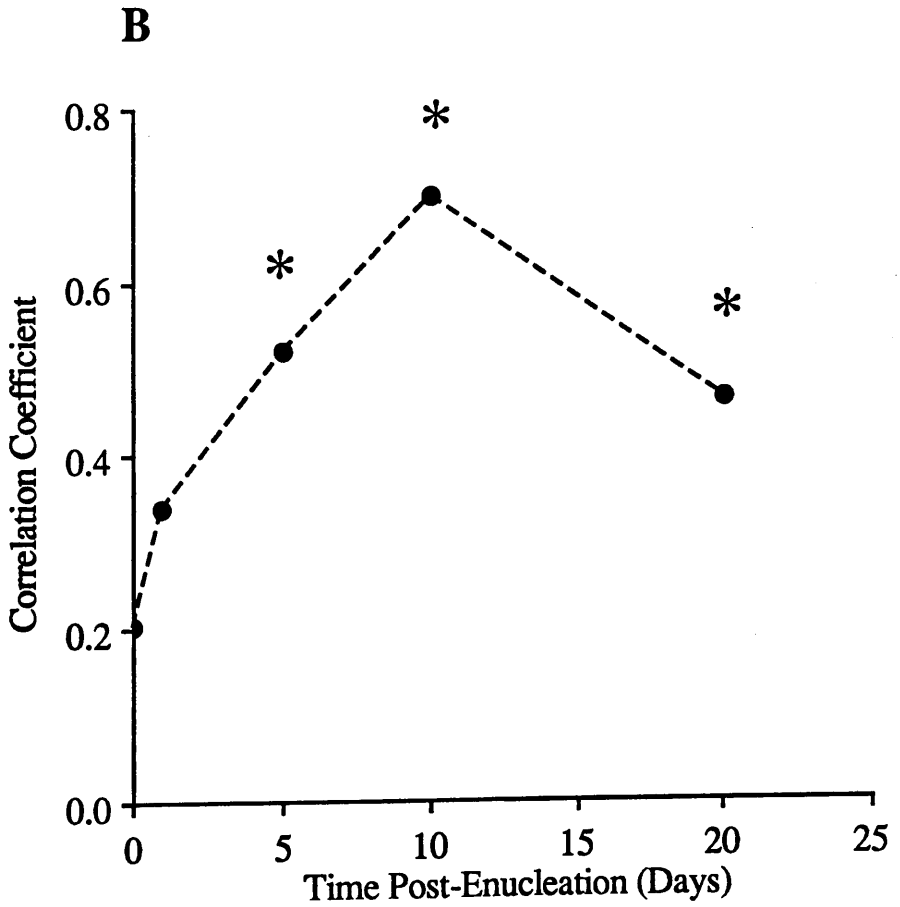
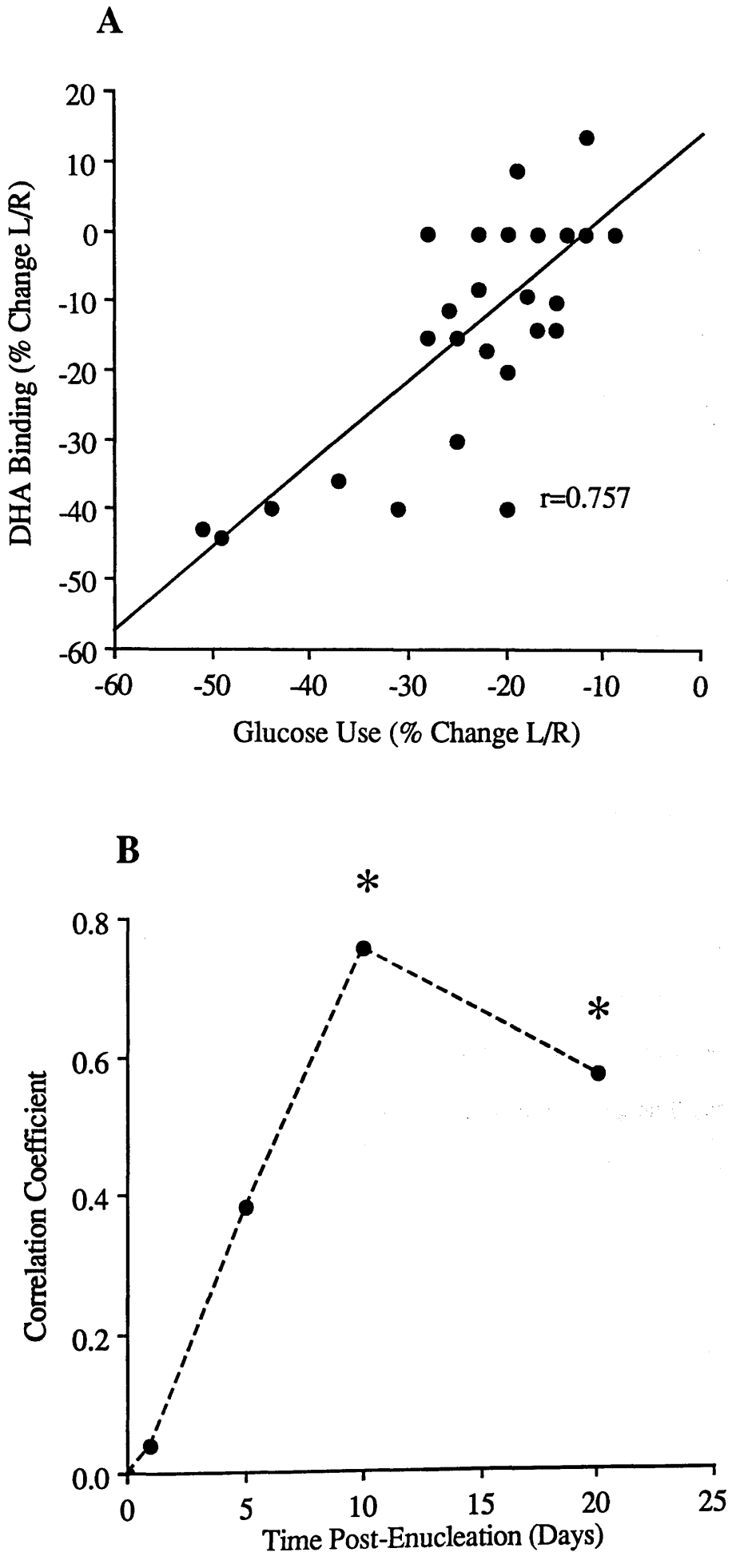


FIGURE 35 RELATIONSHIP BETWEEN DHA BINDING AND GLUCOSE USE POST-ENUCLEATION



3.3 COMMENTARY: DISCUSSION OF ALTERATIONS IN GLUCOSE UTILISATION AND LIGAND BINDING IN THE RAT VISUAL SYSTEM AFTER UNILATERAL ORBITAL ENUCLEATION.

In addition to the major role played by the glutamatergic system, the serotonergic, noradrenergic, GABAergic and cholinergic neurotransmitter systems are all implicated in visual processing. Serotonergic and noradrenergic input to the dorsal lateral geniculate nucleus and superior colliculus originate in the dorsal raphé nucleus and locus coeruleus respectively (Steinbusch, 1984; Swanson and Hartman, 1975). The noradrenergic and serotonergic projections to the dorsal lateral geniculate nucleus modulate relay cell excitability in a reciprocal fashion, serotonin being a potent depressant and noradrenaline facilitating evoked activity (Rogawski and Aghajanian, 1980). In the superior colliculus, both monoamines possess an inhibitory action (Sato and Kayama, 1983; Lai et al. 1978). Visual cortex also receives an extensive monoaminergic innervation and morphological examination of synaptic contacts indicates that both noradrenaline and 5-hydroxytryptamine (5HT) form asymmetric contacts with dendritic shafts or spines in this region (Parnavelas et al. 1985).

Intrinsic inhibitory GABAergic neurons are present in the superior colliculus, dorsal lateral geniculate nucleus and within visual cortex (Mize et al. 1982; Fitzpatrick et al. 1984: Houser et al. 1984). In addition, the local projection from the reticular thalamic nucleus to

the dorsal lateral geniculate nucleus utilises GABA and acts to inhibit relay cells in this region (Kayama, 1985).

The superior colliculus, dorsal lateral geniculate nucleus and visual cortex all exhibit cholineacetyltransferase and acetylcholinesterase activity (Fibiger, 1982). These cholinergic projections are likely to originate in the basal forebrain or nucleus cuneformis (Butcher and Woolf, 1984). Cholinergic fibres modulate activity in the geniculo-cortical projection (Singer, 1977) and may also facilitate excitatory transmission within visual cortex (Bear and Singer, 1986).

The present study has relied on established ligand binding protocols to examine specific serotonergic, noradrenergic, GABAergic and cholinergic receptors in anatomically discrete components of the visual system after unilateral orbital enucleation. The combination of <u>in vivo</u> [^{14}C]-2-deoxyglucose autoradiography and <u>in vitro</u> ligand binding autoradiography has allowed simultaneous measurement of local cerebral function and neurotransmitter receptors within the same brain.

<u>Ligand Binding</u>. The principle finding of the present study was the heterogeneous response of neurotransmitter receptors to unilateral orbital enucleation. $5HT_1$ and β-adrenergic receptor binding were selectively reduced in the visually-deprived superior colliculus and dorsal lateral geniculate nucleus from 5 days post-lesion, but unaltered

in visual cortex up to 20 days post-enucleation. By contrast, GABA receptor binding was specifically reduced only at 20 days post-enucleation in the visually-deprived dorsal lateral geniculate nucleus and visual cortex, whilst $5HT_2$ receptors and cholinergic muscarinic receptors were unaltered by unilateral enucleation. Thus, following lesioning of this polysynaptic system, specific neurotransmitter receptors have occurred at a primary and/or secondary synapse and with differential temporal progression.

Interpretation of these receptor responses is dependent on their cellular localisation. A loss of presynaptic receptors or degenerating retinal projection fibres would be the most obvious explanation for reduced receptor binding in regions receiving primary projections from the retina, e.g. superficial layer of the superior colliculus and dorsal lateral geniculate nucleus. The existence of a population of $5HT_1$ receptors on other non-serotonergic fibres (Cross & Deacon, 1985; Quirion and Richard 1987) indicates the possibility of a presynaptic population of $5HT_1$ receptors on glutamatergic retinal afferents. Indeed, Segu and colleagues (1986) have proposed that 5HT receptors exert a pre-synaptic control on visual input to the colliculus. However, the anatomical selectivity of receptor binding alterations within retinorecipient areas (i.e. lack of binding asymmetries in pretectal nuclei) and the differential temporal progression of the responses, $5HT_1$ and β-adrenergic at 5 days and $GABA_A$ at 20 days, argues against a wholly presynaptic localisation for all receptor changes.

Central serotonin receptors were first classified into 2 receptors subtypes; $5HT_1$, with high affinity for [^{3}H]-5HT and $5HT_2$ with high affinity for [^{3}H]-spiperone (Peroutka and Snyder, 1979). More recent pharmacological studies indicate heterogeneity within the $5HT_1$ sites and have provided evidence for the existence of 4 $5HT_1$ receptor subtypes; $5HT_{1,\ A,\ B,\ C}$ and $_D$ (Pazos et al, 1984; Pedigo et al. 1981; Waeber et al. 1988).

In the present study there was no differentiation of $5HT_1$ receptor subtypes; $5HT_1$ receptors were labelled with high affinity [^{3}H]-5HT binding and $5HT_2$ receptors with [^{3}H]-ketanserin, a selective $5HT_2$ antagonist (Leysen et al. 1981). Although [^{3}H]-5HT labels all four $5HT_1$ receptor subtypes with high affinity, the $5HT_{1A}$ and $5HT_{1B}$ subtypes predominate in the rat superior colliculus and dorsal lateral geniculate nucleus (Pazos et al. 1988). $5HT_{1A}$ receptors appear to be located on postsynaptic targets of serotonergic neurons (Verge et al. 1986), whilst $5HT_{1B}$ receptors act as presynaptic autoreceptors on serotonergic terminals (Engel et al. 1986). Decreased [^{3}H]-5HT binding in superior colliculus and dorsal lateral geniculate nucleus may thus reflect a presynaptic and/or postsynaptic receptor deficit. However it is conceivable that an increase in one of these receptor populations may be masked by a proportionally larger decrease in the other.

As [^{3}H]-DHA has similar affinities for both β_1 and β_2 receptors (Palacios and Wamsley, 1984), the specific subtype components of total β-adrenoceptor binding were not differentiated in the present study. Thus, reduced [^{3}H]-DHA binding in the superior colliculus and dorsal lateral geniculate nucleus in the visually-deprived hemisphere may have reflected an alteration in β_1 and/or β_2 receptors. In this respect, however, it is of interest that the great majority (90%) of β-adrenoceptors in the rat dorsal lateral geniculate are of the β_1 subtype, whilst the β_2-adrenoceptor predominates in the superior colliculus (Rainbow et al. 1984). It would thus seem likely that unilateral enucleation induced reductions in both β_1 and β_2 adrenoceptor binding.

[^{3}H]-5HT and [^{3}H]-DHA binding deficits in the visually-deprived superior colliculus and dorsal lateral geniculate progressed *pari passu* post-enucleation. Both structures exhibited $5HT_1$ and β-adrenergic receptor deficits at 5 days post-enucleation and these responses were maximal at 10 days post-lesion. As β-adrenoceptor ligands also bind $5HT_{1B}$ receptors with relatively high affinity (Pazos et al. 1985), it is possible that reductions in [^{3}H]-DHA and [^{3}H]-5HT binding both reflect a loss of $5HT_{1B}$ receptors in these areas. The maximal deficit in [^{3}H]-5HT and [^{3}H]-DHA binding in the colliculus and geniculate at 10 days post-enucleation, coincides with the loss of adenosine A_1 receptors in these structures (2.3), suggesting that the putative $5HT_{1B}$ receptor loss could be presynaptic, on degenerating retinal fibres. However, the

relatively more pronounced deficits in [^{3}H]-DHA binding in the superior colliculus post-lesion and the lack of symmetrical inter-hemispheric changes in [^{3}H]-DHA and [^{3}H]-5HT binding in other visual structures (e.g. visual cortex), indicates that these two ligands do not bind a single site and that β-adrenoceptor binding is reduced post-lesion.

An alternative explanation for the simultaneous changes in [^{3}H]-DHA and [^{3}H]-5HT binding post-lesion may lie in the proposed functional link between serotonergic and noradrenergic systems in the brain (Ragowski and Aghajanian, 1980; Morrison et al. 1982; Bear and Singer, 1986). More specifically, serotonin axons have been proposed to regulate β-adrenergic receptor number and function on postsynaptic target cells (Stockmeier et al. 1985). The mechanism of this interaction is unknown but may be related to an influence of serotonin on the postsynaptic turnover of β-adrenoceptors (Stockmeier et al. 1985). As both the superior colliculus and dorsal lateral geniculate receive inhibitory serotonergic input from the dorsal raphé nucleus (Steinbusch, 1984), altered neuromodulatory serotonergic input post-enucleation may be responsible for simultaneous changes in both $5HT_1$ receptors and β-adrenoceptors in these regions. However, serotonin levels in both structures have been found to be unaltered post-enucleation (Lai et al. 1978).

The mechanism and location of $5HT_1$ and β-adrenergic changes post-enucleation is thus unclear but it seems likely that a proportion of, at least, $5HT_1$ receptors sites are lost on degenerating retinal fibres. Such a location for $5HT_1$ receptors would be consistent with the proposed presynaptic inhibitory role for these sites in depressing the spontaneous or synaptically evoked activity of dorsal lateral geniculate cells (Rogawski and Aghajanian, 1980).

In contrast to [^{3}H]-5HT and [^{3}H]-DHA binding, [^{3}H]-muscimol binding in the visually-deprived hemisphere was only significantly altered at 20 days post-enucleation. At this time point, [^{3}H]-muscimol was reduced in both the dorsal lateral geniculate and visual cortex, but unaltered in superior colliculus. Thus, both the anatomical specificity and temporal progression of [^{3}H]-muscimol binding alterations post-enucleation varied from those of [^{3}H]-5HT and [^{3}H]-DHA.

[^{3}H]-Muscimol labels low affinity and high affinity $GABA_A$ receptors with a K_D of approximately 50nM and 5nM respectively (Beaumont et al. 1978). At 10nM, the ligand concentration in the present study, [^{3}H]-muscimol is likely to label predominantly high affinity $GABA_A$ receptors, although a small proportion of binding may be to low affinity sites. The lack of correlation between high-affinity GABA binding sites and GABA-stimulated flunitrazepam binding (Unnerstall et al. 1981) suggests that only a low affinity subpopulation of $GABA_A$ receptors are linked to benzodiazepine receptors within the GABA-

chloride ionophore complex.

The loss of [^{3}H]-muscimol binding in visually-deprived visual cortex was laminar specific for layers IV-VI. Cortical layer IV is the major termination layer for geniculo-cortical projection fibres, whilst pyramidal cells in layers V/VI are the cells of origin for cortico-fugal projections to superior colliculus and dorsal lateral geniculate (Sefton and Dreher 1985). Unilateral orbital enucleation is thus associated with $GABA_A$ receptor binding deficits in both input and output layers of visually-deprived visual cortex. The sensitivity of intracortical GABAergic mechanisms to visual deprivation has also been confirmed in monkey visual cortex where reduced numbers of immunostained GABAergic neurons are found in the deprived-eye dominance columns of enucleated animals (Hendry and Jones, 1986). Within visual cortex, a number of intrinsic interneurons, including aspinous and sparsely spinous stellate cells and chandelier cells (Houser et al. 1984), utilise GABA as an inhibitory transmitter. $GABA_A$ receptors are likely to exist on the postsynaptic targets of GABAergic innervation (pyramidal cells and other interneurons) where they mediate GABA inhibition (Sillito, 1984). Reduced $GABA_A$ receptor binding in visually-deprived visual cortex may reflect a disinhibition of cortical neurons as a consequence of reduced $GABA_A$ receptor number/affinity. The loss of cortical $GABA_A$ receptors post-enucleation contrasts with the stability of these sites after early monocular deprivation (Mower et al. 1986) and suggests that $GABA_A$ receptor plasticity in visual cortex

is not a continuum of the involvement of these sites in cortical development. In a similar fashion to visual cortex, the loss of $GABA_A$ receptor binding in the visually-deprived dorsal lateral geniculate nucleus may indicate a reduction in the inhibitory influence of both intrinsic GABAergic neurons and/or GABAergic input from the reticular thalamic nucleus. In view of the lack of retinal input, the functional importance of a selective loss of $GABA_A$ receptor binding at the primary and secondary synapses of the retino-cortical axis is unclear. These specific adjustments in inhibitory receptor parameters were, however, accompanied by functional re-organisation post-enucleation.

Glucose Use and Receptor Plasticity. The cellular functions and energy requirements of cerebral tissue are intimately related. As energy requirements are derived from oxidative catabolism of glucose, measurement of local rates of glucose utilisation provide direct insight into the level of functional activity. Although all structural elements within any anatomical region will contribute to its rate of glucose utilisation, alterations in glucose metabolism appear to predominantly reflect activity in neuronal terminals (Mata et al. 1980; Sejnowski et al. 1980).

At 1 day post-enucleation there was an immediate and maximal deficit in glucose utilisation in both primary and secondary visual structures in the visually-deprived hemisphere. The degree of metabolic depression within visual structures was directly related to

the level of retinal input. Thus, primary visual structures receiving direct retinal projects (superior colliculus, dorsal lateral geniculate) exhibited more prominant deficits in glucose use than, for example, visual cortex, which receives no direct retinal input. The degree of metabolic depression in the visually-deprived superior colliculus varied as a function of post-enucleation survival time. Thus, by 20 days post-enucleation, glucose utilisation in the visually-deprived superior colliculus was 23% greater than that at 1 day post-lesion. In contrast to a previous semi-quantitative study (Cooper and Thurlow, 1985) there was no statistically significant recovery in metabolic activity in any other visual structures in the visually-deprived hemisphere. Increased glial cell metabolism due to reactive gliosis or artefactual increments in optical density measurements due to increased cellpacking, would be possible interpretations of this apparent functional diaschisis. However, the reported attenuation of this response after visual cortex ablation (Thurlow and Cooper, 1985) is supportive of a compensatory polysynaptic mechanism. Thus it is apparent that enhanced cortico-tectal activity can act to increase local cerebral function in the deafferentated superior colliculus. A reorganisation of polysynaptic functional activity is suggestive of plasticity in visual system circuitry.

The time courses of functional and receptor responses post-enucleation were clearly different. Local cerebral function was maximally reduced in both primary and secondary visual structures at

1 day post-enucleation, whilst the earliest receptor alteration was not apparent until 5 days post-lesion. Although delayed responses, receptor alterations occurred only in those areas exhibiting functional deficits, at both primary and secondary synapses. Furthermore, the direct correlation of $5HT_1$ and β-adrenergic receptor deficits with the level of metabolic depression in the visually-deprived hemisphere indicates that those visual structures expressing the greatest functional deficits exhibit the most prominent alterations in these receptors. In view of the differential localisation of receptors (pre- or postsynaptic), their physiological function (inhibition or excitation) and their influence on transynaptic events, such as relationship is not predictable. Nevertheless, a correlation of functional deficit and receptor response indicates that these site specific receptor alterations are dependent, to some degree, on the level of local cerebral function. It is, however, curious that, within this system, disturbed visual input and loss of visual input can induce qualitatively different effects on the same receptor population: decreased [^{3}H]-DHA binding in the visually-deprived superior colliculus and dorsal lateral geniculate nucleus after unilateral orbital enucleation (this study) contrasts with the reported increase in [^{3}H]-DHA binding in these structures after monocular deprivation (Schliebs et al. 1982). This suggests that these receptors may be differentially regulated by both the nature and degree of a functional loss within the system.

The degree of functional deficit in the deafferentated superior colliculus was significantly reduced at 10 and 20 days post-enucleation. The precise mechanism for this metabolic recovery is unknown but may be related to reorganisation of synaptic input (sprouting) (Rhoades, 1984) and/or alterations in CNS receptor function. The selective alterations in neurotransmitter receptors in the present study conincide with the onset of metabolic recovery in the deafferentated superior colliculus. At 10 days post-enucleation, $5HT_1$ and β-adrenergic receptor alterations are maximally expressed in both the superior colliculus and dorsal lateral geniculate nucleus and increased metabolic recovery in the deafferentated superior colliculus at 20 days post-enucleation is accompanied by $GABA_A$ receptor loss in both the dorsal lateral geniculate and visual cortex. As the superior colliculus receives afferents from both the dorsal lateral geniculate and visual cortex, it is possible that the polysynaptic adjustment in selective neurotransmitter receptors may contribute to the functional reorganisation post-enucleation. Selective changes in the adenylate cyclase system in the visually-deprived superior colliculus and dorsal lateral geniculate post-enucleation (Horsburgh et al. 1988) indicates altered receptor transduction mechanisms in these regions post-enucleation.

In conclusion, unilateral enucleation induced selective alterations in neurotransmitter receptors in visual structures exhibiting functional deficits. The temporal and regional heterogeneity of the $5HT_1$, β-

adrenergic and $GABA_A$ receptor changes and the stability of $5HT_2$ and cholinergic muscarinic receptors indicates that the integrity of some, but not all, receptors is controlled by activity-dependent mechanisms and is supportive of a selective adjustment of visual system circuitry post-enucleation. These plastic receptor responses may contribute to the reorganisation of polysynaptic function post-enucleation.

CHAPTER IV

DISCUSSION

Microdissection techniques in combination with specific ligand binding procedures (see Bennett and Yamamura, 1985) have allowed pharmacological and kinetic characterisation of individual neurotransmitter receptors and provided some information on organ and region distribution of these sites. However, the lack of anatomical resolution and sensitivity of tissue homogenate methods has limited their usefulness in studies of the brain, an organ of complex cellular composition and organised anatomical interconnectivity. Only with the development of in vitro ligand binding autoradiography (Kuhar, 1981; Unnerstall et al. 1982; Rainbow et al. 1982) has the examination of neurotransmitter receptors in CNS tissue retaining its anatomical and cellular composition been possible. In view of the fundamental structure-function relationships existing in CNS tissue, receptor autoradiography provides an invaluable technique for brain receptor studies.

As receptor autoradiography allows visualisation of receptors, the most obvious, and common, application of the technique has been in establishing receptor maps, for example for glutamate receptors (Greenamyre et al. 1984a; Monaghan and Cotman, 1985). This has allowed correlation of physiological/pharmacological effects and specific receptor distributions. The sensitivity of the technique had also been instrumental in the derivation of receptor subtypes, some of which may be concentrated in selective, anatomical regions, as is the case for 5HT (Cortés et al. 1984) and NMDA (Monaghan et al. 1988) receptors. However, perhaps the most insightful aspect of in vitro receptor autoradiography is the ability to investigate receptor dynamics within individual components of CNS systems. Studies of receptor regulation after brain lesions have provided information

on both the cellular localisation of receptors and their involvement in specific brain pathways (Chang et al. 1980; Quirion et al. 1985; Pan et al. 1985; Wenk and Engisch, 1986; Cross and Deakin, 1985; Mantyh and Hunt, 1986). The applicability of receptor autoradiography to human postmortem brain tissues has now made it possible to directly study receptor distribution and densities in patients dying with neuropsychiatric or neurodegenerative diseases, such as A.D., potentially providing information of aetiological and/or therapeutic relevance.

Previous hypotheses implicating the glutamatergic system in the aetiology of A.D. have emphasised the excitotoxic properties of glutamate, proposing that neuropathological changes and neuronal death result as a consequence of glutamatergic hyperactivity (Maragos et al. 1987; Greenamyre et al. 1988). Consistent with this pathogenetic mechanism, the distribution of senile plaques and neurofibrillary tangles within the cerebral cortex have been proposed to correlate with the terminal fields of glutamatergic association pathways (Pearson et al. 1985; Rogers and Morrison, 1985) and glutamate receptor-mediated cortical neurotoxicity induces subcortical pathological changes in experimental animals reminiscent of those in A.D. (Sofroniew and Pearson, 1985). However, an inevitable consequence of such an excitotoxic mechanism should be the loss of glutamate receptors as the cortical neurons on which they are located progressively degenerate. This was not a general feature of the present investigations in A.D. cerebral cortex. Indeed, in both frontal and temporal cortex from A.D. patients, there was an overall stability of glutamate receptor binding, with only NMDA receptors exhibiting a slight deficit in the most outer layers of frontal cortex.

Furthermore, it is possible that, in the presence of reduced presynaptic binding ([^{3}H]-D-aspartate) in frontal cortex, the increase in kainate receptor numbers in this region may be the result of a denervation supersensitivity reaction, indicative of glutamatergic hypofunction, rather than hyperfunction. In this respect, it is of interest that removal of glutamatergic retinal input in the rat visual system was shown to result in a selective increase in kainate receptor numbers in the visually-deprived superior colliculus post-lesion. Although the visual system is by no means a model of intracortical excitatory transmission, this response does exemplify the plastic capabilities of the kainate receptor under conditions of presynaptic glutamatergic deficit. Thus, it is conceivable that loss of glutamatergic input, whether occurring slowly during a disease process or rapidly through lesioning, could produce a similar postsynaptic receptor response. It is noteworthy that quantitative autoradiography was crucial in the detection of increased kainate receptor binding restricted to deep layers of A.D. frontal cortex. A recent study using homogenate preparations of frontal cortex from A.D. brains failed to demonstrate any significant alteration in kainate receptor binding (Cowburn et al. 1989).

At face value, the stability of glutamate receptor binding in A.D. cerebral cortex is not supportive of preceding cortical glutamatergic hyperactivity. However, results indicating the existence of "thresholding" phenomena for the maintenance of glutamate receptor densities in the A.D. brain (Geddes et al. 1986) and the involvement of glutamate receptors in synaptic plasticity (Geddes et al. 1985), suggest that excitotoxic interpretations of all glutamate receptor alterations in

A.D. postmortem tissue may be myopic. Hence, at end-stage of the disease process, glutamate receptor alterations may be a consequence of a neurodegenerative and/or neuroregenerative process. The ability of receptors to respond to the neurodegenerative process in A.D. does not appear to be specific to the glutamatergic system. Probst and colleagues (1988) used quantitative autoradiographic techniques to simultaneously measure both cholinergic muscarinic receptors and pyramidal cells in A.D. hippocampus. The results of these studies indicated that the ratio of muscarinic receptors per neuron in A.D. brains tended to be higher than in both young and old controls, with decreases in receptor numbers only apparent in those cases showing severe neuronal fall-out. This response was also evident for benzodiazepine, $GABA_A$, $5HT_2$ and somatostatin receptors. An up-regulation of cortical glutamate receptors on surviving pyramidal cells may, therefore, explain the relative stability of glutamate receptor binding in A.D. temporal cortex in the present studies. If such a compensatory receptor mechanism is ubiquitous in neuropathologically-affected areas of the Alzheimer brain, however, future receptor studies must also incorporate neuronal quantification, as damaged circuitry may not always be detectable by simple comparison of control and A.D. receptor densities.

Although it is possible that receptors responses may be "masked" by increased receptor/neuron ratios in A.D. temporal cortex, the stability of [^{3}H]-D-aspartate binding in this region suggests that presynaptic glutamatergic terminals are unaltered. A loss of presynaptic glutamatergic sites in frontal but not temporal cortex in A.D. brains in not entirely concordant with the proposal that

neuropathological changes in cerebral cortex are propagated along cortico-cortical fibres (Pearson et al. 1985). Association fibres arise from glutamatergic pyramidal cells in cortical layers II/III and V and terminate in the same laminae of connected areas (Jones, 1981). The sequence of cortico-cortical links begins in sensory areas and passes through the polymodal association areas of parieto-temporal and frontal cortex to the cingulate cortex and hippocampus (Jones and Powell, 1970). Successive steps in the parietotemporal lobe are reciprocally connected with the corresponding step in the frontal lobe. The lack of alterations in both pre-and postsynaptic glutamatergic sites in A.D. temporal cortex suggests that intracortical transmission is unaltered in this region. This may be interpreted as indicating that only selective association fibres are affected by the neurodegenerative process, allowing "sparing" of some association areas of cortex. However, assuming that senile plaques are distributed at the terminal regions of degenerating cortico-cortical fibres (Pearson et al. 1985), this explanation is at odds with the comparable numbers of senile plaques in A.D. frontal and temporal cortex in the present investigations. It can be concluded that glutamatergic dysfunction need not be a primary event in the development of neuropathological changes in A.D. cerebral cortex but may contribute to the pattern of change as the disease progresses. The direct correlation of kainate receptor up-regulation and senile plaque numbers in A.D. frontal cortex suggests that kainate receptor plasticity and structural changes are linked in this region.

Frontal cortex has widespread connections with other cortical areas (Jones and Powell, 1970) and is fundamental to the integration of higher mental functions (Milner and Petrides, 1984). Although temporal cortex and hippocampus have been the anatomical foci of most neurochemical studies in A.D. because of their role in learning and memory, patients with A.D. may also show patterns of cognitive decline which are similar to those found in patients with focal frontal lesions (Hart et al. 1988). Alterations in pre-and postsynaptic glutamatergic sites in A.D. frontal cortex may represent the neurochemical basis for the disruption of cortical processing in this region and contribute to the functional changes found here in PET studies of Alzheimer patients (Foster et al. 1983; Horowitz et al. 1987).

Unlike frontal cortex, cerebellar cortex is not a brain area of obvious pathophysiological importance in A.D. Cerebellar "signs" are not generally a prominent feature of A.D. symptomatology (Huppert and Tym, 1986) and neuropathological changes, such as senile plaques, are not found in great numbers within the cerebellum. In view of this, the functional significance of reduced quisqualate receptor numbers in this region of the A.D. brain is not immediately obvious. However, retrospective examination of clinical notes revealed that four of the six A.D. patients used in these studies exhibited physical signs indicative of possible cerebellar impairment. Three patients were noted to have "wide" or unsteady gait and one showed impaired heel-shin co-ordination. If these cerebellar signs are a consequence of reduced excitatory input to cerebellar Purkinje cells, it indicates that functionally relevant alterations in glutamatergic transmission may

precede any gross manifestation of neuropathological changes in A.D. cerebellum. Alterations in neurotransmitter receptors preceding a structural change have also been reported in Huntington's disease (Walker et al. 1984). Thus it is apparent that changes in neurotransmission parameters in human neurodegenerative diseases may not always occur simply as a consequence of cellular degeneration. It is, however, unlikely that loss of quisqualate receptors from A.D. cerebellum is a general feature of the disease, but rather a result of a late-stage progression of the pathophysiological process.

Although the use of human postmortem brain tissue provides a means of directly assessing the integrity of neurotransmitter parameters in A.D., it presents potential problems in relation to time- and temperature-dependent changes in membrane receptor sites. It is apparent from studies in rat brain that the susceptibility of neurotransmitter receptors to postmortem changes is not homogeneous. Following two hours at 25°C, Kuhar et al. (1973) found a significant decrease in brain dihydromorphine binding whilst time-dependent increases in both [^{3}H]-diazepam and [^{3}H]-flunitrazepam have been observed postmortem (Syapin et al. 1987; Whitehouse et al. 1984). In view of the significantly shorter postmortem delay time in the A.D. patient group in the present study, 8 ± 2 hours, in comparison to the control group, 15 ± 2 hours, it is possible that time-dependent changes in glutamate receptors prior to freezing may have influenced results. Thus it could be proposed that increased kainate receptor numbers in A.D. frontal cortex are merely a consequence of greater preservation of the sites relative to controls and that quisqualate receptor

* Linear correlation co-efficients for control and A.D. groups respectively were; r=0.118 and r=0.684 for kainate receptor binding in deep layers of frontal cortex and r=0.667 and r=0.284 for quisqualate receptor binding in the molecular layer of the cerebellum.

numbers in the cerebellum increase with postmortem delay. Several factors, however, argue against such explanations. Firstly, both the kainate and quisqualate receptor responses were anatomically specific, which would not be consistent with a general postmortem phenomenon. Moreover, in each case, the receptor change was confined to a particular cortical or cerebellar layer, indicating that any postmortem influence on receptor integrity would have to be laminar specific. Secondly, there was no significant relationship between either kainate or quisqualate receptor binding and postmortem delay in control or A.D. patient groups*. Finally, results from rat models, in which the postmortem cooling curve approximated to that observed in human tissue, indicate that two glutamate receptor subtypes, NMDA and kainate, are stable up to 72 hours postmortem (Geddes et al. 1986; Geddes et al. 1985). The stability of kainate receptors has also been demonstrated in the human hippocampus up to 22 hours postmortem (Tremblay et al. 1985). All of the above evidence indicates that postmortem delay is likely to have minimal influence on the receptor changes observed in these postmortem studies.

It is evident from investigations in the rat visual pathway that receptor plasticity within a polysynaptic system may occur at a primary or secondary synapse after an initial lesion. Moreover, in addition to changes in glutamate receptors (kainate, quisqualate), alterations in receptors for functionally related but non-lesioned neurotransmitter systems may also occur ($5HT_1$, β-adrenergic, $GABA_A$) and receptor responses may be of pre- and/or postsynaptic localisation. Extrapolation of these results to receptor studies in human postmortem tissue suggests that the

stimulus for any receptor plasticity in A.D. brain may be multifactorial and of local or distant anatomical origin. Such influences may obviously complicate mechanistic interpretation of receptor changes in A.D., especially in cases where a specific postsynaptic receptor site is studied in isolation. The simultaneous assessment of both pre- and postsynaptic glutamatergic sites in the present studies has allowed identification of local and possible transynaptic influences on receptor responses. Kainate receptor up-regulation in A.D. frontal cortex was intimately related to local neuropathology and accompanied by a presynaptic binding deficit, but quisqualate receptor loss in cerebellum occurred in the absence of presynaptic glutamatergic change or significant neuropathological lesions. While it seems likely that the kainate response in frontal cortex is related to cortical glutamatergic changes, quisqualate receptor alterations in cerebellum may result from dysfunction in an anatomically related area, (possibly cerebral cortex via cortico-pontine and ponto-cerebellar pathways), or functionally related system, cerebellar GABAergic pathways. Although some receptor responses in A.D. postmortem tissue may result from alterations in related CNS systems, the present results strongly suggest a primary deficit in cortical glutamatergic transmission.

Alterations in glutamatergic sites in A.D. postmortem tissue indicates that glutamatergic neurotransmission is disrupted at end-stage of the disease process. This "snapshot" of glutamatergic function, however, provides limited information on the mechanism of preceding changes in excitatory transmission in relation to the progression of the disease process. The inherent interpretative difficulties

in extrapolating from postmortem receptor data to the in vivo situation are perhaps best illustrated by the contradictory proposals for both glutamate agonist (Deutsch and Morihisa, 1988) and glutamate antagonist (Woodruff et al. 1988) therapy in A.D. Although resulting in essentially opposite effects in relation to glutamatergic transmission, the basis for both proposals may be intrinsically logical as a glutamatergic deficit at end-stage of the disease could be subsequent to glutamatergic hyperactivity. Thus administration of L-glutamate may act to aid the symptoms of the disease but amplify excitotoxic mechanisms whilst glutamate antagonists may prevent neuronal loss but exacerbate clinical signs.

In an attempt to more clearly define the status of glutamatergic transmission in vivo, several laboratories have measured free levels of amino acids in brain biopsy material and spinal fluid. Smith and colleagues (1985) reported a 22% reduction in the CSF levels of glutamine in a group of Alzheimer patients as compared with controls and although mean glutamate levels were unaltered in the Alzheimer group in comparison to controls, they correlated significantly with measures of cognitive function in a subset of nine Alzheimer patients. In contrast, glutamate levels were found to be reduced and aspartate levels increased by similar magnitudes in biopsy samples of cerebral cortex from Alzheimer patients, obtained approximately 3 years after the emergence of symptoms (Procter et al. 1988). However, in a separate study, glutamate levels in cortical biopsy samples from two young Alzheimer patients were found to be no different from controls (Perry et al. 1987). Although it is apparent that studies of this kind can detect alterations in free levels of glutamate in vivo, the value of such investigations is

limited as changes may reflect abnormalities in metabolic and/or transmitter pools of the amino acid. The use of labelled glutamate ligands with PET scanning may provide a potential future alternative means of examining the status of glutamatergic transmission in Alzheimer patients, allowing determination of receptor changes in vivo.

The present autoradiographic studies in rat brain and human postmortem tissue have revealed anatomically-selective alterations in neurotransmitter receptor sites. While it is clear that receptor plasticity in the rat visual system is confined to those areas exhibiting functional deficits, definitive statements regarding the functional relevance of altered glutamatergic sites in the A.D. brain must await a clearer mechanistic understanding. In this respect, the ever accelerating progress in receptor purification and cloning may allow future analysis of receptors using in situ hybridisation and cDNA, cRNA or oligonucleotide probes. Using such techniques in combination with receptor autoradiography, it will be possible to analyse neurotransmitter receptors with cellular resolution and determine the dynamics of receptor synthesis and metabolism in the brain.

APPENDIX I

RECALIBRATION OF [^{3}H]-MICROSCALES FOR QUANTITATIVE AUTORADIOGRAPHY

Absolute quantification of [^{3}H]-ligand bound/g tissue from receptor autoradiograms is dependent on the veracity of tissue equivalent values employed in the optical density/tissue equivalent calibration curve. Commercially available Amersham autoradiographic [^{3}H]-microscales (Batch 1 and 3) have been recalibrated against Sokoloff's tritium standards for the auto-absorptive properties of intact brain grey matter.

Optical density values for all three sets of standards, after three weeks exposure using Amersham Hyperfilm, were obtained using a Quantimet 970 image analysis system. The subsequent plot of optical density against tissue equivalent for Sokoloff's tritium standards, using a third order polynomial function to describe the relationship (y=loge Sokoloff tissue equivalent and X=OD), allowed the direct conversion of optical densities for Amersham standards 1-5 (Batch 1 and 3) to Sokoloff's tritium equivalents. Values for Amersham standards 6-8 were obtained by extrapolation from a log/log linear plot of Amersham tissue equivalents against Sokoloff tissue equivalents.

APPENDIX I (a)

RECALIBRATION OF AMERSHAM BATCH 1 [^{3}H]-MICROSCALES

AMERSHAM STANDARD	PUTATIVE TISSUE EQUIVALENT (nCi/g)	SOKOLOFF TISSUE EQUIVALENT(nCi/g)	
1	1.09	1.06	
2	4.15	3.33	
3	6.60	4.53	
4	10.41	6.68	
5	15.90	9.32	
			95% CONFIDENCE INTERVAL
6	20.73	11.56	(10.8 - 12.4)
7	24.36	13.20	(12.2 - 14.3)
8	27.90	14.71	(13.7 - 15.7)

APPENDIX I (b)

RECALIBRATION OF AMERSHAM BATCH 3 [^{3}H]-MICROSCALES

AMERSHAM STANDARD	PUTATIVE TISSUE EQUIVALENT (nCi/g)	SOKOLOFF TISSUE EQUIVALENT(nCi/g)	
1	1.3	1.43	
2	2.1	1.84	
3	3.1	2.64	
4	5.3	3.78	
5	8.8	5.55	
			95% CONFIDENCE INTERVAL
6	12.7	7.77	(7.7 - 9.3)
7	21.6	12.66	(11.6 - 15.3)
8	30.8	17.72	(16.7 - 20.6)

APPENDIX II

EXCITATORY AMINO ACID RECEPTOR BINDING IN HUMAN FRONTAL, TEMPORAL AND CEREBELLAR CORTEX

[^{3}H]-D-Aspartate, [^{3}H]-kainate, [^{3}H]-AMPA and NMDA-sensitive [^{3}H]-glutamate binding were measured at single ligand concentrations in middle frontal gyrus, inferior temporal gyrus and cerebellar cortex in control (n=6 in frontal and cerebellar cortex, n=5 in temporal cortex) brains.

In Tables II(a) - II(h) and II(m) -II(p) data are presented as mean ligand bound (pmoles/g tissue) ± S.E.M. *P<0.05, **P<0.01, unpaired t-test.

Simple linear regression analysis of ligand binding with senile plaque numbers and cholineacetyltransferase activity in both frontal and temporal cortex is presented in Tables II(i) - II(l). *P<0.05 for linear correlation coefficient, $r \geq 0.811$.

LIGAND BINDING IN FRONTAL CORTEX

APPENDIX II (a)

[^{3}H]-D-ASPARTATE BINDING IN CONTROL AND A.D. FRONTAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	74 ± 7	34 ± 4 **
III	81 ± 12	40 ± 3 **
IV	60 ± 10	37 ± 6 *
V/VI	76 ± 12	43 ± 5 *
White Matter	20 ± 1	21 ± 2

APPENDIX II (b)

[^{3}H]-KAINATE BINDING IN CONTROL AND A.D. FRONTAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	9 ± 3	10 ± 3
III	7 ± 2	9 ± 4
IV	9 ± 3	17 ± 5 **
V/VI	13 ± 3	22 ± 5 **
White Matter	2 ± 1	2 ± 1

APPENDIX II (c)

[^{3}H]-AMPA BINDING IN CONTROL AND A.D. FRONTAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	39 ± 6	34 ± 5
III	30 ± 3	31 ± 4
IV	22 ± 3	29 ± 3
V/VI	29 ± 3	35 ± 4
White Matter	6 ± 1	7 ± 1

APPENDIX II (d)

NMDA-SENSITIVE [^{3}H]-GLUTAMATE BINDING IN CONTROL AND A.D. FRONTAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	15 ± 2	11 ± 1 *
III	15 ± 2	13 ± 1
IV	6 ± 1	5 ± 1
V/VI	12 ± 1	10 ± 2
White Matter	2 ± 0	2 ± 0

LIGAND BINDING IN TEMPORAL CORTEX

APPENDIX II (e)

[^{3}H]-D-ASPARTATE BINDING IN CONTROL AND A.D. TEMPORAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	121 ± 12	140 ± 20
III	186 ± 24	187 ± 22
IV	104 ± 13	116 ± 15
V/VI	183 ± 21	160 ± 36
White Matter	44 ± 6	48 ± 5

APPENDIX II (f)

[^{3}H]-KAINATE BINDING IN CONTROL AND A.D. TEMPORAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	27 ± 2	27 ± 3
III	25 ± 2	24 ± 2
IV	34 ± 2	34 ± 2
V/VI	37 ± 2	36 ± 3
White Matter	5 ± 1	6 ± 1

APPENDIX II (g)

[^{3}H]-AMPA BINDING IN CONTROL AND A.D. TEMPORAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	46 ± 8	32 ± 5
III	37 ± 8	27 ± 4
IV	33 ± 8	23 ± 4
V/VI	40 ± 7	34 ± 4
White Matter	6 ± 1	6 ± 2

APPENDIX II (h)

NMDA-SENSITIVE [^{3}H]-GLUTAMATE BINDING IN CONTROL AND A.D. TEMPORAL CORTEX

CORTICAL LAYER	CONTROL	A.D.
I/II	26 ± 1	25 ± 4
III	26 ± 1	25 ± 3
IV	20 ± 1	19 ± 3
V/VI	21 ± 1	20 ± 3
White Matter	2 ± 1	2 ± 1

CORRELATIVE ANALYSIS IN FRONTAL CORTEX

APPENDIX II (i)

CORRELATIVE ANALYSIS OF LIGAND BINDING AND SENILE PLAQUE NUMBERS IN ALZHEIMER FRONTAL CORTEX

	LINEAR CORRELATION COEFFICIENT			
	CORTICAL LAYER(S)			
LIGAND	I/II	III	IV	V/VI
[^{3}H]-D-Aspartate	0.001	0.281	0.122	0.197
[^{3}H]-Kainate	0.089	0.148	0.901*	0.914*
[^{3}H]-AMPA	0.001	0.363	0.167	0.291
[^{3}H]-Glutamate	0.104	0.654	0.187	0.582

APPENDIX II (j)

CORRELATIVE ANALYSIS OF LIGAND BINDING AND CHOLINEACETYLTRANSFERASE ACTIVITY IN ALZHEIMER FRONTAL CORTEX

	LINEAR CORRELATION COEFFICIENT			
	CORTICAL LAYER(S)			
LIGAND	I/II	III	IV	V/VI
[^{3}H]-D-Aspartate	0.001	0.662	0.524	0.398
[^{3}H]-Kainate	0.585	0.200	0.458	0.151
[^{3}H]-AMPA	0.746	0.031	0.031	0.325
[^{3}H]-Glutamate	0.181	0.457	0.342	0.328

CORRELATIVE ANALYSIS IN TEMPORAL CORTEX

APPENDIX II (k)

CORRELATIVE ANALYSIS OF LIGAND BINDING AND SENILE PLAQUE NUMBERS IN ALZHEIMER TEMPORAL CORTEX

	LINEAR CORRELATION COEFFICIENT			
	CORTICAL LAYER(S)			
LIGAND	I/II	III	IV	V/VI
[^{3}H]-D-Aspartate	0.044	0.238	0.246	0.031
[^{3}H]-Kainate	0.611	0.409	0.219	0.225
[^{3}H]-AMPA	0.063	0.248	0.414	0.487
[^{3}H]-Glutamate	0.569	0.300	0.230	0.031

APPENDIX II (l)

CORRELATIVE ANALYSIS OF LIGAND BINDING AND CHOLINEACETYLTRANSFERASE ACTIVITY IN ALZHEIMER TEMPORAL CORTEX

	LINEAR CORRELATION COEFFICIENT			
	CORTICAL LAYER(S)			
LIGAND	I/II	III	IV	V/VI
[^{3}H]-D-Aspartate	0.694	0.001	0.031	0.644
[^{3}H]-Kainate	0.293	0.250	0.176	0.447
[^{3}H]-AMPA	0.264	0.700	0.454	0.054
[^{3}H]-Glutamate	0.077	0.044	0.122	0.141

LIGAND BINDING IN CEREBELLAR CORTEX

APPENDIX II (m)

[^{3}H]-D-ASPARTATE BINDING IN CONTROL AND A.D. CEREBELLAR CORTEX

LAYER	CONTROL	A.D.
Molecular Layer	89 ± 3	93 ± 5
Granule cell/Purkinje cell layer	112 ± 4	114 ± 5
White Matter	18 ± 5	25 ± 4

APPENDIX II (n)

[^{3}H]-KAINATE BINDING IN CONTROL AND A.D. CEREBELLAR CORTEX

LAYER	CONTROL	A.D.
Molecular Layer	31 ± 3	32 ± 1
Granule cell/Purkinje cell layer	40 ± 3	39 ± 1
White Matter	10 ± 4	7 ± 1

APPENDIX II (o)

[^{3}H]-AMPA BINDING IN CONTROL AND A.D. CEREBELLAR CORTEX

LAYER	CONTROL	A.D.
Molecular Layer	95 ± 10	28 ± 3 **
Granule cell/Purkinje cell layer	23 ± 2	21 ± 2
White Matter	14 ± 2	12 ± 2

APPENDIX II (p)

NMDA-SENSITIVE [^{3}H]-GLUTAMATE BINDING IN CONTROL AND A.D. CEREBELLAR CORTEX

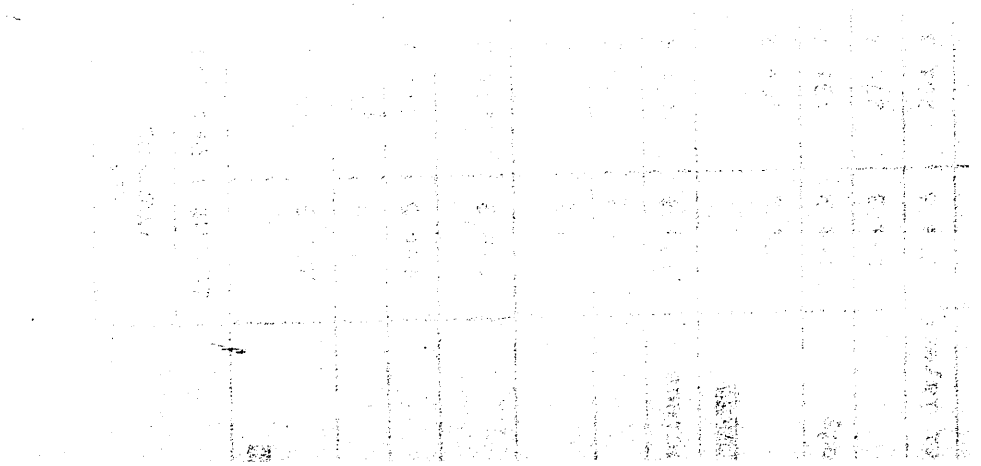
LAYER	CONTROL	A.D.
Molecular Layer	19 ± 2	16 ± 3
Granule cell/Purkinje cell layer	26 ± 2	25 ± 3
White Matter	6 ± 2	5 ± 1

APPENDIX III

EXCITATORY AMINO ACID AND ADENOSINE A_1 RECEPTOR BINDING FOLLOWING UNILATERAL ORBITAL ENUCLEATION.

[^{3}H]-Kainate, [^{3}H]-AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid), NMDA-sensitive [^{3}H]-glutamate and [^{3}H]-MK-801 were measured at four time points up to 20 days post-enucleation. [^{3}H]-CHA (cyclohexyl-adenosine) binding was measured at three time points up to 20 days post-enucleation.

In the following five tables, data are presented as mean ligand bound (pmoles/g tissue) ± S.E.M. *P<0.01. Roman numerals indicate cortical layer examined.



APPENDIX III(a)
[^{3}H]-KAINATE BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=5 CONTROL		n=6 1 DAY		n=6 5 DAY		n=6 10 DAY		n=6 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	28± 3	27± 2	25± 2	24± 2	24± 2	25± 2	27± 3	28± 3	28± 3	28± 3
Visual Cortex IV	25± 3	26± 3	19± 2	19± 1	23± 2	23± 2	24± 2	24± 1	26± 4	26± 3
Visual Cortex V/VI	46± 5	47± 5	41± 4	41± 5	46± 5	45± 5	47± 3	47± 3	44± 5	43± 4
Superior Colliculus (Superficial Layer)	21± 2	21± 2	18± 2	19± 2	18± 1	20± 1	20± 3	23± 3	21± 3	25± 3*
Dorsal Lateral Geniculate Body	14± 3	14± 3	11± 2	10± 1	12± 1	12± 1	13± 1	12± 1	14± 2	13± 1
Pretectal Nuclei	17± 3	17± 3	11± 1	10± 1	11± 2	12± 2	13± 1	12± 1	14± 2	15± 2
Lateral Posterior Nucleus	17± 4	17± 4	13± 2	12± 1	16± 2	16± 1	15±2	16± 1	18± 2	19± 3
NON-VISUAL STRUCTURES										
Inferior Colliculus	17± 2	17± 2	12± 1	12± 1	12± 2	12± 2	16± 2	16± 2	17± 3	18± 3
Medial Geniculate Body	18± 2	19± 2	14± 1	14± 1	15± 2	15± 2	19± 2	19± 2	17± 3	18± 3
Auditory Cortex IV	26± 3	27± 3	20± 1	21± 2	25± 3	26± 2	32± 3	32± 3	34± 5	34± 6
Hippocampus(Molecular layer)	21± 3	20± 3	19± 2	18± 1	18± 2	19± 2	20± 1	19± 1	20± 3	19± 3
Amygdala	44± 6	44± 6	34± 5	33± 5	35± 2	35± 3	37± 1	37± 1	41± 4	40± 3

APPENDIX III(b)
[^{3}H]-AMPA BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=5 CONTROL		n=6 1 DAY		n=6 5 DAY		n=6 10 DAY		n=6 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	108±20	110±21	95±14	95±14	110±17	106±15	96±13	94±14	109±13	108±13
Visual Cortex IV	100±18	101±19	86±13	85±13	108±16	106±14	98±13	97±13	109±12	108±13
Visual Cortex V/VI	117±14	117±15	101±16	102±16	123±17	125±17	96±13	97±13	118±16	116±17
Superior Colliculus (Superficial Layer)	82±13	81±13	71± 8	71± 7	71±12	74±14	78± 8	63± 7*	74± 8	56± 6*
Dorsal Lateral Geniculate Body	61±11	60±11	57± 4	56± 4	66± 8	64± 8	65± 7	65± 6	70± 9	71±10
Pretectal Nuclei	47±12	46±12	44± 4	43± 4	52± 9	54±10	46± 5	43± 6	48± 7	46± 7
Lateral Posterior Nucleus	65±12	65±12	62± 5	62± 5	80±13	79±13	65±5	66± 5	72± 9	69±10
NON-VISUAL STRUCTURES										
Inferior Colliculus	44± 9	44± 9	41± 3	41± 4	43± 3	44± 3	41± 9	41± 8	39± 3	41± 4
Medial Geniculate Body	66±10	67±10	70±11	70±12	79±11	80±12	69± 6	71± 6	73± 7	73± 7
Auditory Cortex IV	83±20	83±20	92±16	91±16	94±14	95±14	94±15	92±14	98±14	99±14
Hippocampus(Molecular layer)	167±15	166±15	173±15	174±15	173±20	171±21	148±17	151±19	140±11	142±11
Amygdala	91±18	91±19	94± 4	94± 4	102±11	102±12	97±14	98±14	109±17	107±17

APPENDIX III(c)
NMDA-SENSITIVE [^{3}H]-GLUTAMATE BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=5 CONTROL		n=6 1 DAY		n=6 5 DAY		n=6 10 DAY		n=6 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	65± 7	65± 6	56± 3	56± 3	55± 8	55± 8	54± 5	54± 5	59± 9	59± 7
Visual Cortex IV	46± 3	46± 3	43± 3	43± 3	39± 5	38± 5	41± 4	41± 3	48± 6	48± 5
Visual Cortex V/VI	42± 4	43± 4	43± 3	43± 3	42± 4	42± 5	40± 2	40± 2	46± 6	46± 6
Superior Colliculus (Superficial Layer)	26± 5	26± 5	25± 1	25± 1	26± 2	27± 3	24± 3	23± 2	29± 3	29± 4
Dorsal Lateral Geniculate Body	35± 5	34± 4	33± 3	32± 3	35± 5	35± 6	36± 3	33± 3	45± 6	44± 6
Pretectal Nuclei	21± 4	20± 4	18± 1	18± 2	17± 3	18± 3	17± 3	18± 3	26± 5	26± 5
Lateral Posterior Nucleus	37± 4	38± 4	34± 1	34± 1	35± 4	34± 4	36± 3	35± 3	42± 7	43± 6
NON-VISUAL STRUCTURES										
Inferior Colliculus	17± 2	17± 2	17± 2	17± 3	17± 4	17± 4	11± 2	11± 2	23± 3	23± 3
Medial Geniculate Body	37± 4	36± 4	40± 3	40± 3	32± 4	32± 3	34± 4	33± 4	47± 4	47± 4
Auditory Cortex IV	44± 6	44± 6	37± 2	37± 2	37± 4	37± 4	45± 3	44± 3	57± 7	57± 8
Hippocampus(Molecular layer)	66±12	66±12	63± 7	64± 7	64±10	66±10	70± 3	71± 3	71±10	73±10
Amygdala	58± 9	57± 9	56± 3	55± 4	52± 6	51± 6	58± 4	59± 4	55± 5	54± 5

APPENDIX III(d)
[^{3}H]-MK-801 BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=5 CONTROL		n=6 1 DAY		n=6 5 DAY		n=6 10 DAY		n=6 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	107± 7	105± 6	130±16	134±16	135±18	133±16	109±16	109±15	87± 6	87± 6
Visual Cortex IV	70±10	71±10	101±16	98± 7	100±14	101±14	99±14	100±14	71± 6	72± 5
Visual Cortex V/VI	68± 6	66± 7	95±20	95±19	93±13	93±13	81± 8	81± 8	64± 5	66± 5
Superior Colliculus (Superficial Layer)	65± 4	65± 3	59± 9	59± 9	50± 5	48± 5	62± 9	59±10	56± 4	56± 4
Dorsal Lateral Geniculate Body	67± 8	68± 8	64± 8	64± 8	63±13	62±13	75±12	75±13	51±10	55± 9
Pretectal Nuclei	40± 8	41± 8	40± 7	41± 7	34± 5	33± 5	52± 6	50± 5	40± 6	60± 6
Lateral Posterior Nucleus	62± 7	66± 8	64±11	66±10	57± 8	60± 7	77± 9	79± 9	55± 7	56± 7
NON-VISUAL STRUCTURES										
Inferior Colliculus	47± 3	50± 3	52± 8	53± 7	41± 5	42± 5	62± 4	61± 4	46± 9	45± 9
Medial Geniculate Body	70±16	69±18	77±10	74± 8	67± 7	67± 8	70± 8	70± 9	62± 4	66± 5
Auditory Cortex IV	100±18	101±18	121±22	119±23	121±18	121±19	106±15	106±14	80± 6	81± 7
Hippocampus(Molecular layer)	122±15	122±15	143±16	142±18	135±18	139±19	130±20	130±21	102± 7	102± 7
Amygdala	115±25	114±25	136±16	132±16	199±22	120±24	121±22	121±22	78± 9	78± 9

APPENDIX III(e)
[^{3}H]-CHA BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=5 CONTROL		n=5 5 DAY		n=4 10 DAY		n=5 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES								
Visual Cortex II/III	42± 3	43± 3	48± 1	47± 2	42± 3	40± 3	49± 2	47± 3
Visual Cortex IV	53± 4	53± 4	57± 0	58± 1	52± 3	51± 3	58± 4	56± 5
Visual Cortex V/VI	44± 4	45± 4	45± 2	46± 2	44± 3	43± 4	46± 2	47± 3
Superior Colliculus (Superficial Layer)	64± 7	64± 7	55± 6	45± 4	57± 3	29± 3*	61± 1	34± 1*
Dorsal Lateral Geniculate Body	47± 2	47± 2	47± 2	47± 2	41± 4	36± 3	52± 3	46± 3
Pretectal Nuclei	23± 3	24± 3	26± 3	26± 3	20± 3	21± 2	31± 1	25± 2
Lateral Posterior Nucleus	67± 5	65± 5	55± 2	56± 2	50± 5	48± 4	59± 3	56± 4
NON-VISUAL STRUCTURES								
Inferior Colliculus	18± 2	18± 2	22± 3	23± 3	18± 1	18± 1	22± 1	22± 1
Medial Geniculate Body	54± 6	54± 6	53± 2	53± 2	49± 5	51± 7	59± 5	56± 5
Auditory Cortex IV	63± 5	63± 5	63± 3	64± 3	55± 4	54± 4	65± 3	64± 4
Hippocampus(Molecular layer)	69± 5	70± 5	80± 3	78± 4	71± 4	73± 6	75± 3	74± 4
Amygdala	25± 2	25± 2	26± 2	26± 2	23± 2	23± 2	32± 2	31± 2

APPENDIX IV

GLUCOSE UTILISATION, $5HT_1$, $5HT_2$, β-ADRENERGIC, $GABA_A$ AND MUSCARINIC RECEPTOR BINDING FOLLOWING UNILATERAL ORBITAL ENUCLEATION.

The effect of unilateral orbital enucleation on [^{3}H]-5-hydroxytryptamine (5HT), [^{3}H]-ketanserin, [^{3}H]-dihydro-alprenolol (DHA), [^{3}H]-quinuclidinyl-benzilate (QNB) and [^{3}H]-muscimol binding has been examined at four time points up to 20 days after the lesion. Glucose utilisation was measured in the same animals using quantitative [^{14}C]-2-deoxyglucose autoradiography.

In Table IV(a) data are presented as mean glucose use ($\mu mol.100g^{-1}min^{-1}$) ± S.E.M.; in the subsequent five tables data are presented as mean ligand bound (pmoles/g tissue) ± S.E.M. *$P<0.01$ paired t-test; † ipsilateral variance (ANOVA). Roman numerals indicate cortical layer examined.

APPENDIX IV (a)

GLUCOSE UTILISATION FOLLOWING UNILATERAL ENUCLEATION

	n=6 CONTROL		n=4 1 DAY		n=5 5 DAY		n=4 10 DAY		n=4 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	105± 4	105± 3	103± 4	78± 4*	99± 4	77± 3*	99± 4	79± 4*	100± 2	82± 3*
Visual Cortex IV	114± 6	116± 5	109± 3	76± 4*	109± 6	80± 3*	112± 5	89± 3*	113± 5	87± 4*
Visual Cortex V/VI	104± 4	105± 4	98± 3	77± 3*	99± 5	75± 2*	102± 4	88± 4*	103± 3	83± 2*
Superior Colliculus (Superficial Layer)	102± 2	103± 3	96± 3	53± 1*	96± 3	63± 2*	109± 5	76± 7*	101± 3	79± 3*
Dorsal Lateral Geniculate Body	126± 7	126± 7	102± 3	65± 3*	104± 7	66± 5*	117±16	75± 5*	127± 5	85± 2*
Pretectal Nuclei	107± 5	108± 6	91± 4	69± 4*	91± 7	71± 6*	101± 8	81± 7*	104± 4	91± 3*
Lateral Posterior Nucleus	118± 5	120± 6	102± 5	80± 5*	104± 8	76± 8*	115±10	91± 6*	118± 5	93± 6*
NON-VISUAL STRUCTURES										
Inferior Colliculus	145±15	141±17	132±13	136±15	144± 4	140± 3	125±12	121±11	118± 7	120±10
Medial Geniculate Body	132± 7	130± 7	118± 8	118± 8	124±11	127±11	137±10	134±11	142±13	141±12
Auditory Cortex IV	132± 9	132±10	132± 7	134±10	135±12	134±10	145±14	138±12	148±14	147±11
Hippocampus(Molecular layer)	88± 4	86± 4	88± 4	88± 4	88± 6	86± 6	94± 7	89± 5	94± 3	92± 1
Amygdala	68± 3	66± 5	63± 3	63± 4	63± 6	60± 4	66± 5	67± 6	68± 5	67± 5

APPENDIX IV (b)
[^{3}H]-5HT BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=4 CONTROL		n=4 1 DAY		n=5 5 DAY		n=3 10 DAY		n=4 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	40± 4	41± 5	49± 7	44± 5	38± 2	35± 2	40± 2	39± 3	31± 4	34± 3
Visual Cortex IV	61± 7	65± 7	65± 5	63± 3	53± 2	49± 3	50± 3	52± 3	48± 7	51± 9
Visual Cortex V/VI	61± 9	60± 8	63± 5	64± 7	50± 3	49± 4	51± 4	50± 4	46± 6	51± 9
Superior Colliculus (Superficial Layer)	102±10	102±11	96± 8	98± 8	93± 9	77± 7*	89± 3	65± 3*	81±12	59± 8*
Dorsal Lateral Geniculate Body	50± 3	52± 3	45± 2	44± 4	48± 3	32± 2*	45± 2	25± 2*	42± 6	24± 3*
Pretectal Nuclei	76± 5	72± 6	60± 3	56± 4	61± 5	56± 4	55± 4	50± 8	55± 8	50± 6
Lateral Posterior Nucleus	55± 4	55± 3	55± 4	51± 5	54± 4	51± 3*	51± 7	48± 5	46± 8	45± 7
NON-VISUAL STRUCTURES										
Inferior Colliculus	47± 7	46± 7	49± 6	45± 8	49± 5	49± 2	44± 5	44± 7	43± 8	43± 9
Medial Geniculate Body	24± 7	25± 5	18± 3	25± 5	22± 1	24± 1	23± 5	26± 7	23± 4	27± 4
Auditory Cortex IV	63±10	59±11	76± 8	70±12	60± 2	62± 2	59± 3	52± 4	60±11	65±11
Hippocampus(Molecular layer)	146±11	145±13	163±10	173±17	136±12	146±15	120±10	135±13	137±21	149±27
Amygdala	96± 5	98±14	97± 2	95± 5	73± 6	76± 5	75± 4	74± 6	76±13	74±11

APPENDIX IV (c)

[^{3}H]-KETANSERIN BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=6 CONTROL		n=4 1 DAY		n=4 5 DAY		n=4 10 DAY		n=4 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	18 ± 4	17± 3	18± 3	18± 4	19± 3	19± 1	14± 1	15± 2	16± 2	16± 3
Visual Cortex IV	24± 4	25± 4	22± 4	23± 3	24± 1	22± 1	22± 7	20± 6	23± 2	23± 3
Visual Cortex V/VI	18± 4	18± 5	17± 3	18± 4	18± 3	17± 2	15± 4	17± 7	15± 2	15± 3
Superior Colliculus (Superficial Layer)	21± 5	21± 5	16± 4	16± 3	22± 6	20± 6	15± 3	15± 4	17± 3	17± 3
Dorsal Lateral Geniculate Body	14± 3	14± 3	8± 2	9± 2	13± 1	12± 2	12± 4	12± 5	8± 2	8± 1
Pretectal Nuclei	17± 4	17± 3	11± 1	11± 2	9± 3	8± 3	11± 4	9± 4	10± 1	9± 1
Lateral Posterior Nucleus	15± 4	17± 3	11± 4	9± 1	10± 1	10± 2	6± 1	7± 1	10± 1	8± 2
NON-VISUAL STRUCTURES										
Inferior Colliculus	12± 1	12± 1	9± 3	9± 3	7± 2	7± 2	8± 2	8± 2	11± 1	11± 1
Medial Geniculate Body	13± 3	13± 3	10± 4	11± 4	13± 3	13± 3	12± 3	12± 3	14± 3	14± 3
Auditory Cortex IV	44± 9	46±10	36± 2	37± 3	36± 2	35± 2	44±11	39± 9	47± 6	41± 4
Hippocampus(Molecular layer)	11± 3	11± 2	13± 4	11± 4	11± 2	11± 2	13± 4	12± 5	16± 4	15± 3
Amygdala	23± 3	22± 3	17± 1	14± 1	18± 4	18± 4	18± 4	17± 5	18± 5	18± 4

APPENDIX IV (d)
[^{3}H]-DHA BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=6 CONTROL		n=3 1 DAY		n=5 5 DAY		n=4 10 DAY		n=4 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	13± 0	13± 0	12± 1	12± 1	15± 1	14± 1	12± 0	11± 0	14± 0	15± 1
Visual Cortex IV	10± 1	10± 1	11± 1	12± 1	11± 1	11± 1	8± 0	8± 1	9± 0	8± 1
Visual Cortex V/VI	10± 0	11± 1	11± 2	11± 1	9± 1	9± 1	8± 0	8± 0	8± 0	9± 0
Superior Colliculus (Superficial Layer)	16± 1	16± 1	14± 1	14± 2	17± 1	13± 0*	11± 1	8± 0*	12± 1	8± 1*
Dorsal Lateral Geniculate Body	10± 1	10± 0	11± 0	10± 0	10± 0	7± 0*	7± 1	4± 0*	8± 1	5± 0*
Pretectal Nuclei	11± 1	11± 1	12± 1	12± 1	11± 0	11± 0	6± 0†	5± 0	8± 1†	8± 2
Lateral Posterior Nucleus	12± 1	12± 1	17± 2	15± 2	13± 0	12± 1	10± 1	9± 1	12± 2	12± 1
NON-VISUAL STRUCTURES										
Inferior Colliculus	8± 1	8± 1	9± 1	9± 1	7± 1	8± 0	7± 0	6± 1	6± 1	6± 0
Medial Geniculate Body	10± 1	10± 0	10± 1	11± 3	8± 1	10± 0	8± 0	8± 0	8± 2	7± 1
Auditory Cortex IV	14± 2	13± 1	15± 4	15± 3	14± 1	14± 1	10± 1	11± 0	11± 1	12± 1
Hippocampus(Molecular layer)	15± 2	15± 2	11± 1	12± 1	14± 1	14± 1	10± 1	10± 1	13± 2	13± 3
Amygdala	11± 1	11± 1	9± 1	9± 1	11± 1	11± 1	8± 0	8± 0	9± 1	9± 1

APPENDIX IV (e)
[^{3}H]-MUSCIMOL BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=6 CONTROL		n=4 1 DAY		n=4 5 DAY		n=4 10 DAY		n=4 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	133± 6	133± 7	163±14	155±15	179±17	182± 6	185±13	163± 7	185±11	145±17
Visual Cortex IV	189±11	186±11	200±13	195±14	225± 9	221± 9	217±37	214±16	236±12	185± 6*
Visual Cortex V/VI	130± 7	132± 7	136± 8	141± 10	165± 4	156± 7	148±14	142±13	144± 4	114± 5*
Superior Colliculus (Superficial Layer)	108± 6	110± 4	130± 5	132± 6	136± 3	132± 6	123± 8	116± 5	114± 3	105± 9
Dorsal Lateral Geniculate Body	181±12	178± 9	172±33	170±33	208±13	194± 8	237± 6	202± 9	233±18	163±12*
Pretectal Nuclei	55± 3	58± 4	57± 5	55± 9	67± 6	65± 4	64± 3	63± 5	69± 5	71± 5
Lateral Posterior Nucleus	140± 7	149±10	142±27	155±33	144± 2	153± 5	160±13	163±14	151± 4	144± 3
NON-VISUAL STRUCTURES										
Inferior Colliculus	82± 7	79±11	69±16	65±12	63± 2	63± 3	88±12	88±14	72± 6	72± 6
Medial Geniculate Body	204±14	204±18	208±24	219±30	215± 9	208±12	220±20	226±19	218±13	208±16
Auditory Cortex IV	203±17	204±11	190±19	196±13	235± 3	223±11	193±26	205±20	208±21	202±21
Hippocampus(Molecular layer)	112±10	125± 9	133±20	132±20	111±13	109±13	94±17	105±15	108±13	107±13
Amygdala	119± 7	121± 9	149±14	150±13	118± 2	115± 5	107±21	116±26	116± 5	115± 3

APPENDIX IV (f)
[^{3}H]-QNB BINDING FOLLOWING UNILATERAL ENUCLEATION

	n=6 CONTROL		n=4 1 DAY		n=4 5 DAY		n=3 10 DAY		n=4 20 DAY	
	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived	Intact	Deprived
VISUAL STRUCTURES										
Visual Cortex II/III	323±20	318±17	343±40	331±45	358±19	332±12	361±48	365±52	330±23	333±28
Visual Cortex IV	274±20	269±22	295±26	298±37	310±19	309±22	308±30	320±37	303±25	304±22
Visual Cortex V/VI	294±24	294±28	315±39	322±36	336±21	329±20	321±36	326±34	321±24	321±23
Superior Colliculus (Superficial Layer)	267±22	271±22	282±24	309±26	291±12	296± 7	278±24	295±13	316±26	310±37
Dorsal Lateral Geniculate Body	122± 9	122± 6	122±12	124±15	120±20	113±12	117± 6	113± 7	142±12	139± 8
Pretectal Nuclei	110± 6	107± 7	101±15	100±18	120±11	117±10	106±11	101± 7	118± 7	119± 9
Lateral Posterior Nucleus	196±19	192±18	216±35	218±37	248±20	233±19	202±26	184±14	247± 8	247± 9
NON-VISUAL STRUCTURES										
Inferior Colliculus	99±13	94±10	84±13	77±16	104±18	105±19	98± 8	97±11	107±17	104±19
Medial Geniculate Body	103±12	108±12	98±11	94±14	116±14	106±12	112±19	114±23	125±16	125±15
Auditory Cortex IV	273±24	282±17	294±40	322±54	313±18	309±21	301±35	313±25	297±10	308±12
Hippocampus(Molecular layer)	287±16	286±14	306±42	319±58	324±20	326±22	343±25	354±26	309±13	308±12
Amygdala	283±15	291±16	347±42	351±33	306±34	290±25	315±15	317± 5	315±28	328±34

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