GABAergic Activity Influencing Cerebral Function in the Rat

by

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To the Memory of

Leonard Kelly

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SUMMARY

Currently available evidence suggests that γ-aminobutyric acid is the major inhibitory neurotransmitter in the mammalian central nervous system. As such, a fuller knowledge of the functional role played by GABAergic neurotransmission would increase our understanding of the complexities of brain activity. Three approaches were employed to analyse GABAergic influences in cerebral function; 1) systemic administration of GABA agonists (muscimol and THIP) or related compounds (benzodiazepines), 2) specific lesioning of a known GABAergic pathway (from caudate nucleus) with subsequent pharmacological challenge and 3) local intracerebral injection of a GABA agonist (muscimol) into a region with known GABA synaptic mechanisms (caudate nucleus). All experiments were performed on restrained, conscious animals, using principally the 2-deoxyglucose quantitative autoradiographic approach to measure cerebral function (as it is reflected in rates of glucose utilisation).

The intravenous administration of muscimol and THIP resulted in a heterogeneous pattern of significantly reduced glucose utilisation in the CNS. The regional hierarchy of changes in glucose utilisation was similar for both muscimol and THIP in all regions (with the exception of the superior colliculus), with muscimol being approximately six times more potent in all regions investigated. The regions in which glucose utilisation was extremely sensitive to change,

displaying reductions of approximately 40% following muscimol (1.5 mg/kg) or THIP (10 mg/kg), included the neocortex and thalamic areas. In contrast, in a large number of areas, including cerebellum and related motor nuclei, hypothalamus, lateral habenula and amygdala, there were only minimal reductions in glucose use following muscimol and THIP. Regions displaying moderate reductions in glucose utilisation included most extrapyramidal regions and a number of cortical and subcortical limbic areas. In no region of the 60 CNS areas measured was a significant increase in glucose utilisation observed with any concentration of either muscimol or THIP. The regional distribution of alterations in glucose utilisation following muscimol and THIP, which do not correspond to the known topography of GABAergic neurons and receptors, provided a comprehensive description of the functional alterations, as reflected in rates of glucose utilisation, which occur in conscious rats following systemic administration of these two putative GABAergic agonists.

Although benzodiazepines are thought to be active at the GABA receptor site, the response in terms of glucose use following diazepam was markedly different from that following GABA agonists. In particular, the degree of heterogeneity of depression in glucose use was less and the hierarchy of responsiveness in diverse brain areas was different, suggesting that the responses to GABA agonists were not merely a function of susceptibility to depression in general.

The relationship between local cerebral blood flow (measured with iodoantipyrine) and glucose use was analysed in two groups of rats. Both blood flow and glucose use decreased in parallel in the 38 areas of the brain analysed following muscimol administration i.v., thus maintaining the relationship observed in control rats. These studies offered no evidence for a direct vasodilatatory action for muscimol (reported by others in vitro) in the intact, conscious rat, but pointed to underlying metabolic activity as the primary determinant of cerebral blood flow.

Local cerebral glucose use was measured 10 days after the unilateral injection of the neurotoxin, kainic acid, into the striatum. The stereotactic infusion of kainic acid resulted in lesions localised to the caudate nucleus. Rates of cerebral glucose use were most markedly affected ipsilateral to the infusion site in areas which normally receive input from the caudate nucleus. In globus pallidus and substantia nigra pars reticulata, increases in glucose use of 82% and 74%, respectively, were measured when compared with controls. Significant increases were also measured in contralateral pallidus and substantia nigra pars reticulata (16% and 20%, respectively). Of the brain structures examined, significant unilateral increases from control were observed in ipsilateral habenula (23%), ventrolateral thalamus (13%), contralateral substantia nigra pars compacta (14%) and sensory-motor cortex (15%). Symmetrical, bilateral

increases in glucose use were found in the nucleus accumbens (15%), ventral tegmental area (24%) and red nucleus (17%). Only in the ipsilateral caudate nucleus was any decrease in glucose use observed, although the association of this change with an area of histologically definable cell damage demands that this result be treated with caution.

The increases in functional activity following striatal lesion provide an insight into the mechanisms by which overt motor behaviour returns to normal a short time after the removal of striatal interneurons and efferent perikarya. Of particular interest are the responses observed contralateral to the affected striato-nigral system in view of the proposed interaction between the two sides of the brain.

Following systemic administration of muscimol (1.5 mg/kg) the increases in functional activity observed following striatal lesions in both hemispheres were either substantially attenuated or completely eliminated, suggesting that the effects of lesioning were due to loss of mainly GABAergic projection neurons. The loss of these neuronal systems also markedly altered the responses to the dopaminergic agonist, apomorphine, observed in the conscious animal. Thus, within the ipsilateral extrapyramidal system, striatal lesions attenuated the apomorphine-induced increases in glucose use observed in entopeduncular nucleus, but potentiated the increase observed in substantia nigra pars reticulata. In

areas outwith the extrapyramidal system, the loss of striatal efferents apparently resulted in attenuation of apomorphine-induced increases in parafascicular-ventromedial complex in the thalamus as well as attenuation of the decreases in lateral habenula following apomorphine. Thus, it would appear that many of the effects of dopaminergic manipulation in intact, conscious animals require integrity of cellular function in the striatum.

Localised unilateral application of the GABA agonist, muscimol (500 ng), into the striatum resulted in decreased glucose use within this nucleus (-33%). This apparent reduction in striatal functional activity resulted in increased rates of glucose use in ipsilateral areas receiving primary striatal projections; globus pallidus (+29%), entopeduncular nucleus (+33%) and pars reticulata of substantia nigra (+46%). Within the regions to which these extrapyramidal nuclei in turn send projections, decreases in glucose use were measured in subthalamic nucleus (-18%), superior colliculus stratum profundum (-18%) and ventrolateral thalamus (-40%). In contrast, a significant increase in glucose use was observed within the lateral habenula (+16%). Thus, it would appear that intrastriatal GABAergic neurons may, by their activation, affect functional activity in striatal projection pathways, and these effects may be manifest also in secondary projection areas.

The data from this thesis provide a comprehensive

description of GABAergic influences in the brain in general and the extrapyramidal system in particular, and provide some insight into the role of GABAergic inhibition in the organisation of integrated cerebral function.

PREFACE AND DECLARATION

This thesis presents the results from studies of cerebral GABA systems using, exclusively, quantifiable autoradiographic techniques. As these methods are relatively new, the methods section describes in some detail both practical and theoretical considerations. Possible modifications to the 2-deoxyglucose technique are critically examined.

Three methods of stimulating GABA systems were used: systemic drug administration; striatal lesion followed by systemic drug administration; intra-striatal drug injection. These three approaches are presented and discussed individually.

In the final discussion I have attempted to show that the 2-deoxyglucose technique, which is used extensively in these studies, provides insight into not only specific cerebral GABA systems, but also into the general principles of functional organisation in the mammalian brain.

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

CHAPTER I

INTRODUCTION

1. Principles of Cerebral Organisation

Almost a century has elapsed since Cajal embarked upon the elegant microscopic analysis of the nervous system which culminated in his two-volume opus describing, in exhaustive detail, the complexity of cellular organisation in the mammalian brain (Cajal, 1911). Whilst most organs of the body are made up of cells which are relatively simple in form, and are similar, or at least complementary in the roles which they subserve, the cellular units of which the brain is comprised present a bewildering array of cytoarchitectural differences whose relation to different functional activities is not immediately apparent. The regional heterogeneity of cerebral function associated with complex cytoarchitectonic subdivisions of the brain has constituted a considerable barrier to the elucidation of general principles underlying integrated cerebral activity.

The integrated function of any bodily organ must inevitably depend upon the co-ordinated action and interaction of its constituent cells. From the histological observations of interneuronal connections made by Cajal and his contemporaries and the classical

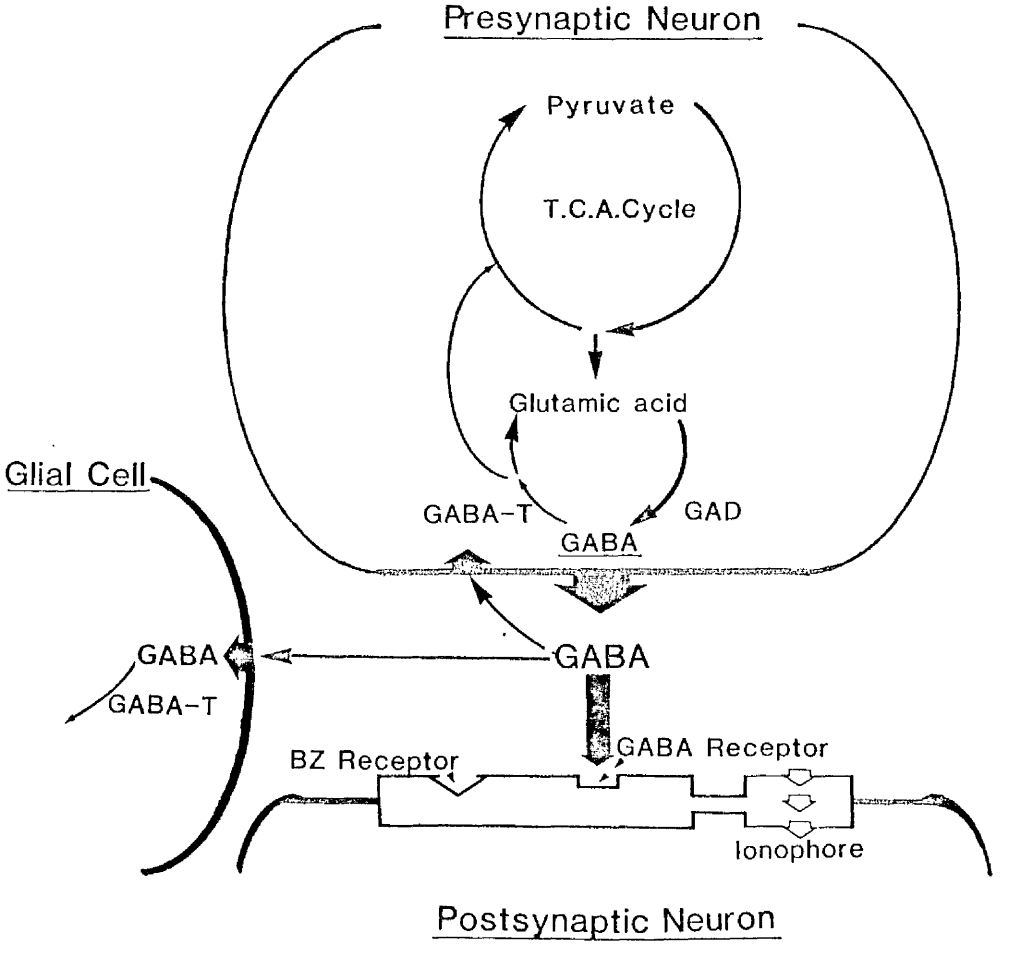
description of the reflex arc, upon which Sherrington based his book "The Integrative Action of the Nervous System" (1906), have arisen modern concepts of neuronal organisation. In the brain, neurons constitute the functionally operative cellular units, but it is the synapse, in allowing neuronal interaction to take place, which represents the essential structural unit of cerebral function. Moreover, despite the profound inter-regional differences in structure and function found in the brain, the synapse, with certain similarities in structural patterns and functional properties, provides a common background against which the welter of regional dissimilarities may be assessed and possibly rationalised. Perhaps the greatest single factor common to synaptic function is provided by the chemical nature of synaptic transmission (Krnjevic, 1974) and, although the number of putative neurotransmitter substances is ever widening (Iversen, 1978), those candidates continue to form a very small fraction of the variety of neurochemicals conceivably available for use in this way. Thus, experimental analysis of single neurotransmitter systems in regions of the brain with diverse functional roles has added further to our understanding of the unifying principles of organisation common to all brain regions.

Almost without exception, neurons in every part of the mammalian brain have been shown to be sensitive to some degree to the inhibitory action of the short chain α, ω-amino acid, γ-aminobutyric acid (GABA) (Krnjevic, 1974, for review). Since GABA is ubiquitously distributed

in the brain in relatively high concentrations (Fahn , 1976) but is required in only minute amounts to elicit neuronal inhibition (Salmoiraghi, 1967), it is reasonable to postulate, in the light of accumulating evidence (Krnjevic, 1974), that GABA is likely to be an inhibitory neurotransmitter used extensively in the brain (Curtis, 1979; Enna and Maggi, 1979). The concept of disinhibition as a governing principle in cerebral function, which has recently been proposed, requires the widespread distribution of tonic inhibitory neurons (Maynard, 1972; Roberts, 1976). The potency and ubiquity of GABA systems could meet the requirements necessary for such a mechanism (Roberts, 1976). Certainly, the apparent unbridled excitation which results from release from endogenous GABA influences (Roberts, 1976) lends support for such a view.

Whether GABA releasing neurons form only one part of the many putative neurotransmitter systems, or are indeed responsible for the integration of cerebral function in a wider, global sense, analysis of GABA-mediated neurotransmission is undoubtedly of interest in furthering our comprehension of the workings of the brain. The importance attached to the examination of GABA influences in the brain is reflected in the number of studies of the mechanisms of GABA neurotransmission and their consequences (Roberts et al. 1976; Krøgsgaard-Larsen et al. 1979; Lal et al. 1980). Some salient features from currently available data are presented in the following short review (Fig. 1), but are also

Figure 1



The GABAergic synapse. A schematic representation of the salient features of GABAergic synaptic neurotransmission.

discussed, where applicable, in the commentaries on the experiments which make up this thesis.

2. GABA Synthesis and Release

The main metabolic pathway for the biosynthesis of GABA in nervous tissue involves the decarboxylation of L-glutamate. This transformation is catalysed by the enzyme, glutamic acid decarboxylase (GAD), with a co-enzyme requirement for pyridoxal phosphate (Wu and Roberts, 1974). In vitro studies have shown that GAD is subject to substrate induced inactivation through the formation of the apoenzyme (Miller et al. 1978). A supply of pyridoxal phosphate is necessary to allow continuous regeneration of the haloenzyme and, therefore, maintenance of a constant level of enzyme activity. Since GAD is the rate-limiting enzyme in the synthesis of GABA, these enzymic processes determine the steady state levels of GABA in brain.

There is considerable evidence that GAD activity may act indirectly as a regulatory mechanism of excitation in the brain (Wood and Peesker, 1974). Effective GAD activity may be reduced by competitive inhibition by substrate analogues of glutamate with, for example, mercapto-propionic acid (Horton and Meldrum, 1973; Tapia, 1975) or by interference with the co-enzyme system by hydrazides and other carbonyl trapping agents (Wu and Roberts, 1974; Tapia, 1975). Reduction of GAD activity below threshold levels results in a convulsive response in vivo (Tapia and Sandoval, 1975), suggesting a relatively high turnover rate for GABA

available for neurotransmission. It is perhaps worth emphasising, however, that the results of experiments in which GABA metabolism is manipulated must be interpreted with a degree of caution, bearing in mind the dual role of this pathway in intermediary metabolism as well as in neurotransmitter synthesis (van der Berg et al. 1975).

Although GAD has been localised to nerve terminals where it is associated with the synaptosomal fraction (Iversen and Neal, 1968), following axotomy GAD accumulates in the proximal portion of putatively GABAergic neurons (Storm-Mathisen, 1975). Similarly, colchicine treatment, by inhibiting microtubule formation, leads to an accumulation of GAD in neuronal cell bodies (Ribak, 1978). These observations are consistent with the synthesis of GAD being localised to the cell body before subsequent removal to terminal sites by axoplasmic transport systems.

A specific synaptosomal population for the storage of GABA has been identified (Kuhar et al. 1971) and, although GABA levels in nerve terminals may not be extensive, the amounts present in cortical synaptosomes are sufficient to produce significant inhibition when released from suspensions directly on to cortical cells (Krnjevic and Whittaker, 1965). Moreover, the in vivo release of endogenous GABA from cerebral cortex has been shown to be markedly increased by electrical stimulation (Iversen et al. 1971) at a rate which elicits an inhibition of cortical neurons (Mitchell and **Srinivasan** , 1969).

Similarly, stimulation of inhibitory input arising from distant sites also increased the measured GABA efflux (Iversen et al. 1971). Reinforcement of the suggestion that synaptic GABA release was responsible for the inhibitory responses was provided by the action of Ca^{++}-free superfusion medium in abolishing the stimulus-evoked efflux (Iversen et al. 1971). The results of these studies must, however, be viewed with a degree of circumspection. GABA is accumulated by cells other than neurons, and release from these elements may also be produced by depolarisation. Recently, however, using pure cultures of dissociated cortical neurons, a K^{+}-evoked release of GABA was observed which was significantly reduced by the removal of Ca^{++} from the bathing medium (Hauser et al. 1980). This highly controlled in vitro study confirmed the conclusions of the in vivo work that GABA release was mediated by the mechanisms usually associated with synaptic transmission. The mechanisms by which GABA efflux is coupled to the neuronal stimulus in vivo remain to be fully determined.

Several agents have been demonstrated to have an effect upon the synaptic release of GABA. Imipramine, chlorpramazine, diazepam, haloperidol and baclofen inhibit the stimulus-evoked release of GABA from synaptosomes (Olsen et al. 1977) whilst, in certain regions of the brain at least, dopamine enhances synaptic GABA release (Reubi et al. 1977). Elucidation of the mechanisms underlying these interactions may allow a greater

understanding of the stimulus-coupling of GABA release.

3. The Ionic Basis of GABA Action

The electrophysiological basis of GABAergic action in synaptic neurotransmission was initially most successfully characterised in the invertebrate, crayfish stretch receptor preparation (see Otsuka, 1976, for historical review). In this preparation the post-synaptic response to the application of mammalian brain extracts containing GABA closely paralleled that observed following activation of inhibitory neurons (Florey and McLennan, 1959). Subsequently, synaptic release of endogenous GABA was found in response to stimulation of inhibitory neurons (Iversen et al. 1966). Techniques of microapplication and simultaneous post-synaptic electrophysiological recording within the mammalian brain have also shown a close relationship between the actions of exogenous GABA and naturally occurring inhibition (Salmoiraghi and Steiner, 1967).

Initial experiments reported that iontophoretic application of GABA at synaptic sites induced a hyperpolarisation of the post-synaptic cell from its resting potential level (Curtis and Johnston, 1974). If the negativity of the resting potential was artificially increased, or if the post-synaptic cell was subject to IPSPs induced by pre-synaptic inhibitory cell activity, then the hyperpolarising response to exogenous GABA administration was reduced until the response was reversed and GABA elicited a depolarising response (Levy, 1977). A purely depolarising post-synaptic

action of GABA has also been reported (Levy, 1977; Misgeld et al. 1982). Recent investigations have described a biphasic (hyperpolarisation followed by depolarisation) response to a single GABA administration in tissue slice preparations (Alger and Nicoll, 1979; Mayer et al. 1981), but both phases are inhibitory. It would appear, therefore, that the inhibitory action of GABA is mediated not merely by the induction of hyperpolarising currents, but rather by the clamping of the membrane potential at a level just below the resting potential. In neurons with high resting negativity, the effects of clamping produce a depolarising effect. The reversal level at which the action of GABA switches from a hyperpolarising to a depolarising influence corresponds closely to the equilibrium potential for chloride ions. This evidence, together with the observed attenuation of GABA effects in preparations with artificially reduced extracellular, or increased intracellular levels of chloride (Curtis and Johnston, 1974), indicate that the post-synaptic action of GABA is mediated via an increased membrane permeability to chloride ions (Curtis and Johnston, 1974).

Although some controversy surrounded initial reports of a pre-synaptic electrophysiological action of GABA (Curtis and Ryall, 1966; Galinda, 1969), evidence continues to accumulate that GABAergic effects may be manifest pre-synaptically (Pickles, 1976, 1979). The underlying ionic mechanism again appears to be an increased membrane permeability for chloride which results,

in this instance, in an efflux of the anion from the terminal region. Action potentials arriving at the already depolarised terminal are reduced in the magnitude of their effects and, therefore, the quantal release of transmitters is correspondingly lower.

4. Post-synaptic GABA Binding - The GABA Receptor

The triggering of post-synaptic electrophysiological events by chemical neurotransmitters is dependent upon the reversible binding with sub-synaptic membrane recognition sites which are associated with transmitter receptors (Krnjevic, 1974). In mammalian CNS, receptors for acetylcholine (Burgen et al. 1974; Yamamura et al. 1974), serotonin (Bennett and Snyder, 1975) and glycine (Young and Snyder, 1973) have been characterised biochemically. A complete chemical characterisation of GABA receptors is not yet available. Currently, information must be derived from binding techniques or electrophysiological analysis (Nistri and Constanti, 1979).

In the absence of selective post-synaptic GABA receptor antagonists which could be readily radiolabelled to high specific activities, labelled GABA itself was used as the ligand in initial receptor binding studies (Zukin et al. 1974; Enna and Snyder, 1975). The main problem to be circumvented with this approach is that GABA binding to crude synaptic membrane preparations reflects the total population of recognition sites, only a fraction of which are receptor associated. To study the GABA receptor in isolation

requires the membranes to be repeatedly frozen, thawed and washed (Enna and Snyder, 1977) before the binding studies are performed in Na^+-free medium (Young et al. 1976). The concentration of specific binding sites apparent from these preparations (0.7 pmole/mg protein) was approximately equal to non-specific binding, but whilst the former system was saturable by increasing GABA concentrations, with a dissociation constant of 0.37 μM, non-specific binding was not saturable (Young et al. 1976).

Further analysis of the membranes, prepared as described above but with an incubation with Triton X-100, revealed a population of GABA recognition sites which had an affinity for GABA ten times greater than that observed previously (Massotti, 1979). The demonstration of this second recognition site led to speculation that the GABA receptor formed a complex which included a protein entity capable of modulating high affinity GABA binding (Toffano et al. 1976). Electrophysiological evidence also suggested that the complex is operationally coupled to a Cl^- ionopore by means of which Cl^- conductance could be influenced (Nistri et al. 1980). Of these subunits, only the modulator protein (GABA-modulin) has been isolated and characterised (Toffano et al. 1978).

The mechanism of interaction between the GABA receptor complex and the ionopore remains to be fully elucidated. However, results from invertebrate species suggest that more than one GABA molecule

binding to the active site is required before the GABA receptor is functionally effective in eliciting conductance changes (Brookes and Werman, 1973; Takeuchi, 1976). Although a similar phenomenon is likely to be involved in higher phyla, such a mechanism has not been demonstrated to date.

The use made of radioligand binding to GABA receptors has allowed their cellular localisation to be visualised with both light and electronic microscopic analysis of autoradiographic emulsions (Chan-Palay, 1978). Within the cerebellum, GABA receptor sites were localised to the plasma membranes between synaptic elements of both axodendritic and axosomatic synapses (Chan-Palay, 1978). Using this technique, it proved impossible to determine whether the radioligand binding was associated with pre- or post-synaptic membranes. With a more biochemical approach in which post-synaptic membranes were separated by density gradient centrifugation, the experimental data was concluded to be in keeping with a predominantly post-synaptic GABA receptor localisation (Bittiger, 1979).

The somewhat disruptive approaches used to prepare GABA receptor sites (Enna and Snyder, 1977) may result in the loss of some of their native properties. Thus, the observation of an apparent activation of GABA receptor binding by diazepam in fresh brain membranes (Guidotti et al. 1978) could not be repeated with frozen and thawed, thoroughly washed preparations (Olsen et al. 1978a,b). Current evidence suggests

that the benzodiazepine receptor is yet another fraction of the GABA receptor complex (see Olsen, 1981, for review), although the possibility that these drugs may interact with a modulator protein to regulate GABA binding remains to be fully verified (Toffano et al. 1978; Olsen et al. 1980). In contrast, the reverse interaction of some GABA agonists in promoting benzodiazepine binding is well documented (see Olsen, 1981). Although there is considerable overlapping of GABA and benzodiazepine receptor distributions in the brain (Mohler and Okada, 1977; Squires and Braestrup, 1977; Young and Kuhar, 1979), the patterns are not identical. Whether benzodiazepine receptors are to be found in locations which are lacking in GABA receptors remains an open question.

5. GABA Receptor Pharmacology

The two most extensively studied antagonists of GABAergic effects upon neurons are the potent convulsants, bicuculline and picrotoxin (Johnston, 1976). Although both agents antagonise GABAergic inhibition of neuronal firing rates (Curtis et al. 1971; Curtis and Johnston, 1974), they appear to have different mechanisms of action (Olsen et al. 1978). Competitive inhibition of GABA binding by bicuculline has been observed with various brain cell membrane preparations (Zukin et al. 1974; Enna and Snyder, 1975). The concentrations of bicuculline required to inhibit GABA binding in these studies were considerably higher than those reported to antagonise GABA inhibitory effects.

Moreover, as the regional distributions of GABA and bicuculline binding are not identical, it seems likely that not all agonist and antagonist sites are identical (Enna and Snyder, 1977). In contrast to the action of bicuculline at the GABA recognition site on the receptor, picrotoxin does not influence the binding of GABA to synaptic membranes, although it does inhibit GABA-induced increases in chloride permeability (Ticku and Olsen, 1977). Thus, it appears that the antagonist effects of picrotoxin are mediated via interaction with the mechanism controlling chloride permeability, the ionophore, or with the functional linkage between receptor and ionophore. It is interesting, in view of the differential actions of the "classical" GABA antagonists, that the antagonism of GABA-induced chloride conductance by penicillin compounds is due to an action both at the receptor recognition site for GABA (weak) and at the ionophore (strong) (Hochner et al. 1976).

Three criteria have been clearly established as requisite properties of true GABA agonists: they must evoke a bicuculline-sensitive inhibition of neuronal firing; they must compete in a competitive manner with labelled GABA for Na^+-independent binding sites in brain membrane preparations; they must produce the same neurological effects as GABA itself when applied locally to neurons. If, as appears likely, more than one population of GABA receptors co-exist in the brain (Olsen et al. 1979) with different patterns of regional distribution (Guidotti et al. 1979), one might expect

agents which meet the preconditions as agonists for one receptor type to be inappropriate at other receptors. To date such possible diversity of receptor-agonist characteristics remains poorly determined.

The naturally occurring psychotomimetic agent, muscimol, has proved to be a potent GABA agonist (Curtis et al. 1971; Johnston, 1976). This confirmationally restricted structural analogue of GABA shows a remarkable selectivity, binding to GABA receptors with an affinity greater than that of the endogenous ligand (Enna et al. 1977). Whilst muscimol inhibits Na^+-independent binding in nanomolar concentrations (Enna et al. 1977; Krøgsgaard-Larsen and Johnston, 1978; Olsen et al. 1978), in contrast, millimolar concentrations are required to inhibit GABA transport systems (Krøgsgaard-Larsen and Johnston, 1978; Olsen et al. 1978). A good correlation exists between the potency of muscimol in binding to GABA receptors and its activity in inhibiting neuronal cell firing (Krøgsgaard-Larsen and Johnston, 1978).

The potential use which may be made of muscimol as a pharmacological tool in manipulating central GABA systems is enhanced by its ability to cross the blood-brain barrier, which is relatively impermeable to GABA itself (Naik et al. 1976). Within the brain, muscimol is only slowly metabolised (Baraldi et al. 1979), but it is rapidly broken down in the periphery (Baraldi et al. 1979; Maggi and Enna, 1979) such that, following systemic administration, only 0.02% of the initial

amount injected can be recovered from brain tissue in its native form. It has been suggested that this amount of muscimol would be sufficient to elicit GABA mimetic effects (Baraldi et al. 1979), although the possibility exists that the total response observed could also be due, to some extent, to active metabolites. No such GABA mimetic metabolites have, however, been identified. Recent investigations have suggested that the anticonvulsant effects observed following systemic administration of muscimol are due solely to the unmetabolised agonist in the brain (Enna at al. 1980; Matthews et al. 1981), and pharmacological inhibition of peripheral degeneration of muscimol has been proposed as a method of enhancing the GABA-mimetic properties of muscimol (Enna et al. 1980). Although muscimol metabolites do not exert any influence at the GABA receptor, it is possible that they are responsible for the side effects of this drug reported in humans (Waser, 1967; Tamminga et al. 1978). Reduced levels of circulating metabolites could conceivably expand the therapeutic uses to which muscimol may be applied (Enna et al. 1980).

The extremely potent GABA-agonist properties of muscimol have provided a yardstick against which other laboratory-synthesised, putative agonists may be measured, as well as providing a structural model from which a variety of related mono- and bicyclic compounds have been systematically developed (Krøgsgaard-Larsen et al. 1979). Dihydromuscimol and thiomuscimol are approximately

equipotent with muscimol in inhibiting GABA binding to rat brain membranes (Krøgsgaard-Larsen et al. 1979), but the structures of these compounds render them as susceptible to metabolic transformation as the parent compound. Of the many other compounds examined, to date only 4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol (THIP) and isoguvacine have proved to be specific and potent GABA agonists (Krøgsgaard-Larsen et al. 1981). Although both of these GABA agonists have been shown to inhibit neuronal firing in vivo (Polc, 1979), isoguvacine had no effect upon seizure responses when delivered systemically (Anlezark et al. 1977). THIP, in contrast, appears to be pharmacologically active in a variety of animal models (Christensen et al. 1979; Fuxe et al. 1980; Meldrum and Horton, 1980). It is of interest that, in spite of the structural similarities between the two GABA agonists, THIP apparently penetrates the blood-brain barrier more readily than isoguvacine.

As well as providing a possible therapeutic agent (Hill et al. 1981), examination of the properties of THIP has been useful to further elucidate the biochemical characteristics of the GABA receptor. A recent study of labelled THIP binding to synaptic membranes in vitro suggested that the experimental data best fitted a receptor model based on three receptor types (Falch and Krøgsgaard-Larsen, 1982). Moreover, the density of THIP-receptive GABA receptor sites was remarkably low in all of the brain regions studied,

with the exception of cerebellum. One possible explanation is that THIP may bind selectively to only one receptor-type population (Falch and Krøgsgaard-Larsen, 1982), a suggestion which could open up possibilities for differential, possibly regionalised, GABA receptor activation.

6. GABA Uptake and Degradation

Evidence is accumulating that termination of the interaction of GABA and its post-synaptic receptor is brought about by high affinity GABA transport mechanisms located in pre-synaptic nerve terminals and peri-synaptic astroglial cells (Schousboe, 1980). The GABA recognition sites for these uptake processes are both sodium dependent and temperature sensitive (Martin et al. 1976). Astrocytic GABA transport is dependent upon the simultaneous exchange of one sodium ion per molecule of GABA, but is independent of potassium and calcium ions (Hertz et al. 1978). The neuronal GABA transport system displays both a greater affinity for GABA and a higher capacity for GABA uptake than is found in the glial system (Hertz, 1979; Schousboe, 1979; Hertz and Schousboe, 1980). On the basis of in vitro studies, some recent experiments in vitro have suggested that high affinity GABA uptake into synaptosomal fractions is due mainly to a homoexchange on a one for one basis with the endogenous pool (Levi and Raiteri, 1974). In vivo, however, there does appear to be a net inward transport of GABA (Sellstrom et al. 1976), with a translocation of two or more sodium

ions per GABA molecule (Simon et al. 1974). GABA transport across glial membranes represents a net inward transport (Hertz et al. 1978).

By making use of the range of GABA analogues developed to characterise post-synaptic receptor agonists (Schousboe et al. 1979) it has been possible to identify differences in substrate specificity in glial and neuronal transport systems. It is thus possible to selectively inhibit to some degree either of the two systems alone (Schousboe et al. 1980). Using these compounds as pharmacological tools, it may be that future studies will be able to determine the relative importance of the two systems in the termination of post-synaptic GABA effects.

GABA is catabolised in the brain by transamination with α-ketoglutarate to produce succinic semialdehyde, a reaction catalysed by GABA transaminase (GABA-T). Subsequent oxidation catalysed by succinic semialdehyde dehydrogenase returns succinate to the tricarboxylic acid cycle. The predominantly mitochondrial localisation of GABA-T (Hyde and Robinson, 1976) separates the synthetic (GAD) and degradative enzymes in both neuronal and glial elements (Iversen and Kelly, 1975). Moreover, the mitochondria derived from glial cells are much richer in GABA-T than are those derived from pre-synaptic nerve endings, although GABA-T activity in astrocytes represents only a third of that of the whole brain (Schousboe et al. 1980). Thus, not only is GABA breakdown compartmentalised within cellular organelles, but it also appears

to be to some extent separated in different cell types from neuronal GABA synthesis. Again, however, the relative importance of neuronal and glial systems for GABA breakdown remains to be determined. To date no specific antagonists have been developed to distinguish between the two systems, and their similarity probably excludes such a possibility. However, selective inhibition of the specific uptake mechanisms (Schousboe et al. 1980) indicates that both degradative processes contribute significantly to GABA inactivation.

7. Distribution of Cerebral GABA Systems

Several methods exist to measure the relative distributions of GABA neurotransmitter mechanisms in different regions of the brain. In essence, these techniques measure concentrations of the elements at each stage of GABA neurotransmission (Fig. 1), or the rates of activity of the enzymes involved.

Measurement of the concentrations of GABA itself is perhaps the most obvious index of the relative importance of this mode of neurotransmission in any given region (Okada et al. 1971). Recently developed techniques have overcome the problems of post mortem increases in brain tissue GABA content (van der Heyden and Korf, 1978) and have provided a fluorometric assay method which, according to the authors, is quick, simple, and requires little by way of specialised equipment (van der Heyden and Korf, 1978). There are, however, two major limitations to this methodological approach. Firstly, the GABA content of brain is not

necessarily of neuronal origin (Baxter, 1976) and, secondly, GABA content has been shown to be dependent upon the functional state of the brain prior to sacrifice (Killam et al. 1960). Although the inconsistencies in GABA content refer in this instance to convulsive states, it is not unreasonable to assume that less extreme circumstances may also affect GABA levels.

As GAD is the rate limiting enzyme in the synthesis of GABA, it has been proposed that the presence and activity of this enzyme will reflect directly upon the availability of GABA for neurotransmission. GAD activity may be demonstrated using carboxy-labelled glutamate (Albers and Brady, 1959) and measuring the enzymic production of either CO_2 or GABA (MacDonnell and Greengard, 1975; Fahn, 1976; Tappaz et al. 1976; Nieoullon and Dusticier, 1981). The specificity of these assays for neuronal GAD has been enhanced by the recognition of different kinetic properties for GAD from non-neuronal sources (Hoker et al. 1970). Thus, the GAD assays are sensitive and allow quantitative comparisons between brain regions, although these approaches do not allow any insight into the cellular localisation of GAD. To meet this requirement, immunohistochemical methods have been developed which allow visualisation of GAD distribution at both the light and electron microscopic levels (see Roberts, 1978). The antibodies raised specifically against neuronal GAD display no cross reaction with GAD from non-neuronal sources

(Wu, 1980). It should be stressed, however, that the specificity of GAD antibodies may vary in different laboratories, depending upon the rigour with which they are prepared. Moreover, verification of experimental results is not possible unless access is allowed to the same batch of antibodies. Despite these reservations, the studies which have been performed with this approach have provided information about the localisation of this enzyme at the cellular and sub-cellular level (Wood et al. 1976; Ribak, 1978), as well as allowing the mapping of GABA neuron circuitry within and between regions of the brain (Fonnum et al. 1978; Ribak, 1978).

Approaches similar to those used for localising GAD have been employed to study the distributions of the GABA degradative enzyme, GABA-T (Pitts, 1965; Waksman et al. 1968; Barker and Saito, 1976). However, unlike GAD, non-neuronal GABA-T has not been specifically characterised. Although it is possible that some brain regions contain only minimal concentrations of GABA-T from glial sources (Vincent et al. 1981), this has not been proven for all areas. The criticisms of GABA-T as a generalised marker for GABA neurotransmission throughout the brain (Storm-Mathisen, 1976) have not been adequately answered.

Whilst the techniques described so far have been limited by the fact that GABA may be involved in intermediary metabolism, without any neurotransmitter role, the mapping of high affinity GABA receptors reflects directly upon the mechanism of neurotransmission.

The high degree of specificity associated with muscimol binding to these receptors (Snodgrass, 1978) has added a further refinement to the biochemical localisation of GABA systems in the brain (Beaumont et al. 1978). The use of these techniques is limited by the poor spatial resolution resulting from the necessity for large tissue samples to be used (Penney et al. 1981). The use of ^{3}H-labelled muscimol and tritium-sensitive film has not only enabled GABA receptor distributions to be visualised with a high degree of resolution, but also to be fully quantified (Palacios et al. 1980, 1981: Penney et al. 1981).

Although the mapping of cerebral GABAergic markers has undoubtedly provided useful indices of the importance of GABA neurotransmission to the integrated function of different areas of the brain, one must always be careful to avoid attributing a functional role to those mechanisms purely on the basis of their existence within an area. It is becoming apparent that even the presence of specific receptors does not mean that the rest of the transmitter system is available.

8. GABA Systems in Cerebral Function

Specific regulatory functions involving GABAergic neurons have been identified in the control of sleep (Poddar et al. 1980), food intake (Kelly and Grossman, 1980; Meeker and Meyers, 1980), parasympathetic activity (Gale et al. 1980), cardiovascular control (Billingsley et al. 1980; Hamilton and Gillis, 1980; Snyder and Antonaccio, 1980), and hormone release

(Chase and Tamminga, 1978). However, the ubiquitous distribution of GABAergic neurotransmitter systems in the brain suggests a global role for GABA neurons in the control of excitatory influences (Roberts, 1979). A complete understanding of these wider influences of GABA requires not only a full description of the cellular morphology and distributions of GABAergic neurons themselves, but also a detailed knowledge of the organisation of these neurons with respect to other neurotransmitter systems.

Attempts to elucidate the functional role of GABAergic neurons from a synthesis of biochemical and histological data have been concentrated to a large extent upon the cerebellum, hippocampus and extrapyramidal motor system. In the cerebellum the exact distribution of GABAergic inhibitory neurons (Hökfelt and Ljungdahl, 1971; Storm-Mathisen, 1976; Chan-Palay, 1978) has been added to the already detailed knowledge of cellular morphology and interconnections (Eccles et al. 1967) without substantially increasing understanding of the principles of cerebellar functional processes (Roberts, 1979). In contrast, within the caudate nucleus where the cellular organisation is less clearly defined, biochemical analysis of the interaction between GABAergic neurons and cholinergic (Scatton and Bartholini, 1980) and dopaminergic neurotransmitter systems (Racagni et al. 1978) has increased awareness, if not yet understanding, of the complexity of striatal function. Moreover, animal behaviour models which allow motor functions

to be assessed have revealed two distinct GABA-dopamine interactive processes, one of which is responsible for controlling the occurrence of locomotor activity, whilst the other is involved in stereotypy of motor behaviour (Scheel-Kruger et al. 1977). The different anatomical origins of these two related motor activities in nucleus accumbens and caudate nucleus is indicative of the difficulties which may be encountered if the functional activity of a single brain area is studied in isolation, with no knowledge of events occurring elsewhere.

The purpose of the studies which comprise this thesis was to analyse the simultaneous effects of GABA-ergic manipulation upon the integrated function of anatomically discrete and functionally diverse regions of the brain with a view to providing a greater understanding of the importance of GABA systems, both in general and in particular, in the integrated activity of the brain as a whole.

CHAPTER II

METHODS

1. General

Several factors were common to all the experimental approaches used in these studies; the animals and their preparation for experiments, and the form in which experimental data were generated and handled prior to analysis. These elements are dealt with in this first section.

1.1 Animals: All experiments were performed on male rats (Rattus norvegicus, var. albinus) of the Sprague-Dawley strain. The rats used weighed between 300 and 450g, which corresponds to a post-weaning age of approximately 5 to 8 months (cf. 50% survival age of 30 to 32 months). Animals are expected to lose up to 5% of body weight in transit from supplier, and to avoid the possibility of starvation-induced physiological changes, rats were allowed to become re-acclimatised to their new surroundings for at least 2 days (3 nights) before being used for any experimental purposes. Food and water were freely available to all animals until the day of the experiment. Room temperature and lighting cycle were not strictly controlled in the holding area, although ambient temperature was maintained at approximately 21^{o}C. The rats were exposed to a natural

day/night cycle, and all experiments were performed between 1030 and 1530 hours under constant laboratory lighting. Body temperature was maintained at 37°C, using heat lamps where necessary.

1.2 Standard surgical animal preparation: For the necessary surgical preparation the animals were placed in a perspex box into which an anaesthetic gas mixture (70% nitrous oxide: 30% oxygen, containing 5% halothane) was flowing. Anaesthesia was maintained by means of a face mask through which a 1% halothane mixture was delivered.

Small incisions (1 cm, or less) were made at the groin on either side of the animal and, taking care not to injure nerve fibres in this area, the femoral vessels were exposed. Polythene cannulae (Portex; internal diameter 0.58 mm, external diameter 0.96 mm) 15 cm long and filled with heparinised Ringer's solution (10 I.U/ml) were inserted retrogradely into both femoral arteries and one femoral vein to a depth of 3 cm from the point of entry. The patency of the cannulae was tested before the protruding ends were blocked with sealed needles. The cannulae were tied in place and the wounds sutured closed, infiltrated with local anaesthetic, and covered with gauze pads. A plaster of Paris bandage (Gypsona, width 7.5 cm) was applied around the lower abdomen, pelvis and hindquarters, thus immobilising the rear legs. The plaster and feet were taped to a lead weight to completely immobilise the lower parts of the animal. Anaesthesia was

then withdrawn. All experiments were performed on conscious rats.

1.3 Experimental sample analysis: Common to all techniques used is the requirement for liquid scintillation analysis of blood-borne tracer and the measurement of tracer accumulation in brain tissues at the end of the experimental period, for which quantitative autoradiographic techniques were used.

1.3.1 Liquid scintillation analysis (LSA): Plasma samples containing ^{14}C labelled tracer were pipetted into 1 ml of distilled water in plastic scintillation vials. The water was drawn up into the pipette and expelled into the vial several times to rinse out thoroughly any residual tracer and to ensure that any dead space in the tip contained only minimal amounts of dilute plasma.

Whole blood samples containing tracer were bleached with the addition of 0.5 ml hydrogen peroxide (27.5%w/v) to reduce the possibility of colour quenching. Vials were left at room temperature for at least 2 hours for the bleaching to proceed.

To each counting vial, containing either plasma or whole blood samples, was added 10 ml of a proprietary scintillation cocktail (Packard Instagel), chosen for its miscibility with aqueous samples. The vials were placed in a refrigerated scintillation counter and allowed to equilibrate to 4°C before each sample sample was counted for 4 minutes. Three temporally

spaced counting periods were used in the calculation of counts per minute. The raw count data were converted to disintegrations per minute, using the external standard channels ratio method (Peng, 1977) together with a standard quench correction calibration curve. The accuracy of the computerised correction was monitored in two vials from each experiment which were subjected to the internal standard method (Peng, 1977).

1.3.2 Preparation of autoradiograms from brain sections: At the end of the pre-determined period (see appropriate protocol description) the rats were killed by decapitation. Dissecting from the supra-occipital bone, the dorsal cranium was removed, the underlying dura reflected, and the whole brain removed intact to be frozen in isopentane pre-cooled to $-45^{\circ}C$. The time from sacrifice to freezing of the brain did not exceed 3 minutes. The brain was mounted in cryo-matrix by its rostral extremities on to a microtome chuck over a bed of solid CO_2. Sections of the brain, 20 microns thick, were cut on a rotary microtome in a cryostat maintained at a maximum temperature of $-22^{\circ}C$. Of the serial sections cut, 3 consecutive sections were picked up on to thin glass coverslips and rapidly dried on a hotplate ($60^{\circ}C$), whilst the intervening 10 sections were discarded. The coverslips were glued into position on thin card and, together with a set of epoxy resin standards (Spurr's Resin containing 0 - 1880 $\mu Ci/g$ tissue equivalents of ^{14}C-benzoic acid) (see Appendix I), were applied to x-ray film (Kodak SB-5) in a light-tight

X-ray cassette for between 7 and 14 days routinely (longer if tissue ^{14}C levels were predictably low). The exposed plates were processed according to the manufacturer's instructions, following standard radiological practice.

^{14}C was the isotopic label used throughout because the energy of emission is high enough to penetrate to the film emulsion, but low enough to allow good spatial resolution.

1.3.3 Quantitative densitometric image analysis:

Analysis of the resultant images on the X-ray film was performed using a computer-based micro-densitometer (Quantimet 720, Cambridge Instruments). The effective field of the video detection system covered an area of film approximately 18 cm^2 which, magnified by a factor of 5.6, was projected on to a visual display unit. The field could be varied down to an area of 2.5×10^{-5} cm^2 in decrements of 5×10^{-3} cm along each axis. The densitometer digitised the X-ray images in 'picture point' units, one picture point covering 2×10^{-5} cm^2, allocating each picture point to one of 64 grey levels on a densitometric scale according to the optical density of the film in the light path of each picture point. The range of absolute optical densities covered by the 64 levels could be varied. The densities at each picture point within the field were added to give the integrated density of the area and each density measurement was the mean of 3 scans.

For each area of brain, 12 bilateral density readings

were made on 6 consecutive sections in which the structures could be defined anatomically by reference to stereotaxic atlases (Zeman and Innes, 1963; Konig and Klippel, 1967; Pellegrino et al. 1970), with the aid of cresyl violet stained sections where necessary. Where the brain had been subjected to a unilateral stimulus or treatment (e.g., unilateral lesions), 6 readings were taken on each side with care being exercised to avoid side-to-side differences arising from asymmetrical mounting of the brain. The size of the measuring frame was variable and ranged from 0.02 mm^2 to 0.25 mm^2, depending upon the size of the region being examined, but was constant for each region between animals. The optical densities of the images resulting from exposure to the plastic standards were measured, providing a calibration curve of density versus ^{14}C concentration from which the tissue isotope concentration corresponding to the measured optical density could be quantified in absolute terms.

1.4 Statistical analysis: The autoradiographic techniques which are used exclusively in these studies present particular problems for statistical analysis. The multiple, non-independent measurements made in diverse brain areas in each individual animal and the multiple treatments to be compared with a distinct control group demand sophisticated statistical approaches. The Bonferroni correction factor, which has been proposed to eliminate the error involved in using multiple t-testing (Wallenstein et al. 1980), may be used to

allow comparison with control values in many regions and comparisons within these regions across multiple treatments. However, a degree of experimental bias could be introduced merely by reducing the number of structures analysed, to increase the probability of statistical significance. The most appropriate statistical analysis in many of the experiments performed was found to be the Scheffé extension of analysis of variance tests (Scheffé, 1959) which permits multiple treatment comparisons to be made. The analysis of variance is less susceptible than the t-test to errors arising from its repeated use for many brain areas in the same animals. Furthermore, the Scheffé analysis is a rather conservative test and, therefore, statistical significance accrues greater credibility.

The analysis of blood flow and glucose utilisation data together required the development of a novel statistical model (McCulloch et al. 1982) to meet the particular demands of analysis. This innovative statistical test is discussed in the appropriate commentary.

2. Autoradiographic Techniques

As these techniques are relatively recent innovations, some of the theory behind the experimental protocols will be presented. Most of the experiments to be described employed the 2-deoxyglucose (2-DG) technique, and greatest emphasis is placed in that section.

2.1 Measurement of local cerebral blood flow: Local cerebral tissue perfusion was quantified using the [^{14}C] iodoantipyrine technique (Sakurada et al. 1978).

2.1.1 Theory: Iodoantipyrine is a tracer which is freely diffusible across the blood-brain interface in the cerebral vessel wall. The brain tissue to blood partition coefficient is 0.79. Because the tracer has no diffusion limitations, the equation devised by Kety (Kety, 1960) relating tissue accumulation of tracers to blood flow is wholly applicable:-

$$Ci(T) = \lambda K \int_{o}^{T} CA \quad e^{-K(T-t)} \quad dt$$

where Ci is the tissue concentration of tracer at time T, λ is the partition coefficient, and the integral is the area under the curve described by the function; arterial tracer concentrations (C_A) against time from onset of tracer circulation. The constant K is an entity which incorporates tissue blood flow per unit weight and a constant, m, representing the extent of blood-tissue equilibrium which is attained in a single passage:

$$K = {}^{mF}/_{W\lambda}$$

With a freely diffusible tracer m = 1 and K becomes flow (F) per unit weight (W) times the partition coefficient. Thus, by measuring the accumulation of ^{14}C iodoantipyrine in regions of the brain on sacrifice and by sampling the arterial input of tracer adequately throughout the experiment, flow may be derived.

To maximise the power of the procedures employed to resolve differences in regional blood flow, arterial tracer concentrations must not reach constant levels. This is illustrated in the extreme case where a relatively long time is interposed between a pulse of [^{14}C] iodoantipyrine and sacrifice of the animal. Under these circumstances the accumulation of isotope in different brain regions is no longer flow-limited, but becomes limited only by the partition coefficient (indeed, it is by this method that the partition coefficient may be determined). Thus, uniform concentrations of tracer are found in areas known to be served by widely differing perfusion levels, e.g., white matter and neocortex. Although the experimental time interval used in these studies was never sufficient to allow full equilibrium to occur, it was found that if tracer was infused at a constant rate over 1 minute, the plasma history of tracer levels showed a trend towards equilibrium during the last few seconds which could at least result in areas of similarly high perfusion becoming indistinguishable. This problem may be overcome in two ways; either the experimental time can be shortened so that the measurement takes place over the rising phase of the input function, or a continuously increasing rate of infusion may be employed to eliminate the possibility of reaching steady-state blood isotope concentrations. The former option was found to be impractical because of the difficulty in collecting a large enough number

of samples in a short time to adequately define the input function. The latter option proved more acceptable and became the standard approach.

2.1.2 Practice: Rats were prepared in the standard manner for conscious animals and the measurement of local cerebral blood flow was performed, using the freely diffusible tracer $[^{14}C]$-labelled iodoantipyrine in conjunction with quantitative autoradiography (Sakurada et al. 1978). Immediately prior to the measurement, a sample of arterial blood was withdrawn from the femoral artery for the determination of blood gas status. Over a 1 minute period the tracer (50 μCi in 0.5 ml saline) was infused into the femoral vein at a constantly increasing rate while 18 timed sample drops of whole arterial blood were collected from a freely flowing femoral arterial cannula on to pre-weighed filter discs. At the end of the experiment the rats were sacrificed and the brains processed for quantitative autoradiography. The filter discs were tightly capped in scintillation vials to prevent evaporation, and re-weighed. From the weight difference and the specific gravity of whole blood (1.05) the sample volume was calculated. The vials were treated and subjected to LSA as described. The concentration of ^{14}C tracer measured in each sample, corrected for sample volume, permitted an accurate history of arterial tracer levels at each time point of over the minute to be derived.

Local tissue $[^{14}C]$ concentrations were measured

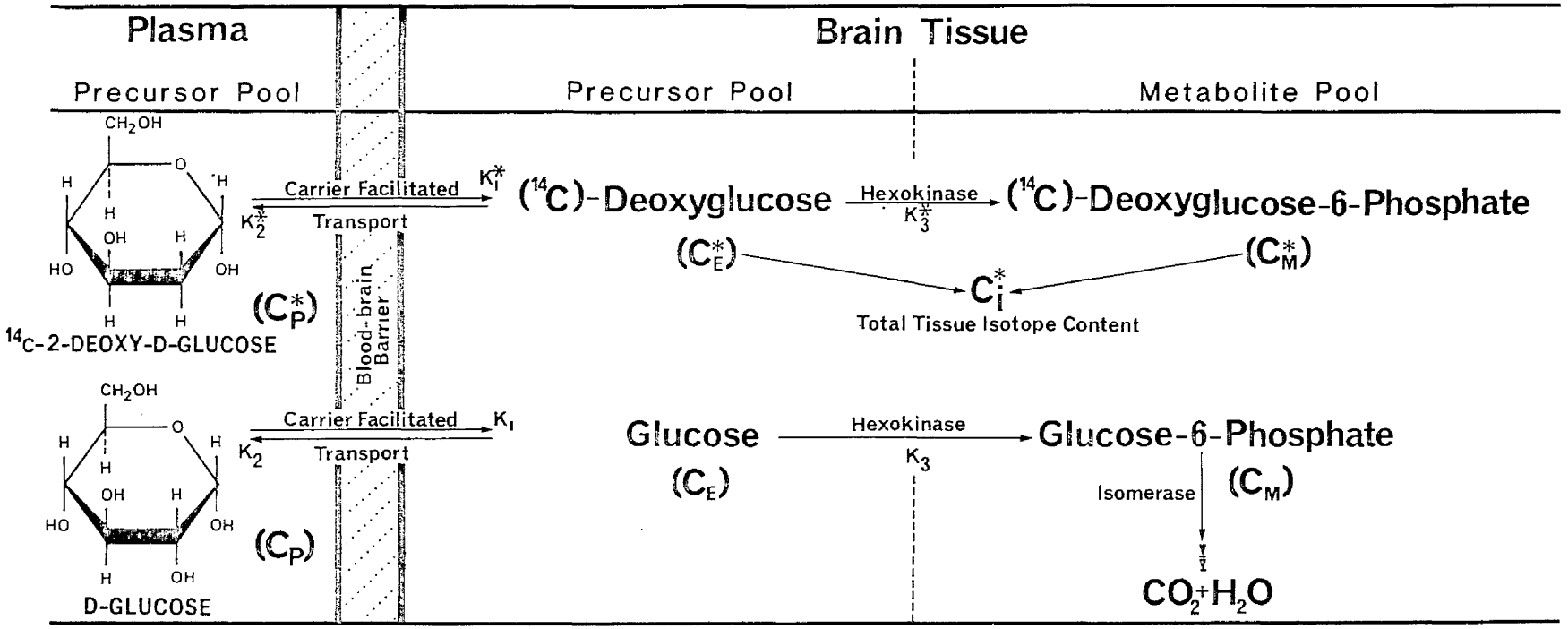
by quantitative densitometric analysis in 39 regions of the brain relative to standards of known $[^{14}C]$ concentration. Blood flow was calculated from the arterial tracer profile and tissue $[^{14}C]$ levels, employing the equation derived by Kety (1960) for the measurement of tissue perfusion, using freely diffusible tracers.

2.2 The measurement of local cerebral glucose utilisation: Local cerebral glucose utilisation was quantified using the $[^{14}C]$-2-deoxyglucose (2-DG) technique (Sokoloff et al. 1977).

2.2.1 Theory: Under normal, non-fasting conditions the adult brain uses glucose as the principal substrate for the production of high energy phosphate molecules with which to fuel the many biochemical reactions necessary for cerebral function (Kety, 1948). Although some glucose is stored within the tissues of the brain in the form of glycogen, the relatively fixed volume afforded by the cranial bones and the requirement for 3g of water for every 1g of glycogen severely limits the availability of this energy source to meet total energy requirements (Cahill and Aoki, 1980). Thus, for the maintenance of function the brain requires the continuous supply of blood-borne glucose.

2-Deoxy-D-glucose, a structural analogue of native glucose differing only in the hydrogen atom attached to the second carbon in place of a hydroxyl group (Fig. 2), was shown many years ago to be a highly successful inhibitor of glucose uptake and phosphorylation in

Figure 2



Diagrammatic representation of the theoretical model with structural formulae of D-glucose and 2-deoxy-D-glucose (Sokoloff et al. 1977).

cerebral tissue slices. This inhibition was found to be accompanied by an accumulation of 2-deoxyglucose-6-phosphate (2-DG-6-P) in the tissue (Tower, 1958). Although the significance of these results was not appreciated at the time, this experiment supplies the basis for the usefulness of the method developed later by Sokoloff and his colleagues (Sokoloff et al. 1977). Sokoloff's technique relies upon the systems of brain uptake and phosphorylation being common to both glucose and deoxyglucose, and the inability of the isomerase enzyme which converts glucose-6-phosphate to act upon the anomalous structure of 2-DG-6-P which, as a result, accumulates in the tissue. If the relevant rate constants are known for the steps which take blood-borne glucose and 2-DG to phosphorylation in the tissue, and the blood concentration of both substances is known, then the rate at which 2-DG-6-P accumulates may be directly related in a predeterminable fashion to the rate at which glucose itself passes through the preliminary stages of the glycolytic pathway, provided that the levels of deoxyglucose present are never sufficient to act as a competitive inhibitor.

On the basis of the theoretical model (Fig. 2) formulated by Sokoloff and his colleagues (Sokoloff et al. 1977), an operational equation was derived (equation 30 in original text) which described rates of cerebral glucose utilisation in terms of the concentration of $[^{14}C]$-2-deoxyglucose and glucose in arterial plasma over the experimental period (C_p^* and C_p) and the concentration

of tracers found within the CNS (C_i^*) (Fig. 3). The distribution of tracer between the plasma and brain tissue compartments is governed by the kinetic rate constants for the movement of $[^{14}C]$-2-deoxyglucose into and out of the CNS (k_1^* and k_2^*), its phosphorylation to 2-deoxyglucose-6-phosphate (k_3^*) and a composite constant which, in simplistic terms, reflects the relative preference of glucose transport and enzyme system for glucose as opposed to 2-deoxyglucose. Despite the rigour with which these constants were derived, a relatively high degree of measurement error is associated with each of them. However, within the confines of the original model and the operational equation, these errors become so small as to be negligible if a suitably long time interval is interspaced between a pulse injection of 2-deoxyglucose and ultimate sacrifice of the animal.

The main limitations of the technique as an approach to the measurement of functional activity are the degree of restraint necessary for plasma sampling and the long time constant required for the experimental measurement. The time between the pulse of labelled deoxyglucose and sacrifice of the animal must be sufficiently long to minimise potential errors arising from uncertainty in the model (particularly the values for rate constants), but must be short enough to limit the depleting effects upon 2-DG-6-P of the small amounts of phosphatase known to be present in cerebral tissue. An experimental time course

Figure 3

The operational equation

$$R_i = \frac{C_i^*(\tau) - k_1^* e^{-(k_2^*+k_3^*)\tau} \int_o^\tau C_p^* e^{(k_2^*+k_3^*)t}\, dt}{K \cdot \left[\int_o^\tau (C_p^*/C_p)dt - e^{(k_2^*+k_3^*)\tau} \cdot \int_o^\tau (C_p^*/C_p)\, e^{(k_2^*+k_3^*)t}\, dt\right]}$$

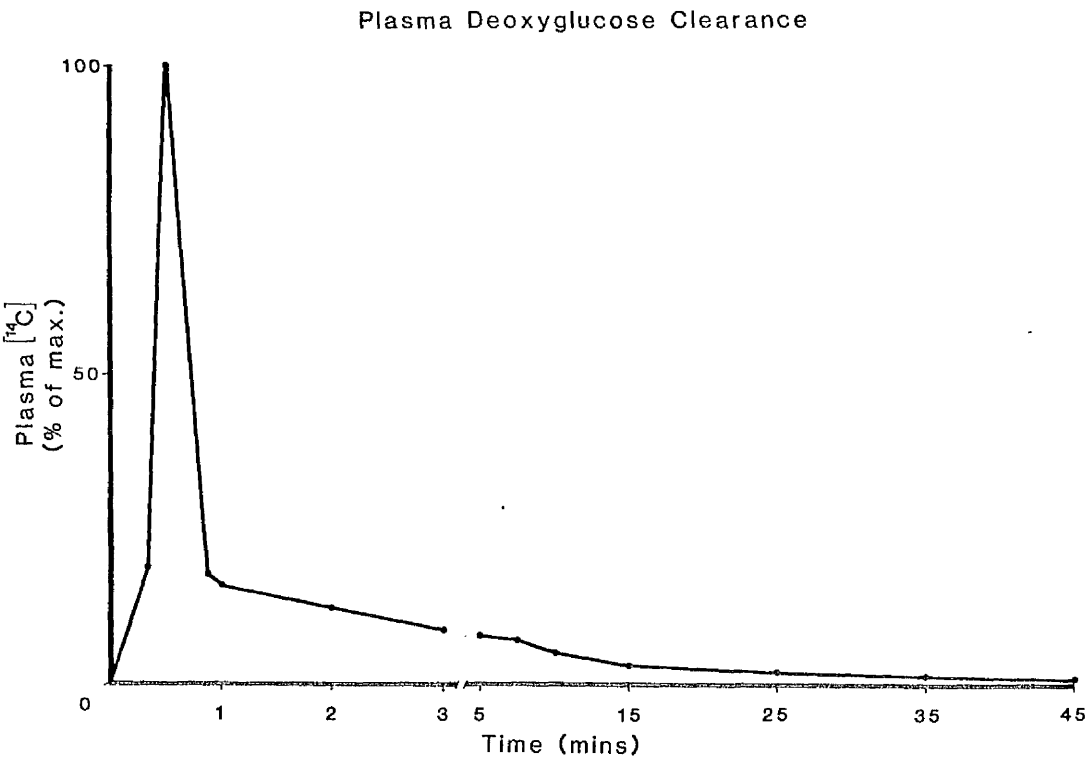
where R_i is the rate of glucose consumption in any tissue sample of total isotope concentration C_i^*, C_p^* and C_p are the concentrations of $[^{14}C]$-deoxyglucose and glucose in plasma; and k_1^*, k_2^* and k_3^* are the enzymatic rate constants for bidirection transport between plasma and tissue precursor pools, and for phosphorylation of deoxyglucose by hexokinase.
The "lumped constant", K, is composed of the relative distribution spaces for deoxyglucose and glucose; the ratio of the Michaelis - Menten constants and maximal velocities of hexokinase for deoxyglucose and glucose and the fraction of glucose-6-phosphate which continues via the glycolytic pathway for further metabolism (Sokoloff et al. 1977).

of 45 minutes has been found to meet both of these requirements. However, the experimental design as described originally by Sokoloff and colleagues is such that events which occur within the first 10 minutes after intravenous isotope delivery carry greater weight in their effects upon measured glucose utilisation by virtue of the shape of the arterial input function (Fig. 4). Thus, for example, drugs need not be infused throughout the whole 45 minutes to ensure steady-state conditions. Nevertheless, great care must be taken to ensure that the relative timings of drugs and tracer administration are meaningful in terms of the previously reported time course of action for the specific agents.

A further limitation of particular relevance in the studies reported here is the inability of the model as it stands at present to accommodate hyperglycaemia as a reaction to stress. As the classical GABA antagonist agents, picrotoxin and bicuculline, are active in promoting seizure-like responses which are undoubtedly stressful, these substances could not be used with the fully quantitative approach in conscious animals.

Efforts have been made to overcome some of the limiting factors in the original experimental protocol and the relatively sophisticated hardware required for analysis. Such modifications are examined critically in a later section.

Figure 4



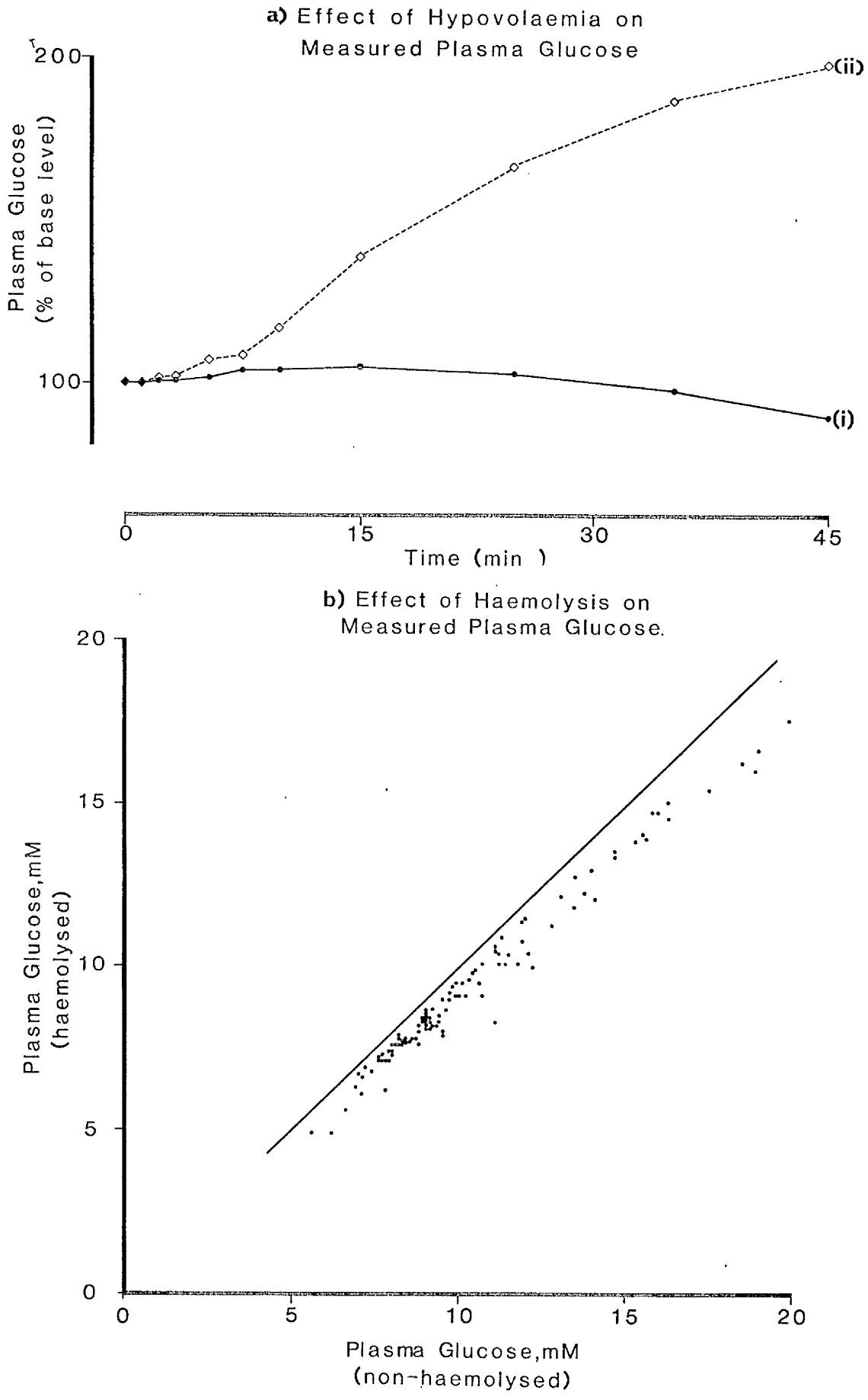
Time course of ^{14}C deoxyglucose concentrations in arterial plasma from a typical control, conscious rat injected with isotope over 30 seconds.

2.2.2 Practice: Measurement of local cerebral glucose utilisation was performed on conscious rats, prepared as described previously, using the [^{14}C]-2-deoxyglucose method in conjunction with quantitative autoradiography (Sokoloff et al. 1977).

Each measurement was initiated with an intravenous infusion of [^{14}C]-2-deoxy-D-glucose (125 μCi/kg) dissolved in saline, injected at a constant rate over 30 seconds. A total of 14 timed samples of arterial blood were withdrawn from the femoral cannula and collected into plastic centrifuge tubes over the subsequent 45 minutes according to a predetermined schedule. Five samples were taken over the first minute of the experiment at 15 second intervals, the third sample being co-ordinated with the end of isotope delivery, thus allowing an accurate determination of the history of label in plasma during the time at which it is changing most rapidly (see Fig. 4). As the rate of clearance of isotope from the plasma becomes progressively less, the time between successive samples was increased with little loss of accuracy to the profile (Fig. 4). In order to minimise hypovolaemic stress resulting from loss of body fluids and the compromise of the 2-DG methodology (see Fig. 5a), each sample was limited to around 75 μl of whole blood and was replaced by a similar volume of Ringer's solution.

The cell fraction of each arterial sample was separated from the plasma by centrifugation as quickly after withdrawal as was possible in order to minimise

Figure 5



For legend see over.

Figure 5a. Plasma glucose levels in conscious rats over 45 minutes where (i) whole blood samples did not exceed 80 µl and (ii) where whole blood samples were 160 µl. Both animals started from a plasma glucose level of 8.5 mM. In rat (i), the plasma glucose remained within 5% of the starting levels, whereas in rat (ii) plasma glucose rose to levels almost twice that of starting levels.

Figure 5b. Measured plasma glucose levels in two consecutive samples of blood taken from conscious rats. One sample was centrifuged immediately (non-haemolysed sample), whilst the other was left for 1 minute at room temperature before centrifugation, resulting in a degree of haemolysis. In every case the measured plasma glucose level of haemolysed blood was less than that in non-haemolysed blood (all points on the plot fall below the line of identity). This potential measurement error could result in an under-estimation in cerebral glucose utilisation.

haemolysis (see Fig. 5b). Aliquots from each plasma sample (20 µl) were pipetted from the centrifuge tubes into 1 ml of distilled water in 14 plastic disposable scintillation vials. A further aliquot of each plasma sample (10 µl) was pipetted into a semi-automated glucose analyser (Beckman). The principle which allows this machine to measure glucose concentrations is based upon a glucose oxidase enzyme assay. A sample is delivered manually into a chamber containing glucose oxidase enzyme reagent and an electrode which is sensitive to oxygen concentrations. The rate at which oxygen is consumed in the reaction

$$\text{glucose} + O_2 \xrightarrow[H_2O]{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

is monitored by the electrode and is proportional to the initial glucose concentration (Kadish, 1965). Glucose levels in the sample may therefore be derived from the measurement of the rate of oxygen depletion. The digital readout indicates plasma glucose levels in µmol/ml.

At the end of the 45 minute period, the rats were killed by decapitation and the brains processed for quantitative autoradiography (see appropriate Methods section).

Rates of glucose utilisation within chosen anatomically discrete and functionally diverse regions of the rat brain were calculated from post-mortem levels of ^{14}C measured in tissue by quantitative autoradiography which, together with the plasma histories of ^{14}C and glucose concentrations, were applied to the operational

equation for the technique as characterised by Sokoloff and his colleagues (Sokoloff et al. 1977) (see Fig. 3). The values for the rate constants k_1^*, k_2^*, k_3^* were 0.189, 0.245 and 0.052, respectively, for grey matter areas, and 0.079, 0.133 and 0.02 for white matter areas. The value for the "lumped constant" was 0.483 (Sokoloff et al. 1977). These values were used throughout the studies cited, where plasma glucose levels were relatively constant throughout the experiments. A protocol was available in cases where the glucose in plasma changed dramatically over the measurement period, but it was never necessary to use it (Savaki et al. 1980).

To illustrate in a simplistic fashion how the data collected during the course of an experiment is applied to the determination of glucose utilisation, the operational equation may be written thus:

$$\text{Rate of glucose utilisation} = \frac{\text{Total Tissue } ^{14}C - \text{Tissue 2-DG}}{\text{Lumped Constant} \times \text{Integrated plasma specific activity}}$$

where total ^{14}C in tissue is measured densitometrically, and tissue 2-DG and integrated plasma activity are calculated from plasma histories of 2-DG and glucose. It is worthy of note that quantification of the methodology by this approach gives an index of the rate of glucose utilisation, and not merely of the accumulation of tracer which, at the instant of kill, will be present both in the form of 2-deoxyglucose and 2-deoxyglucose-6-phosphate (the numerator of the equation). The implications of this are addressed subsequently.

2.2.3 An appraisal of 2-DG methodological modifications: Simplifying technical modifications have been advanced as feasible alternatives to the rigorous 2-DG approach. Of these, the most widely used are a) the use of free-running animals, which often precludes the plasma sampling protocol and involves a different route for tracer administration (Meibach et al. 1980, for review) and b) the use of optical density ratios in the semi-quantitative analysis of autoradiograms (Brown and Wolfson, 1978; Collins, 1978; Meibach et al. 1980). The theoretical justification for these modified approaches rests upon the fundamental assumptions that a) on sacrifice of the animals all isotope in the brain tissue is in the form of phosphorylated 2-DG with no residual free 2-DG, and b) that a simple correlation exists between brain tissue glucose use, total isotope concentrations on sacrifice of the animal, and the optical densities of resultant images on photographic emulsion. These assumptions were tested with a view to evaluating the two simplified approaches for possible use in subsequent studies.

A total of 16 animals were prepared in the standard manner for conscious animals. Following tracer amounts of $[^{14}C]$-2-DG delivered via either the intravenous (n=8), intraperitoneal (n=5) or subcutaneous route (n=3), timed arterial blood samples were withdrawn and treated according to the usual 2-DG experimental protocol. In the i.v. group, 3 animals were hyperglycaemic with plasma glucose levels of 17 $\pm$ 1 mM

compared to a range of 7.2 to 10.8 mM in the normoglycaemic groups. At 45 minutes after the delivery of the isotope the brains were removed from the i.v. group and processed for autoradiography. From the resultant autoradiograms cerebral glucose utilisation was calculated for neocortex (mean of 5 cortical areas) and white matter (mean of 4 areas), as well as several other grey matter areas including median raphe nucleus, substantia nigra pars compacta, and septal nucleus.

Using a simple rearrangement of Sokoloff's operational equation (Fig. 6) and the experimentally generated arterial plasma histories of glucose and $[^{14}C]$-2-DG, levels of unphosphorylated $[^{14}C]$-2-DG in the CNS were calculated and the influences examined of a) the rate of regional glucose utilisation itself, b) the duration of the experiment, c) the route by which the tracer is administered, and d) moderate hyperglycaemia. Under all conditions the values used for the required rate constants were those derived for the Sokoloff model (Sokoloff et al. 1977).

Four identical sets of ^{14}C-containing epoxy resin standards were exposed on X-ray film for 3, 5 and 10 day exposure periods. The standards had been calibrated previously (see Appendix 1) and ranged from 0 to 1880 μCi/g. At the end of the chosen exposure time the plates were processed for tissue autoradiographs, but, in this instance, stricter than usual control was maintained over the temperature of the processing chemicals and the time spent at each of the stages

Figure 6

The concentration in the CNS of unphosphorylated 2-deoxyglucose C_{DG} can be described by

$$C_{DG(T)} = k_1^* e^{-(k_2^*+k_3^*)\tau} \int_o^\tau C_p^* e^{(k_2^*+k_3^*)t}\, dt$$

and simple rearrangement in the "operational equation" (equation 1) yields

$$C_{i(T)}^* = R_i \,.\, K \,.\, \left[\int_o^\tau (C_p^*/C_p)dt - e^{(k_2^*+k_3^*)\tau} \,.\, \int_o^\tau (C_p^*/C_p)\, e^{(k_2^*+k_3^*)t}\, dt\right]$$

$$+ k_1^* e^{-(k_2^*+k_3^*)\tau} \int_o^\tau C_p^* e^{(k_2^*+k_3^*)t}\, dt$$

where the nomenclature is that described in Figure 3. Thus, the proportion of the total amount of radioisotope present as unphosphorylated DG (i.e., $C_{DG}(T)/C_i^*(T)$) can be calculated from the rate of glucose utilisation and the arterial plasma histories of 2-deoxyglucose and glucose over the measurement period, if the values of various constants are known.

(developer, stop bath, fixatives and wash). The optical densities of the resultant images were measured on the densitometer which had been calibrated against a range of filters (Kodak, Wratten gelatin filters) rising from 0.1 to 2.0 in steps of 0.1 optical density units. Optical density ratios of neocortex to white matter were calculated, using the tissue concentrations generated autoradiographically from the normoglycaemic i.v. injected rats and the corresponding densities from the standard isotope functions at the 3 exposure times.

2.3 Measurement of local cerebral protein synthesis: A recently developed autoradiographic technique permits local rates of protein synthesis in the nervous system to be measured (Smith et al. 1980). The use of this method was limited in these studies; therefore, its description will be limited.

2.3.1 Theory: This approach is very similar to the 2-DG technique in that a rigorous, empirically based model has been developed to describe the kinetics of transfer and incorporation of a tracer, in this case $[^{14}C]$ labelled leucine, from blood into tissue. A mathematically derived operational equation defines the rate of leucine incorporation into protein in terms of the relevant rate constants and experimentally measurable plasma and brain tissue variables.

The model is greatly simplified by the fact that the metabolic pathways which determine the fate of the

essential amino acid, leucine, are such that using [carboxyl-^{14}C] leucine ensures that metabolically degraded tracer is lost from the system and the [^{14}C] tissue pool comprises only free leucine and leucine incorporated into protein. By allowing the experiment to run for a relatively long time after the pulse injection of [^{14}C] leucine, free labelled leucine in the tissue approaches zero. As for the 2-DG model, brain uptake of the tracer is intimately linked to the relative concentrations of labelled and endogenous leucine in the blood. For full quantification of the method, therefore, an amino acid analyser is required.

2.3.2 Practice: Conscious animals, prepared in the standard manner, were injected with a pulse of ^{14}C leucine (25 μCi) over 30 seconds. Timed arterial plasma samples were withdrawn over the subsequent 60 minutes and plasma taken for liquid scintillation analysis. No amino acid analysis was available. At the end of the hour the animals were killed and the brains processed for autoradiography. Analysis of the autoradiograms was performed in terms of total tissue isotope concentrations, and these were compared with concentrations of label found after similar treatments in 2-DG experiments.

3. Experiments Performed

The autoradiographic techniques described were used in conjunction with three approaches to allow examination of possible GABAergic influences upon cerebral function:

a) Following systemic administration of agents known to interact pharmacologically with GABA systems.

b) Following specific lesions of a known GABAergic pathway and subsequent pharmacological challenge.

c) Following localised injection of the neurotransmitter analogue into a brain area of known GABAergic output.

3.1 Systemic drug administration: Four pharmacological agents were studied for their central effects when administered intravenously; two GABA agonists, muscimol and THIP, picrotoxin, a potent GABA antagonist, and diazepam which, as a benzodiazepine, interacts with GABA systems. All agents were dissolved in saline directly, with the exception of diazepam which was first dissolved in 0.1 N HCl, the pH adjusted to 7.4, then added to suitably concentrated saline to make the final injectate isotonic.

Muscimol and THIP were injected into the femoral vein 20 minutes prior to the initiation of the 2-DG experiment at doses of 0.15 to 5.0 mg/kg (n=15) and 1.0 to 10.0 mg/kg (n=12), respectively.

Picrotoxin was injected i.v. five minutes prior to

the 2-deoxyglucose experiment at a dose of 2 mg/kg (n=1). The animal which received this dose died at 25 minutes after the delivery of tracer. The convulsions suffered by this conscious animal were deemed to contravene the conditions for experimental practice laid down by the Home Office, and no further such experiments were performed.

Diazepam was injected i.v. 10 minutes before the 2-deoxyglucose experiment at doses of 0.1, 0.3 and 1.0 mg/kg (n=15).

Contemporaneous control experiments were performed in which animals received saline alone. Diazepam controls received appropriate vehicle but, as no difference was discernible from saline controls, statistical comparisons were made to the same saline control group for all systemic drug studies.

Local cerebral glucose utilisation was calculated in 60 functionally diverse regions of the brain. This relatively large number reflects an appreciation of the ubiquity of cerebral GABA systems, all of which will be influenced by systemic administration of pharmacological agents. No quantification of glucose use in the brain of the convulsive animal (picrotoxin 2 mg/kg) was performed because the experimental model was severely compromised by the stress-induced hyperglycaemia and early demise of the animal.

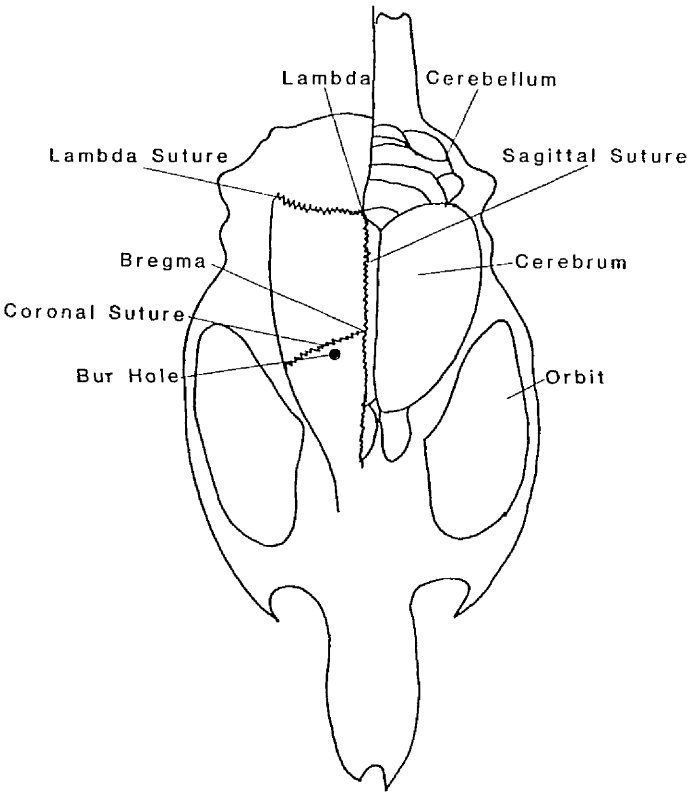
Local cerebral blood flow was measured in a total of 16 rats following 0.5 or 1.5 mg/kg muscimol or 0.5 ml of saline alone injected into the femoral vein 30

minutes prior to the initiation of the measurement. The timing was such that the median point of the blood flow experiments coincided with that of the 2-DG experiments in the time after delivery of muscimol.

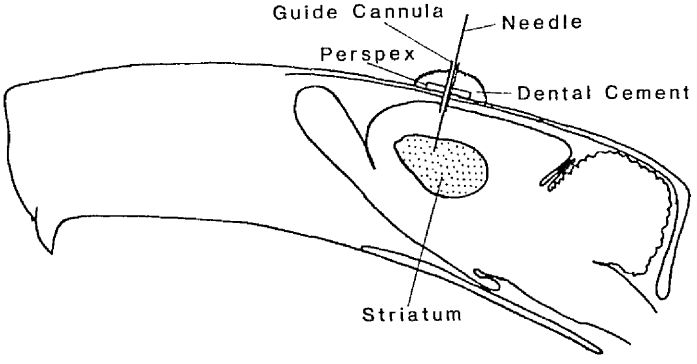
3.2 Striatal lesioning with kainic acid: Rats were anaesthetised with halothane and mounted by ear bars into a Trent-Wells small mammal stereotaxic frame. A midline incision was made to expose the dorsal aspect of the skull. The position of bregma was determined with the aid of an operating microscope (X40) and, using this as a reference point, a small hole 0.9 mm in diameter was drilled 1.5 mm anterior and 2.2 mm lateral from bregma (Fig. 7). The dura was carefully punctured with a hooked needle. A stainles steel cannula, diameter 0.3 mm, was lowered 5.5 mm ventral from bregma (all co-ordinates modified from Divac et al. 1978). In 32 rats, 2 µl of mock CSF (Leusen, 1950) containing 2 µg of kainic acid and adjusted to pH 7.2 was injected into the caudate nucleus from a Terumo microsyringe (10 µl) attached to a Harvard infusion pump at a constant rate over 5 minutes. A small amount of the inert dye, Evans blue, was added to the injectate so that it could be easily visualised at the end of the cannula. In one animal the kainic acid injection was made slightly more medially, although otherwise at the same level, thus injecting the neurotoxin into the septal nucleus. Mock CSF alone, pH 7.2, was injected into 21 animals which formed the control groups. The needle was left in situ for 10 minutes before being slowly withdrawn. The skull

Figure 7

a) Positioning of drill hole for intrastriatal injection of kainic acid in anaesthetised rats



b) Positioning of guide cannula & needle for intrastriatal injection in conscious rats



Positioning of intracranial intervention for lesioning and intrastriatal injection relative to the skull and underlying brain.

was reconstituted with bone cement, the wound held closed with sutures and local anaesthetic applied to the scalp before the animals were left to recover. The cannula was checked before and after the injection to ensure that it remained fully patent.

Four kainic acid-injected rats were taken at 7 days after the lesioning procedure and prepared in the standard manner for conscious rats. At least 2 hours elapsed before the experiments proceeded. Various physiological parameters were measured from these conscious animals; blood gas tension, arterial blood pressure and heart rate, haematocrit, and plasma glucose levels.

Ten days after the intracerebral injections, 6 of the control group together with 7 lesioned animals (including one septal lesion) were prepared as detailed before, and local cerebral glucose utilisation was determined using the 2-DG method. A further group of lesioned animals (n=4) were allowed to recover for 30 days before the 2-deoxyglucose experiments.

Autoradiograms were prepared from frozen tissue section and analysed as described previously. However, the cryostat protocol was altered slightly in that, at each level, a fourth section was taken to be fixed and stained for subsequent histological examination. Glucose use was calculated from the autoradiograms for a number of structures, discrete measurements being made both ipsilateral and contralateral to the site of kainic acid injection. Analysis was performed by comparing

the respective sides of lesioned and control animals.

In a parallel study, 5 animals which had been lesioned and 5 which had received intrastriatal CSF were perfusion-fixed at 10 days with formaldehyde/acetic acid/methanol (1:1:8) and the brains subjected to conventional light microscopy. The cryostat sections from the 2-DG experimental animals allowed a direct comparison to be made between the autoradiograms and the extent of damage, although the resolution was much less than in perfusion fixed material.

Two of the lesioned animals were taken 10 days after the induction of the lesion, and uptake of [1-^{14}C] leucine into various brain regions was analysed densitometrically as described.

In the remaining 10 lesioned and 10 sham-lesioned animals, 5 from each of the two groups were ascribed at random to one of two subgroupings. At 10 days after the neurosurgical intervention, the subgroups were injected with either muscimol (1.5 mg/kg) 20 minutes before the initiation of the 2-DG experiment, or the dopaminergic agonist, apomorphine (1.0 mg/kg), 10 minutes before the start. Thus, four treatments were studied; sham lesion plus muscimol, sham lesion plus apomorphine, striatal lesion plus muscimol, striatal lesion plus apomorphine. Standard 2-DG protocol was followed. Autoradiographs were analysed to differentiate ipsilateral from contralateral effects, and analysis of the data allowed comparisons between control and drug treatment in sham-lesioned and lesioned

groups and within drug treatments between sham and lesioned groups. Both comparisons were made ipsilateral and contralateral to the intervention site.

3.3 Intrastriatal drug administration: A group of 15 rats were prepared in the standard manner for conscious animal preparation to the stage at which the restraining plaster cast was applied. With anaesthesia maintained, they were mounted in a stereotaxic frame as previously and, using bregma as the zero reference point, a hole was drilled in the skull at the same co-ordinates used in the lesion studies. A stainless steel guide cannula, external diameter 0.6 mm, had previously been positioned through a hole in a square of perspex (25 mm^2) such that 2 mm of its length protruded in one direction and 5 mm in the other direction. Glue was amassed to form a mound from the perspex along the longer protrusion (see Fig. 7). For each guide cannula, a needle was made which fitted exactly through the bore of the guide cannula (0.3 mm). When pushed completely home, this needle penetrated 5.5 mm from the base of the perspex platform.

The guide cannula was lowered into the bur hole until the flat perspex base was in contact with the skull. At this point the end of the cannula penetrated less than 1 mm into cerebral cortex. The skull around the perspex was thoroughly dried before dental cement was applied to hold the cannula in position (Fig. 7). Local anaesthetic was administered

to the scalp which was sutured over the cement once it had hardened. Small sponges were used to keep the scalp a little away from the protruding cannula, and to absorb any seepage from the wound which could clot and block the cannula. The animals were mounted on to the lead weights and the anaesthesia was withdrawn. Because the operating time was approximately one hour (cf. less than 30 minutes for standard conscious animal preparation), and because of greater possible surgical trauma, the rats were left for at least 4 hours before further manipulation.

After the recovery period the injection needle was placed in position, attached to a microsyringe. This action comprised two steps. Firstly, the needle was placed in the guide cannula such that it would not protrude into brain. The proximity of the experimenter necessitated by this manoeuvre invariably caused the rat to become agitated; therefore, a period of 10 minutes was allowed for the rat to settle down before the needle was pushed home and the injection started. Over two minutes, 2 µl of CSF alone (n=5), or containing 100 ng muscimol (n=5) or 500 ng muscimol (n=5), was injected by hand at a relatively constant rate. All solutions contained Evans blue to allow visualisation of the injection site in the brain sections cut subsequently. Fifteen minutes after the start of the intrastriatal injection, 2-DG experiments were initiated. Standard 2-DG protocol was followed. When sectioning the brain through the caudate nucleus, a fourth section was taken

at each level for histological examination.

Analysis of the data was performed by comparing the respective sides of CSF control and treated brains.

4. Drugs and Tracers

Several pharmacological agents and radioactive tracers were used in these studies.

The GABA agonist, muscimol, was supplied by Fluka AG, Buchs SG (Switzerland); THIP was a gift from Professor Krøgsgaard-Larsen; diazepam (Ro 05-2807) was a gift from Roche Ltd.; apomorphine-Hcl and kainic acid were supplied by Sigma.

All radiolabelled tracers were supplied by New England Nuclear (Boston, Massachusetts); [^{14}C]-2-iodoantipyrine (specific activity 49.8 mCi/mmol); [^{14}C] leucine (specific activity 54 mCi/mmol).

CHAPTER III

RESULTS AND COMMENTARY

1. An Appraisal of 2-DG Modifications

1.1 General results and observations: Physiological parameters, body temperature, blood gas tensions, haematocrit and mean arterial blood pressure were the same in all groups. Plasma glucose levels varied between individuals (from 7.2 to 10.8 mM), despite the care taken to standardise possible stress factors arising from surgery. However, no pattern of elevated plasma glucose concentration and no statistical significance were found between the designated "normoglycaemic" groups. The handling required for i.p. and s.c. injections of tracer resulted in no apparent stress-related hyperglycaemia.

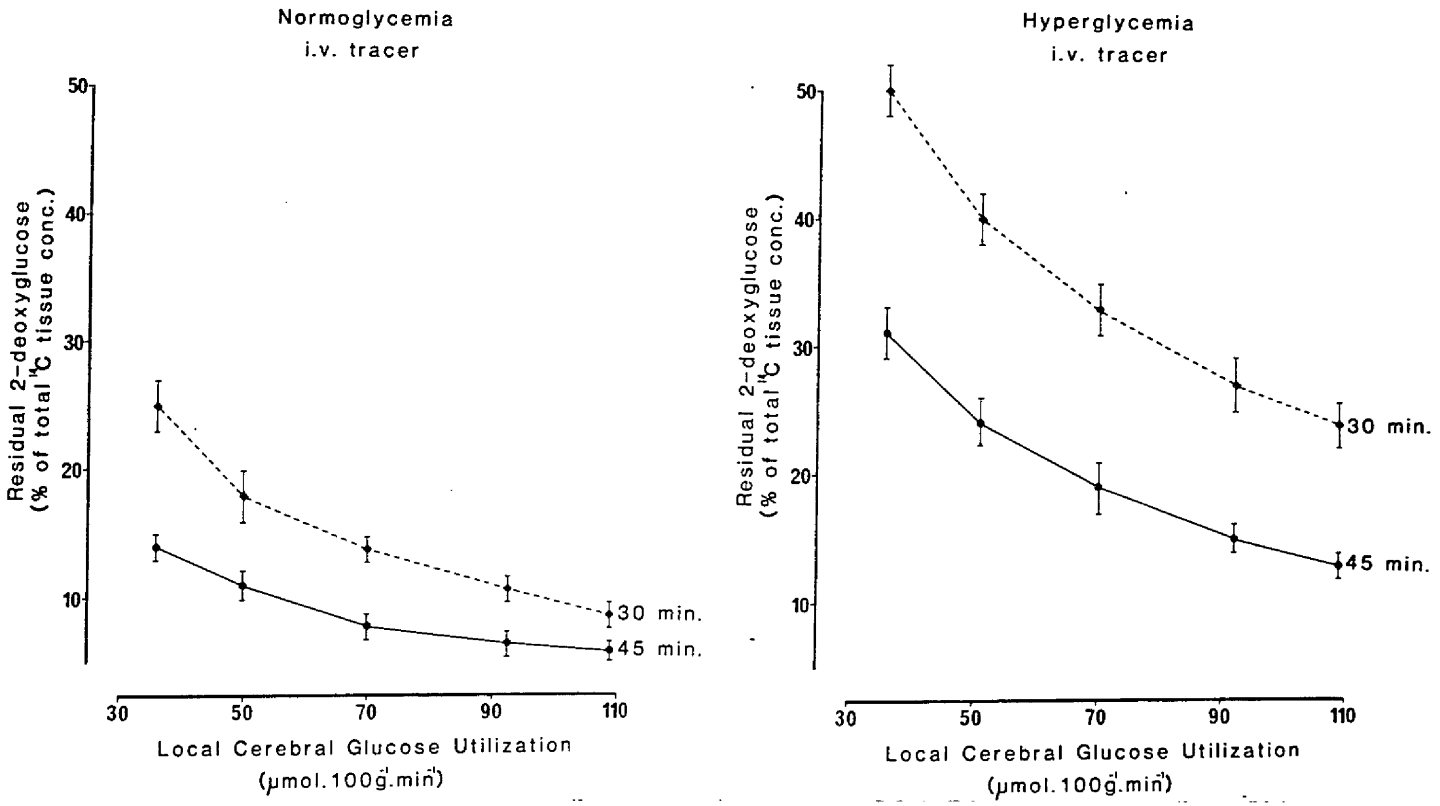
From autoradiographic analysis of the brains, glucose use was calculated using the operational equation and found to be 109 ± 5 μmol/100g/min (mean ± SEM) for neocortex, and 35 ± 2 μmol/100g/min for white matter. The other grey matter areas fell between these two extremes at 92 ± 2, 70 ± 3 and 51 ± 2 μmol/100g/min for median raphe, substantia nigra pars compacta and septal nucleus, respectively.

The total levels of $[^{14}C]$ in the individual brains of the i.v. tracer group, measured by quantitative

autoradiography, ranged from 750 μCi/g in neocortex and 250 μCi/g in white matter areas of the animal with the lowest plasma glucose (7.2 mM) to 520 μCi/g in neocortex and 125 μCi/g in white matter of the animal with the highest plasma glucose (10.8 mM). Despite these marked differences in tissue isotope concentrations, the relatively low variability in mean glucose use calculated for these areas is indicative of a high degree of accuracy and reproducibility in the method when applied in the rigorous manner described originally by Sokoloff (Sokoloff et al. 1977).

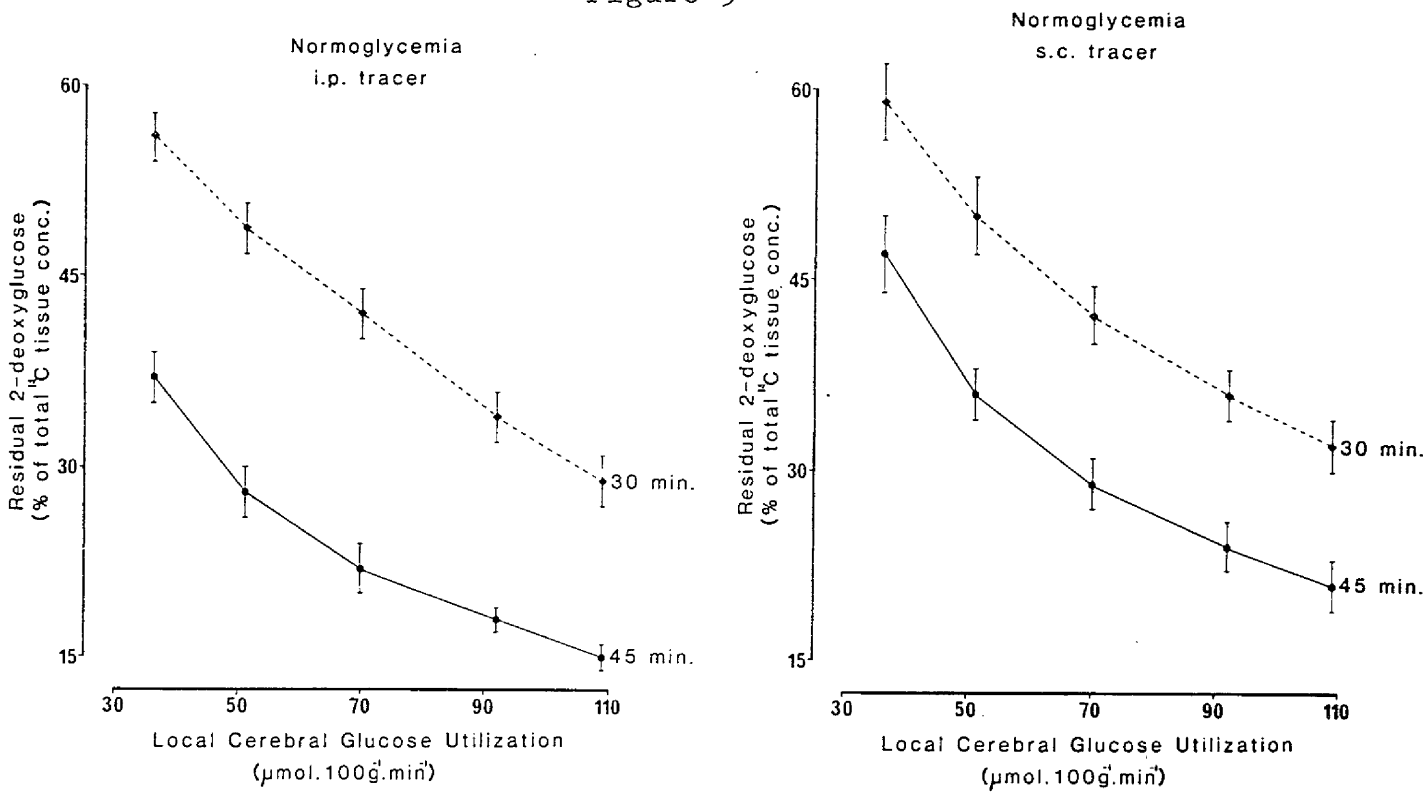
1.2 Errors associated with the assumption of total 2-DG phosphorylation: The calculated fraction of total $[^{14}C]$ present at the time of sacrifice in the form of residual unphosphorylated 2-DG in any given brain region was found to be inversely related to the rate of glucose utilisation in that area. In areas of relatively high glucose use, such as neocortex, only a small fraction of the total radioactivity (6 ± 1%) remained in the form of unphosphorylated 2-DG, 45 minutes after the intravenous injection of the isotope (Fig. 8a). However, in areas of relatively low rates of glucose utilisation - for example, white matter tracts at the lowest extreme - the calculated residual 2-DG concentrations were found to be markedly higher (13 ± 1%) (Fig. 8a). Moreover, in all areas examined, irrespective of rates of glucose use, the fraction of unphosphorylated 2-DG was calculated to increase substantially if the measurement period was shortened from

Figure 8



Calculated levels of unphosphorylated 2-deoxyglucose as a fraction of the total isotope found following intravenous tracer delivery within regions of the rat brain with different rates of glucose utilisation. Solid line, levels calculated to be present 45 minutes after pulse injection of tracer: dashed line, levels calculated 30 minutes after injection in (a) normoglycaemic and (b) hyperglycaemic rats.

Figure 9



Calculated levels of unphosphorylated 2-deoxyglucose as a fraction of the total isotope found following (a) intraperitoneal and (b) subcutaneous tracer delivery within grey and white matter regions of the rat brain. Solid line, levels calculated to be present 45 minutes after pulse injection of tracer; dashed line, levels calculated 30 minutes after injection.

45 to 30 minutes (Fig. 8a) or if the animals were subjected to moderate hyperglycaemia (Fig. 8b). Thus, in white matter areas of moderately hyperglycaemic rats, almost 50% of the total tissue $[^{14}C]$ was calculated to be present in the form of unphosphorylated 2-DG after a shortened 30 minute measurement period (Fig. 8b). If no account was to be taken of these residual 2-DG levels in white matter, and glucose use calculated on the assumption of complete phosphorylation of the tracer, then an over-estimation in the order of 100% would be incurred.

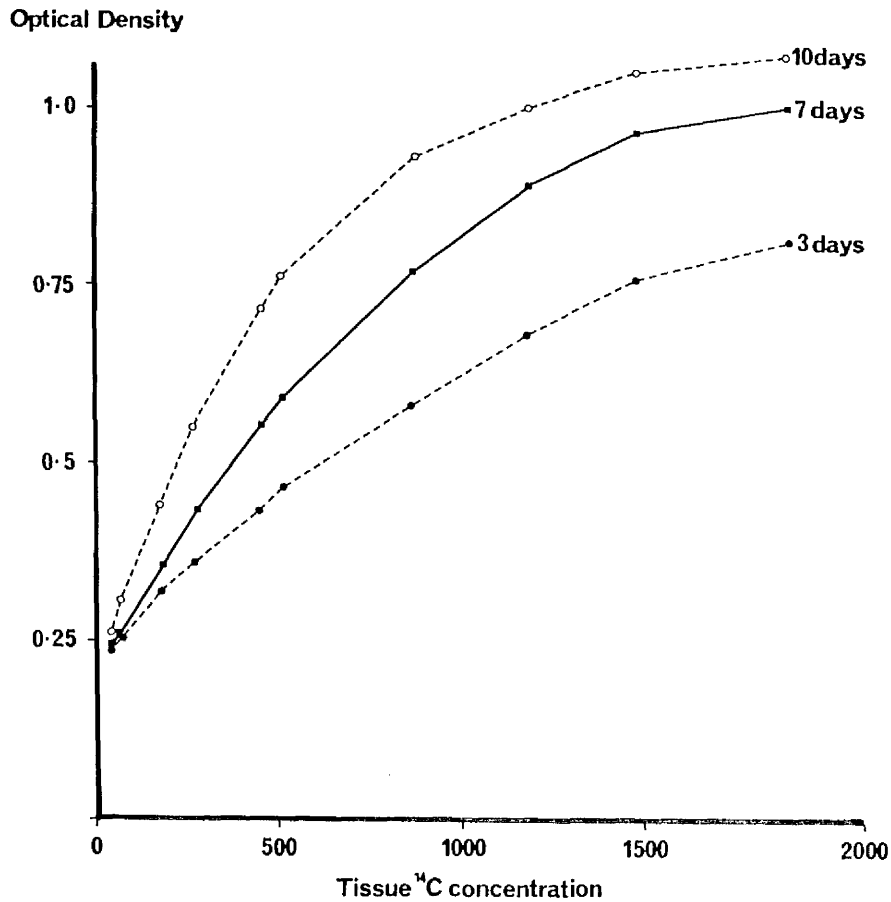
The route by which the isotopic tracer was administered was found to have a profound influence upon the fraction of total $[^{14}C]$ found in brain tissue in the form of unphosphorylated 2-DG. Following both i.p. and s.c. injections of the tracer, a very much larger part of the total tissue $[^{14}C]$ was calculated to be present as residual 2-DG (approximately double) than when the i.v. route was used (Figs. 9a,b), irrespective of rates of glucose utilisation. Once again the fraction was found to be considerably larger when the measurement period was reduced to 30 minutes (Figs. 9a,b).

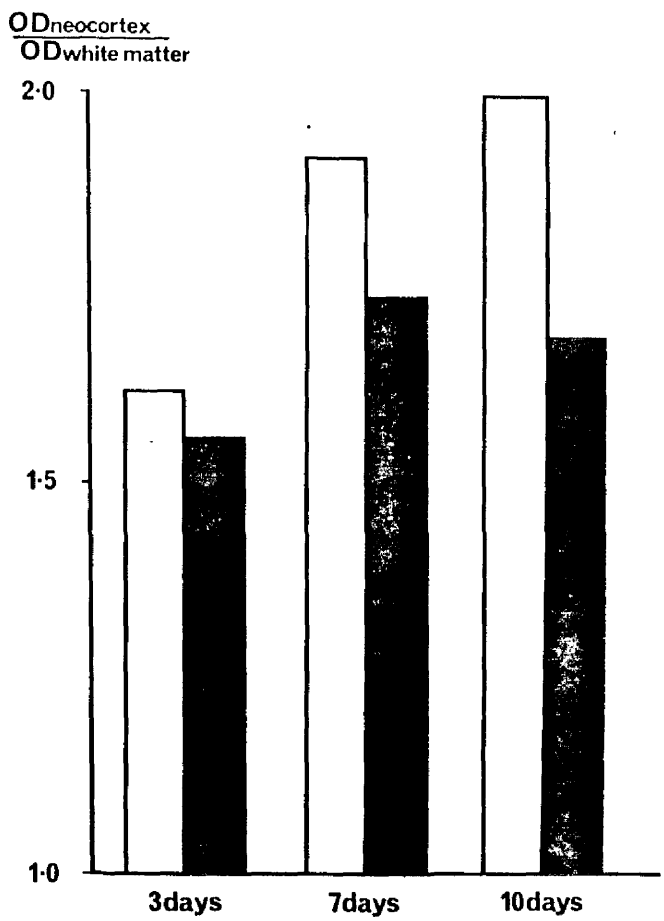
1.3 Errors associated with the use of optical density ratios in autoradiogram analysis: The relationship between $[^{14}C]$ levels in the calibrated epoxy resin standards and absolute optical densities of the photographic images they produce was found to be a non-linear function (Fig. 10a). Furthermore, the characteristics

of the curve, initial slope and point of inflection, were observed to alter radically merely from an increase in the time for which the films were exposed (Fig. 10a). The variability in optical densities measured from the four sets of standards at each time was so small that it could not be represented on the graphs.

The effects of this curvilinear relationship upon optical density ratios of grey matter (neocortex) to white matter were profound. In the animal where plasma glucose concentrations were at the lower end of the normoglycaemic range, the ratios of optical densities resulting from tissue $[^{14}C]$ concentrations of 750 μCi/g (grey matter) and 250 μCi/g (white matter), taken from the standard exposure curves, was increased by around 12% by a lengthening of the exposure time from 3 to 7 days (Fig. 10b). However, the difference between the ratios at 3 and 10 days exposure was lower, at around 8% (Fig. 10b). If tissue isotope concentrations were decreased in a global manner - for example, as the result of slightly elevated plasma glucose concentrations - the pattern of optical density ratios was radically altered. In the animal from the upper end of the normoglycaemic range in these experiments, final tissue isotope levels were lower both in grey matter (520 μCi/g) and white matter (125 μCi/g). The ratio of grey to white matter optical densities taken from the standard exposure curves at these tissue levels of isotope increased by around 19% from 3 to 7 days exposure, and again to 26% between 3 and 10 days exposure (Fig. 10b). Moreover,

Figure 10





For legend see over.

Legend to Figure 10

10a. Optical densities of autoradiographic images resulting from tissue isotope concentrations of 44 to 1880 μCi/g following 3, 7 or 10 days' exposure to X-ray film (Kodak SB-5).

10b. Optical density ratios arising from fixed ratios of tissue isotope content. Hollow bars, tissue isotope ratio of 3:1; filled bars, tissue isotope ratio of 4:1. Both of these ratios were derived from values for neocortex and white matter in normoglycaemic animals.

a comparison of the difference in ratios elicited by changing final tissue isotope levels in tandem showed an increase at each exposure time, from 4% at 3 days to 17% at 10 days (Fig. 10b).

1.4 Commentary: Within the constraints of the model developed by Sokoloff and colleagues (Sokoloff et al. 1977), the 2-DG quantitative autoradiographic method has provided an accurate assessment of functional activity in the brain as reflected in the rate of glucose utilisation, under a variety of experimental conditions (see Sokoloff, 1981 and McCulloch, 1982 for full reviews). However, at the present time, modified forms of the original, rigorous approach to the application of 2-DG methods are in widespread use. Although each of these modifications has sought to reduce the complexity of the experimental protocol and the hardware required for full quantification, or to expand the applicability of the technique to other than restrained animals (Brown and Wolfson, 1978; Nelson et al. 1978; Collins, 1978; Herkenham, 1981; Meibach et al. 1980, for full discussion), it is far from assured that all of these experimental models provide reliable estimates of local cerebral glucose utilisation under the wide variety of circumstances in which they are commonly applied.

The desire to couple glucose utilisation as an index of cerebral function with behaviour studies to examine the externalised consequence of that functional activity is thwarted to a large extent if the restrained animal preparation is used. However, free-running animals make the intravenous injection of tracer and subsequent sampling of plasma glucose and 2-DG concentrations technically very much more difficult. The use

of free-running animals and the abandonment of any effort to sample arterial blood variables is based on the assumption that, at the instant of sacrifice, all tracer found within the CNS is present in the form of 2-DG-6-P, with no residual free 2-DG. Justification for this assumption arises from biochemical analysis of brain tissue subsequent to sacrifice (Meibach et al. 1980; Sagar and Snodgrass, 1980; Dunn et al. 1980). In contrast, the results from the studies described here show that, using radiolabelled 2-DG, the measurement of ^{14}C accumulation, even if the time from a pulse delivery is relatively long, does not totally reflect 2-DG-6-P because unphosphorylated 2-DG in the brain approaches zero in an exponential fashion. The values of residual 2-DG calculated from plasma data, and using the rearranged operational equation, are in excellent agreement with those values derived directly from biochemical analysis of tissue in which chemical activity is stopped at the instant of sacrifice with a "freeze-blower" device (Hawkins and Miller, 1978). Unless an instantaneous method is used, the rapid flux of substrates through the glycolytic pathway which occurs post mortem (Lowry et al. 1964) will tend to result in an underestimation of unphosphorylated 2-DG and, therefore, the evidence upon which the modified approach is based must remain questionable.

The contribution made by unphosphorylated 2-DG present at the instant of sacrifice to total isotope concentrations measured after death is an important

factor which must be accommodated if isotope accumulation is to be related ultimately to functional activity. The original highly rigorous approach allows the levels of residual 2-DG to be calculated from the experimentally generated plasma glucose and tracer profiles and the predetermined rate constants. Once quantified, this element may be subtracted from the total isotope content of tissue and the actual concentrations of 2-DG-6-P determined. However, despite the rigour of the model upon which the technique is based, the rate constants which feature in the operational equation are subject to a degree of error, although the uncertainty is minimised where the time between the pulse injection of 2-DG and sacrifice is relatively long and where residual 2-DG in tissue is relatively low, which is also time-dependent under steady state conditions. If the experimental time is shortened, or if residual 2-DG is elevated as is the case in hyperglycaemia or following i.p. or s.c. delivery of the tracer, then the certainty with which the calculation may be made will diminish. It is worthy of note in this context that if only the route of administration of tracer is altered whilst the requisite measurements are made of the plasma variables over a suitably long time (45 minutes), then the final rate of glucose utilisation calculated from the operational equation is relatively unaltered, particularly for areas of high glucose use. On the other hand, if no attempt is made to evaluate levels of unphosphorylated 2-DG on the assumption that all

tissue tracer is in the form of 2-DG-6-P, then the factors which we have shown to affect residual 2-DG, i.e., the route of tracer delivery, the time between tracer delivery and sacrifice, the intrinsic metabolic rate of the area studied and the relative state of plasma glucose, may singly, or in combination, severely compromise the putative indices of glucose utilisation using such methods.

Although the measurement of total isotope accumulation in tissue is most efficiently made from tissue samples suitably treated for pulse counting (Blackwood and Kapoor, 1979; Glick et al. 1979, 1980), the degree of resolution afforded by such an approach, in terms of discrete brain structures, will depend heavily upon the homogeneity of the sample and the competence and reproducibility of the dissection technique. It could prove extremely difficult, if not impossible, to adequately isolate discrete functional units from contamination of surrounding tissue or to detect heterogeneity of response within anatomical structures as have been described, for example, in the striatum (Edvinsson et al. 1982).

The advantage of autoradiographic procedures is that spatial distributions of isotope are preserved and may be visualised with photographic images, permitting a relatively high degree of resolution. Absolute quantification of autoradiograms is possible by comparing the optical density of images on processed photographic emulsion which arise from exposure to an

isotope source of known activity with the optical density of images arising from isotope held within tissue sections. From the relationship between optical density and the concentrations of the standards, the isotope levels in areas of tissue may be deduced. However, using the 2-DG technique, the general relationships which exist between the optical density of autoradiographic images, tissue isotope concentrations and, ultimately, levels of functional activity as reflected in the rate at which glucose is being used, have apparently been widely misinterpreted.

It has been proposed that the response to any treatment in terms of altered functional activity will be reflected in an altered ratio of optical densities of affected, relative to supposedly non-affected areas, such responses usually being reported as percentage change in the ratio from control (Brown and Wolfson, 1978; Collins, 1978; Herkenham, 1981; Meibach et al. 1980). The relative stability of glucose utilisation in white matter under a variety of circumstances has been exploited to provide the standard against which changes may be measured (Meibach et al. 1980). Two assumptions are implicit to this approach; firstly, that a linear relationship exists between isotope concentrations and the resultant images on processed photographic emulsion; secondly, that relative optical densities remain constant and that changes in relativities are a simple function of changes in the rate at which glucose is being utilised.

As has been shown, the true relationship between isotope concentrations and optical density is curvilinear with only a limited part of the function approximating to linearity. Moreover, the exact limits of this linear portion change with exposure time. In general terms, the ratio between the values of any two points on a curve is determined by the slope and length of the line which may be drawn between them. If, as is the case in this instance, the relationship is described by a non-linear function, optical density ratios will depend upon the position of the data relative to the point of inflection, together with the initial slope of the curve. At lower tissue isotope concentrations, both points will fall within the initial rising phase of the curve, but if tissue concentrations rise in parallel for both points, and that corresponding to grey matter passes the point of inflection, then the optical density ratio will decrease from the previous value. If both points fall above the point of inflection, a further decrease in the ratio is evident. Thus, changing tissue isotope concentrations in tandem, relative to the point of inflection, will in itself radically alter the calculated ratios completely independently of any underlying change in glucose utilisation. It is noteworthy that even if the relationship between isotope levels and image density was indeed linear throughout the entire range, the differential effects of even minimal hyperglycaemia upon uptake of 2-DG tracer into grey and white matter (750 decreasing by around 30% to

520 μCi/g in grey and 250 decreasing by 50% to 125 μCi/g in white matter) could result in a 40% change in uptake ratio without any change in underlying glucose utilisation.

Whilst errors associated with different lengths of film exposure are relatively easy to eliminate by strict experimental control (although this is not always mentioned in methods reports), it could prove much more difficult to control for absolute levels of isotope present in the brain on sacrifice of the animals. As has been illustrated, uptake of labelled 2-DG is influenced by the glycaemic state of the animal, but may also be affected by the metabolic rate of other body tissues and the total quantity of tracer injected. These variables will reflect an inter-animal difference which may be difficult to eliminate, and may even be exacerbated by experimental treatments which may prove to be inherently stressful (Brown and Wolfson, 1978). Furthermore, tissue isotope content comprises both phosphorylated 2-DG and residual 2-DG in the total measured densitometrically, and the changes described previously in the relative fraction of each in different brain areas, and in response to hyperglycaemia, cannot be accommodated within a protocol which uses an unquantified measure of total 2-DG uptake, as does the optical density ratios approach.

Although it is desirable that methods should be under continual scrutiny with a view to their possible improvement, the modifications to the original 2-DG

technique which have been introduced and are now in widespread use are based upon assumptions which, whilst appearing to simplify the approach, in fact introduce errors which are difficult to control, and therefore complicate the interpretation which may be placed on the final analysis. The rigorous experimental methodology developed by Sokoloff and colleagues must therefore remain the most appropriate approach.

2. Systemic Drug Administration

2.1 General results and observations: The administration of muscimol and THIP had little effect upon the measured cardiovascular and respiratory parameters. No significant changes were observed in pCO_2, pO_2, pH or plasma glucose levels. However, mean arterial blood pressure, which was 142 ± 6 mm Hg (mean ± SEM) in control animals, was significantly altered only with the highest concentrations of muscimol when it was reduced to 116 ± 9 mm Hg (mean ± SEM; $p < 0.05$). With increased doses of both agents, spontaneous motor activity and responsiveness to background noise were progressively reduced. The grooming, sniffing, and movement of the unrestrained head and forelimbs, which were displayed by the saline treated rats, were totally absent from animals which had received the highest concentrations of muscimol and THIP. These behavioural changes were relatively constant throughout the measurement period. Even at the highest doses employed the animals retained consciousness and, although sedated, remained capable of responding to tactile and auditory stimuli. The clearance characteristics of $[^{14}C]$ 2-DG from the blood were not systematically altered by either of the drug treatments, and thus the plasma histories of 2-DG levels were essentially similar in all groups.

The intravenous injection of 2 mg/kg of picrotoxin was followed within 2 minutes by the animal

displaying seizure-like activities. The eyes closed, ears flattened against the head and the animal arched its back upward and backward with forelimbs extended forward. These bouts, which lasted between 30 seconds and 2 minutes approximately, were interposed between periods of apparent quiescence of similar duration, but which became less frequent and less prolonged as the experiment progressed. Mean arterial blood pressure was elevated throughout to around 200 mm Hg, although during seizures this was further increased, with spikes of pressure in excess of 300 mm Hg. Plasma glucose levels rose to 19.2 mM during the experiment. The animal died 20 minutes after the injection of picrotoxin. Post mortem examination revealed both pulmonary and coronary congestion.

Although it was appreciated that an understanding of the effects of GABA antagonists upon regional cerebral glucose utilisation would form a necessary part of any comprehensive pharmacological study of GABA systems in the brain, these experiments were abandoned at an early stage on two counts. Firstly, the degree of suffering experienced by the one animal which was used was considered ethically unacceptable. Secondly, the stress-induced hyperglycaemia precluded any accurate quantification of glucose use in this animal model.

Following intravenous diazepam, blood pressure was again the only physiological variable which was found to alter from control levels. Although no change was

observed following 0.3 mg/kg, a dose of 1.0 mg/kg resulted in a reduction to 130 ± 5 mm Hg which was not statistically significant. No overt behavioural changes were observed following the administration of 0.3 mg/kg, but following 1.0 mg/kg sedation, with marked reduction in spontaneous motor activity, was observed. This apparently reduced state of arousal lasted for only around 30 minutes of the experimental measurement period, after which the animals became more responsive to the environment.

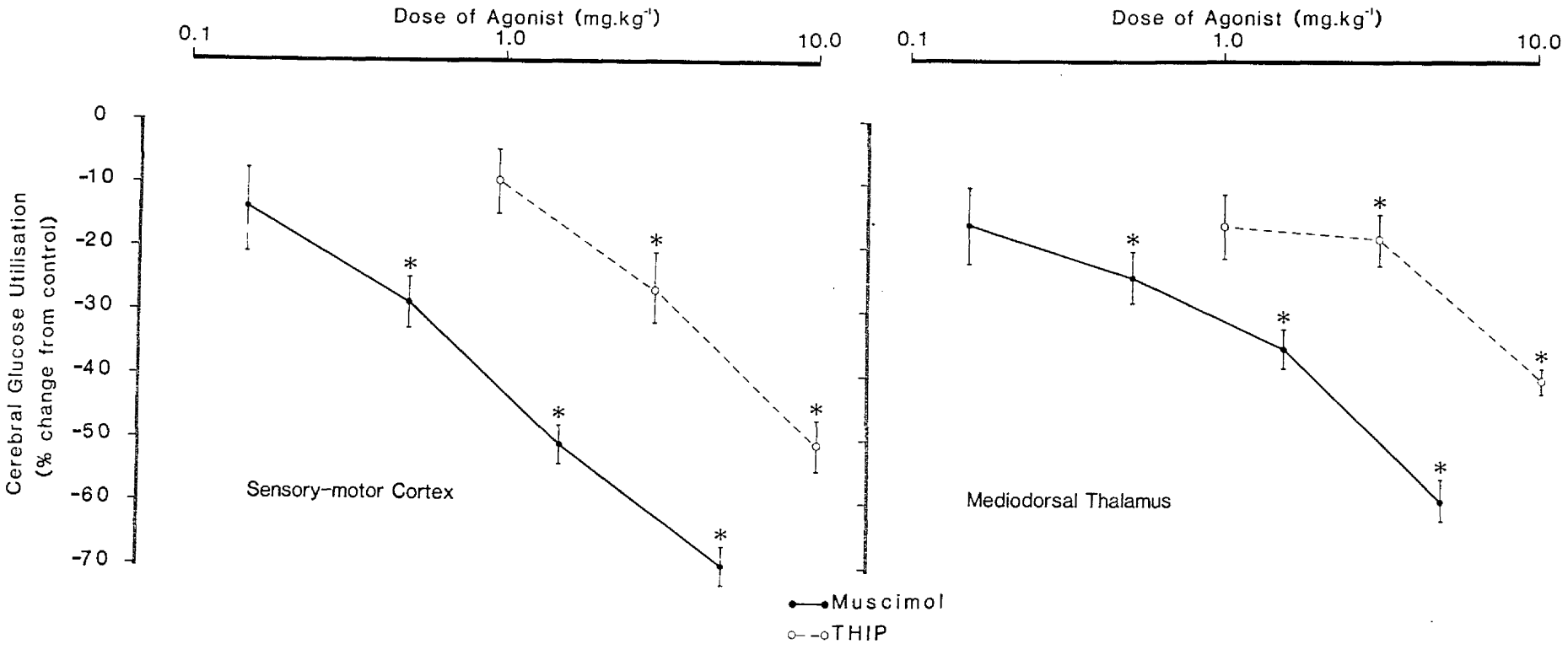
2.2 Local cerebral glucose utilisation following GABA agonist administration: The dose-dependent alterations in local cerebral glucose utilisation found to be associated with the intravenous administration of muscimol and THIP are presented in Appendix II. Following the administration of muscimol and THIP, reductions in glucose utilisation were evident throughout the brain. Muscimol was, in general, five to six times more potent in inducing the observed changes than was THIP. Only one area measured, the cerebellar vermis, failed to display a statistically significant alteration in glucose use with any concentration of muscimol or THIP used. No significant increases in glucose use were observed in any of the 60 regions examined at any dose of either of the two agonists.

Although, in essence, the observed responses were qualitatively similar in all of the brain regions investigated (i.e., generalised reductions in glucose use), analysis of the dose-response relationships

(Appendix II: Figs. 11-13) and visual inspection of the ^{14}C autoradiographic pattern from the brain sections (Fig. 14) provide evidence for pronounced inter-regional differences in the sensitivity of local cerebral glucose use to depression by GABAergic manipulation with muscimol and THIP. Analysis of the regional responses in terms of functional involvement revealed no clear pattern of unified responsiveness between functional sub-units (see Appendix II). Thus, for example, whilst auditory cortex and auditory thalamic nuclei (medial geniculate bodies) display at least a moderate degree of sensitivity to both agonists, more peripherally involved nuclei of the primary auditory pathways - for example, the cochlear nuclei - were very much less sensitive. Only within the primary visual system were all the areas involved prone to the action of the GABA agonists, notwithstanding the differential response of parts of the superior colliculus to muscimol and THIP (see later discussion).

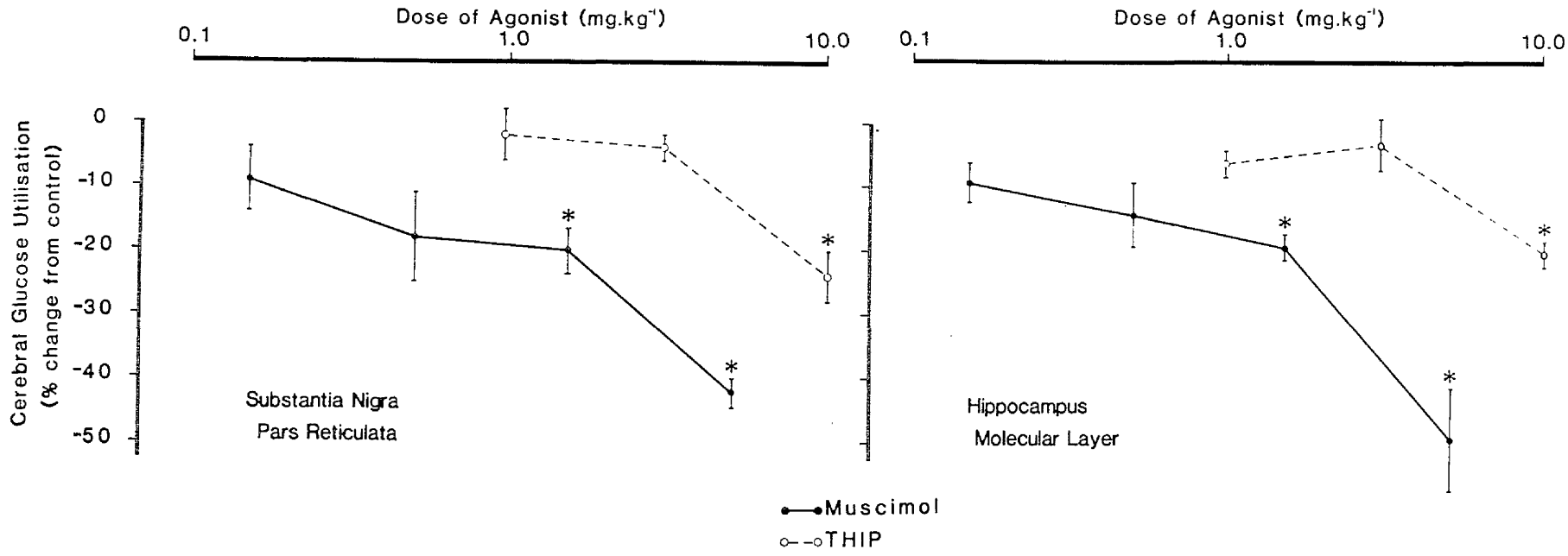
Ranking the 60 structures analysed according to absolute magnitude of response at stipulated dose thresholds (i.e., percentage change from control at 0.5 and 1.5 mg/kg muscimol and 3.0 and 10.0 mg/kg THIP, expressed as a mean of the two doses) (Tables 1 and 3), and also according to the calculated index of deviation from control across the dose groups for each structure (i.e., F ratio generated from analysis of variance) (Tables 2 and 4), revealed a hierarchy of sensitivity to GABAergic manipulation which was

Figure 11



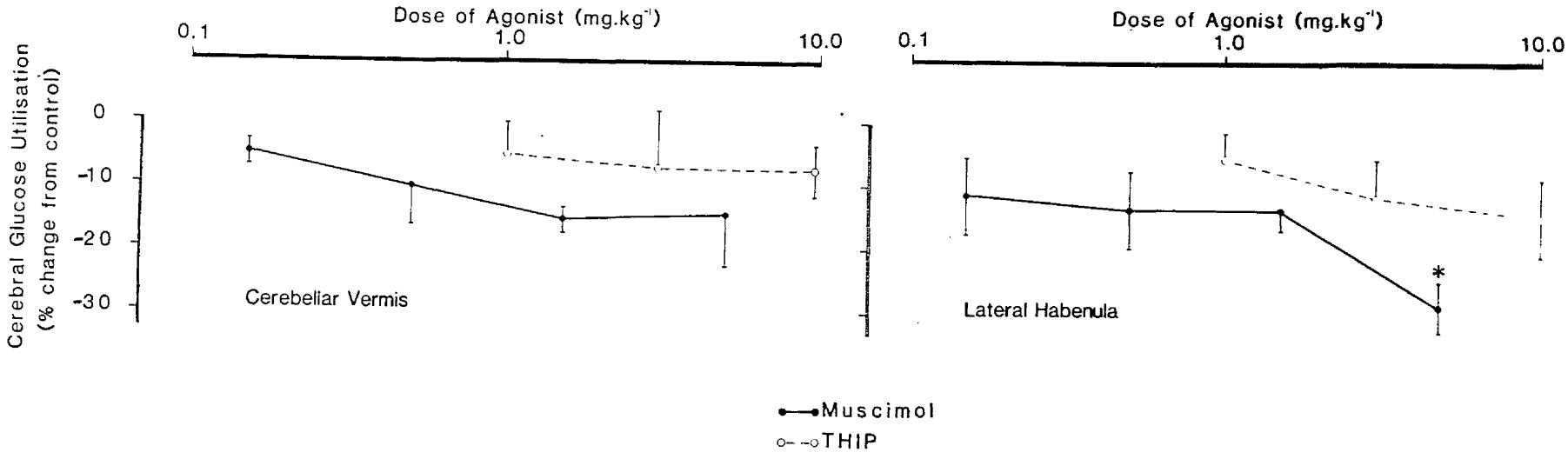
CNS regions in which glucose utilisation was highly sensitive to muscimol and THIP; sensory-motor cortex (left) and mediodorsal thalamus (right). Log dose-response curves of glucose utilisation following the intravenous administration of GABA agonists. Data are presented as mean percentage change from control ± SEM. * $p < 0.05$.

Figure 12



CNS regions in which glucose utilisation was moderately sensitive to muscimol and THIP; substantia nigra pars reticulata (left) and molecular layer of hippocampus (right). Log dose-response curves of glucose utilisation following the intravenous administration of GABA agonists. Data are presented as mean percentage change from control ± SEM. * $p < 0.05$.

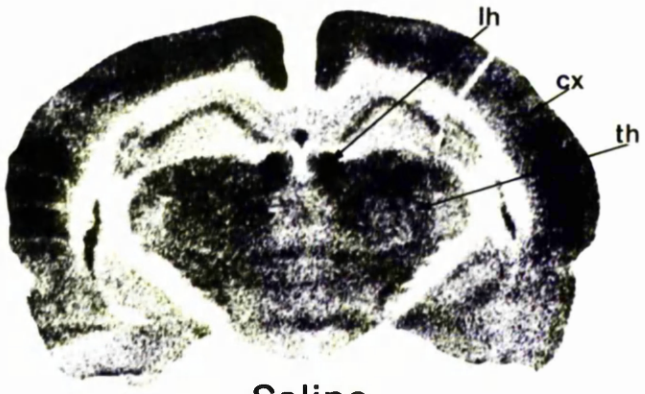
Figure 13



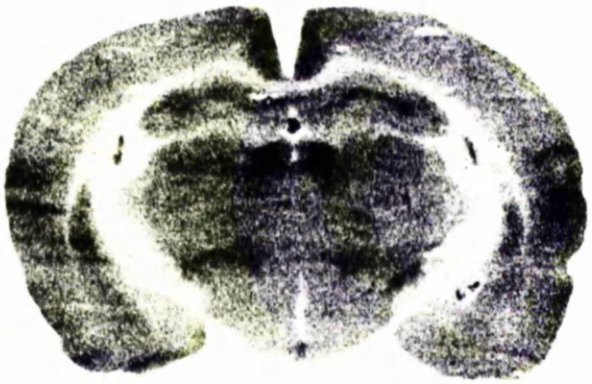
CNS regions in which glucose utilisation is relatively insensitive to muscimol and THIP; cerebellar vermis (left) and lateral habenula (right). Log dose-response curves of glucose utilisation following intravenous administration of GABA agonists. Data are presented as mean percentage change from control ± SEM. * $p < 0.05$.

Figure 14

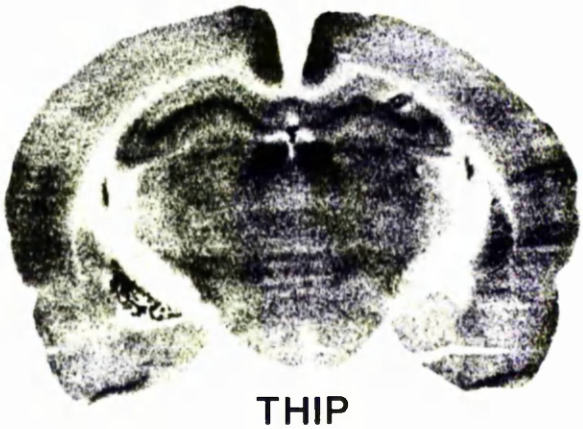
Autoradiograms prepared from coronal sections of rat brain at the level of the habenular nuclei.



Saline



Muscimol



THIP

For legend see over.

Legend to Figure 14

Autoradiograms prepared from coronal sections of rat brain at the level of the habenular nuclei.

Upper: Control animal. Cerebral cortex (cx), thalamus (th) and lateral habenula (lh) display similar levels of density.

Middle: Muscimol (1.5 $mg.kg^{-1}$) treated animal. Cerebral cortex and thalamus, whilst still of similar densities, both appear much less dense relative to lateral habenula.

Lower: THIP (10 $mg.kg^{-1}$) treated animal. A similar pattern to that observed following muscimol, with cortex and thalamus less dense relative to lateral habenula.

Glucose utilisation is proportional to relative optical density in these and subsequent autoradiograms.

Table 1

Hierarchy of Responsiveness to Muscimol.

Magnitude of Depression from Control (see legend).

Extremely Sensitive	Sensitive	Moderately Sensitive	Minimally Sensitive	Insensitive
Sensory-motor Cortex IV	Auditory Cortex IV	Ventrolateral Thalamus	Interpeduncular N.	Lateral Habenula
Parietal Cortex IV	Prefrontal Cortex	Dorsal Tegmental N.	Medial Raphe Nucleus	Vestibular Nucleus
Frontal Cortex IV	Lateral Geniculate	Auditory Cortex II	Medial Habenula	Red Nucleus
Lateral Caudate	Frontal Cortex II	Medial Caudate	Substantia Nigra	Hypothalamus
Sensory-motor Cortex VI	Frontal Cortex VI	Anterior Cingulate Cortex	(compacta)	Pons
Parietal Cortex II	Visual Cortex IV	Medial Geniculate	Genu	Cerebellar Nuclei
Sensory-motor Cortex II	Visual Cortex VI	Cochlear Nucleus	Globus Pallidus	Dentate Gyrus
Superior Colliculus (S)	Parietal Cortex VI	Olfactory Cortex	Hippocampus	Lateral Lemniscus
	Mediodorsal Thalamus	Posterior Cingulate Cortex	Superior Olive	Dorsal Septal Nucleus
	Auditory Cortex VI	Anterior Thalamus	Superior Colliculus (D)	Ventral Tegmental Area
	Visual Cortex II	Nucleus Accumbens	Inferior Olive	Cerebellar Hemisphere
		Substantia Nigra	Subthalamic Nucleus	Inferior Colliculus
		(Reticulata)	Corpus Callosum	Cerebellar Vermis
			Ventral Septal Nucleus	Amygdala
			Internal Capsule	Cerebellar White

Table 2

Hierarchy of Responsiveness to THIP.

Magnitude of Depression from Control (see legend).

Extremely Sensitive	Sensitive	Moderately Sensitive	Minimally Sensitive	Insensitive
Sensory-motor Cortex IV	Frontal Cortex VI	Anterior Thalamus	Superior Colliculus (D)	Lateral Habenula
Parietal Cortex IV	Auditory Cortex IV	Auditory Cortex VI	Interpeduncular N.	Pons
Sensory-motor Cortex II	Visual Cortex IV	Medial Geniculate	Medial Habenula	Ventral Tegmental Area
Frontal Cortex IV	Mediodorsal Thalamus	Ventrolateral Thalamus	Vestibular Nucleus	Hypothalamus
Sensory-motor Cortex VI	Medial Caudate	Auditory Cortex II	Ventral Septal Nucleus	Red Nucleus
Frontal Cortex II	Parietal Cortex VI	Lateral Geniculate	Dentate Gyrus	Inferior Colliculus
Parietal Cortex II	Visual Cortex II	Inferior Olive	Medial Raphe Nucleus	Lateral Lemniscus
Lateral Caudate	Visual Cortex VI	Superior Olive	Cerebellar Nuclei	Dorsal Septal Nucleus
	Prefrontal Cortex	Genu	Hippocampus	Dorsal Tegmental Nucleus
	Olfactory Cortex	Globus Pallidus		Corpus Callosum
	Posterior Cingulate Cortex	Internal Capsule		Cerebellar White
	Subthalamic Nucleus	Nucleus Accumbens		Amygdala
		Substantia Nigra		Cochlear Nucleus
		(Compacta)		Cerebellar Vermis
		(Reticulata)		Superior Colliculus (S)
		Cerebellar Hemisphere		

Table 3

Hierarchy of Responsiveness to Muscimol.

Distribution of F-Values (see legend).

Extremely Sensitive	Sensitive	Moderately Sensitive	Minimally Sensitive	Insensitive
Lateral Caudate	Medial Geniculate	Anterior Thalamus	Medial Raphe Nucleus	Cochlear Nucleus
Mediodorsal Thalamus	Superior Colliculus (S)	Frontal Cortex II	Ventral Septal Nucleus	Amygdala
Sensory-motor Cortex IV	Parietal Cortex IV	Nucleus Accumbens	Superior Colliculus (D)	Interpeduncular Nucleus
Frontal Cortex IV	Ventrolateral Thalamus	Visual Cortex IV	Visual Cortex VI	Inferior Colliculus
	Parietal Cortex VI	Olfactory Cortex	Internal Capsule	Corpus Callosum
	Sensory-motor Cortex II	Anterior Cingulate Cortex	Globus Pallidus	Pons
	Medial Caudate	Parietal Cortex II	Hippocampus	Ventral Tegmental Area
	Sensory-motor Cortex VI	Lateral Geniculate	Genu	Dorsal Septal Nucleus
		Auditory Cortex VI	Dorsal Tegmental N.	Cerebellar Vermis
		Posterior Cingulate Cortex	Auditory Cortex VI	Inferior Olive
		Prefrontal Cortex	Substantia Nigra	Medial Habenula
		Frontal Cortex VI	(Reticulata)	Red Nucleus
		Visual Cortex II	Auditory Cortex II	Lateral Lemniscus
			Subthalamic Nucleus	Superior Olive
			Substantia Nigra	Cerebellar Nuclei
			(Compacta)	Cerebellar White
			Hypothalamus	Lateral Habenula
			Dentate Gyrus	Vestibular Nucleus
			Cerebellar Hemisphere	

Table 4

Hierarchy of Responsiveness to THIP.
Distribution of F-Values (see legend).

Extremely Sensitive	Sensitive	Moderately Sensitive	Minimally Sensitive	Insensitive
Lateral Caudate	Medial Geniculate	Lateral Geniculate	Nucleus Accumbens	Interpeduncular Nucleus
Medial Caudate	Sensory-motor Cortex IV	Visual Cortex II	Vestibular Nucleus	Pons
Visual Cortex IV	Anterior Cingulate Cortex	Parietal Cortex II	Genu	Cerebellar Nuclei
Auditory Cortex IV	Frontal Cortex IV	Posterior Cingulate Cortex	Superior Olive	Cochlear Nucleus
Parietal Cortex IV	Sensory-motor Cortex VI	Frontal Cortex VI	Superior Colliculus (D)	Ventral Tegmental Area
	Sensory-motor Cortex II	Anterior Thalamus	Inferior Olive	Medial Raphe Nucleus
	Mediodorsal Thalamus	Auditory Cortex VI	Prefrontal Cortex	Ventral Septal Nucleus
	Parietal Cortex VI	Visual Cortex VI	Internal Capsule	Lateral Lemniscus
	Frontal Cortex II	Auditory Cortex II	Inferior Colliculus	Corpus Callosum
		Subthalamic Nucleus	Dentate Gyrus	Medial Habenula
		Ventrolateral Thalamus	Cerebellar Hemisphere	Dorsal Tegmental N.
		Substantia Nigra (Reticulata)	Substantia Nigra (Compacta)	Lateral Habenula
			Olfactory Cortex	Hypothalamus
			Hippocampus	Cerebellar White
			Red Nucleus	Amygdala
			Globus Pallidus	Dorsal Septal Nucleus
				Superior Colliculus (S)
				Cerebellar Vermis

LEGENDS TO FIGURES 1-4

Criteria for establishing hierarchies of responsiveness to muscimol and THIP

TABLE 1

Extremely sensitive:	decreases > 20% at 0.5 mg/kg muscimol
Sensitive:	decreases > 15% at 0.5 mg/kg muscimol
Moderately sensitive:	decreases > 20% at 1.5 mg/kg muscimol
Minimally sensitive:	decreases > 15% at 1.5 mg/kg muscimol
Insensitive:	decreases < 15% at 1.5 mg/kg muscimol

TABLE 2

Extremely sensitive:	decreases > 40% at 10 mg/kg THIP
Sensitive:	decreases > 30% at 10 mg/kg THIP
Moderately sensitive:	decreases > 20% at 10 mg/kg THIP
Minimally sensitive:	decreases > 15% at 10 mg/kg THIP
Insensitive:	decreases < 15% at 10 mg/kg THIP

TABLES 3 and 4

F values generated by analysis of dose response curves to muscimol (range 2.5 to 40.0) and THIP (range 0.1 to 35.0) divided into 5 equal interval groups.

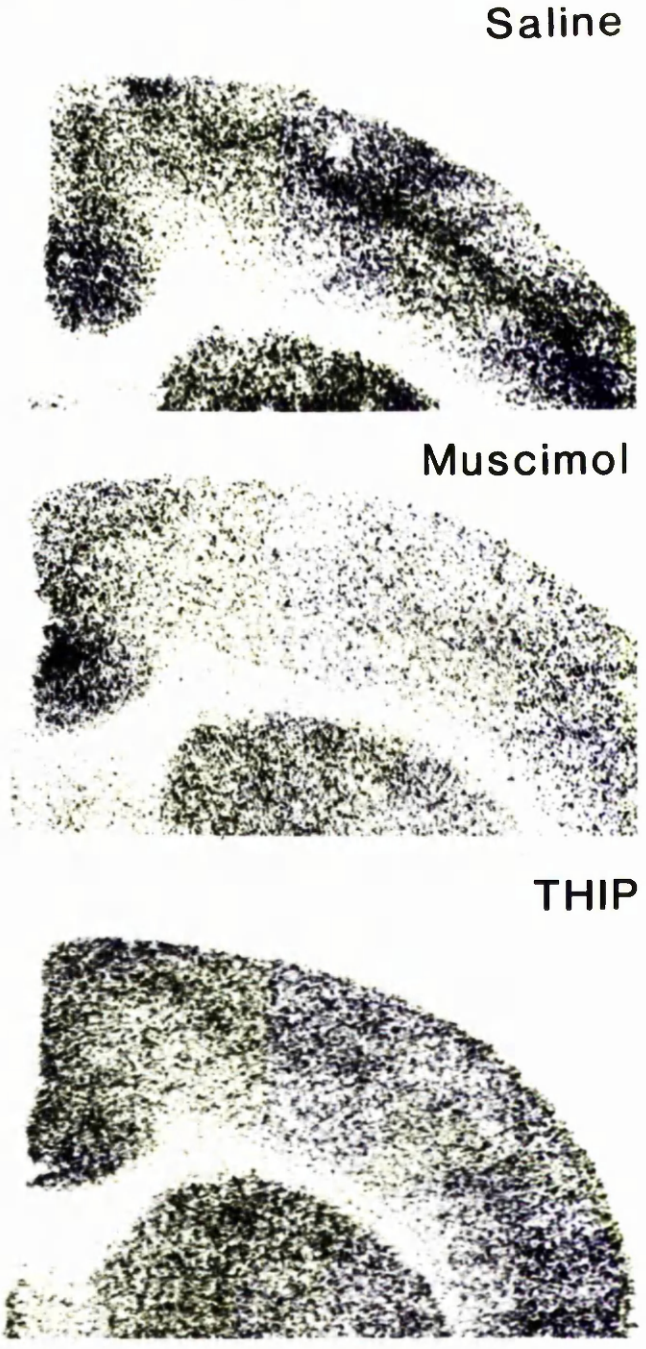
relatively constant for both modes of analysis and for both agonists used (Tables 1-4). Whilst the thresholds for grouping under headings; from extremely sensitive through to insensitive, were somewhat arbitrary for the hierarchies based upon magnitude of depression, the groupings for hierarchies based upon F-ratios were set at equal class intervals across the range of values generated by these experiments.

The regions of the brain in which glucose utilisation was most sensitive to depression by muscimol included most areas of neocortex, primary thalamic nuclei and the lateral portion of the caudate nucleus (see Fig. 1 for typical dose-response relationships). Within neocortex, however, cortical lamina IV was consistently more susceptible to depression than were the immediately surrounding layers at any point of measurement (Fig. 15).

The majority of brain regions analysed displayed only moderate or minimal sensitivity to the action of muscimol. Areas in which this degree of responsiveness was apparent included most of the extrapyramidal motor system (substantia nigra, globus pallidus, and medial portion of the caudate nucleus: Fig. 16), both cortical and subcortical limbic areas (cingulate and olfactory cortex, hippocampus, anterior thalamus and nucleus accumbens), medial habenula, interpeduncular nucleus, dorsal tegmental nucleus and median raphé nucleus (see Fig. 2 for typical dose-response relationships). The contrast in sensitivity between cingulate cortex and

Figure 15

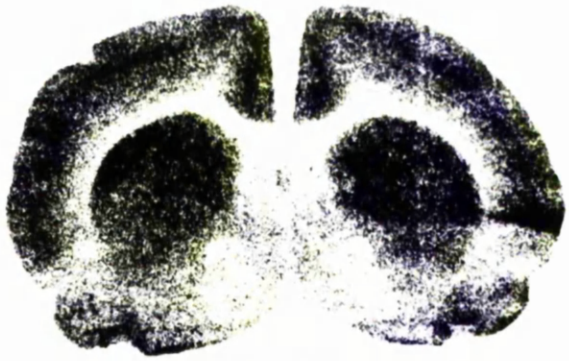
Autoradiograms prepared from coronal sections of rat brain at the level of the caudate nucleus showing dorsal and sensory-motor cortex.



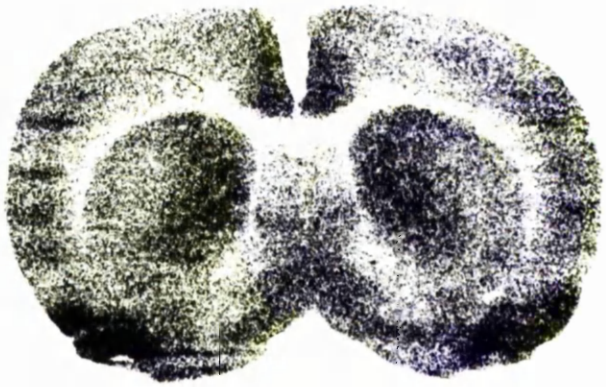
Upper: Control animal. Within cortex the area corresponding to layer IV is evident as a line of elevated optical density relative to the rest of the cortex. Middle: Muscimol (1.5 $mg.kg^{-1}$) and Lower: THIP (10 $mg.kg^{-1}$) treated animals. Layer IV no longer discernible in cortex of relatively homogeneous O.D.

Figure 16

Autoradiograms of coronal sections of rat brain cut at approximately A7500 (König and Klippel).



Saline



Muscimol

Upper: Control animal. No medial-lateral heterogeneity of O.D. in caudate nucleus.

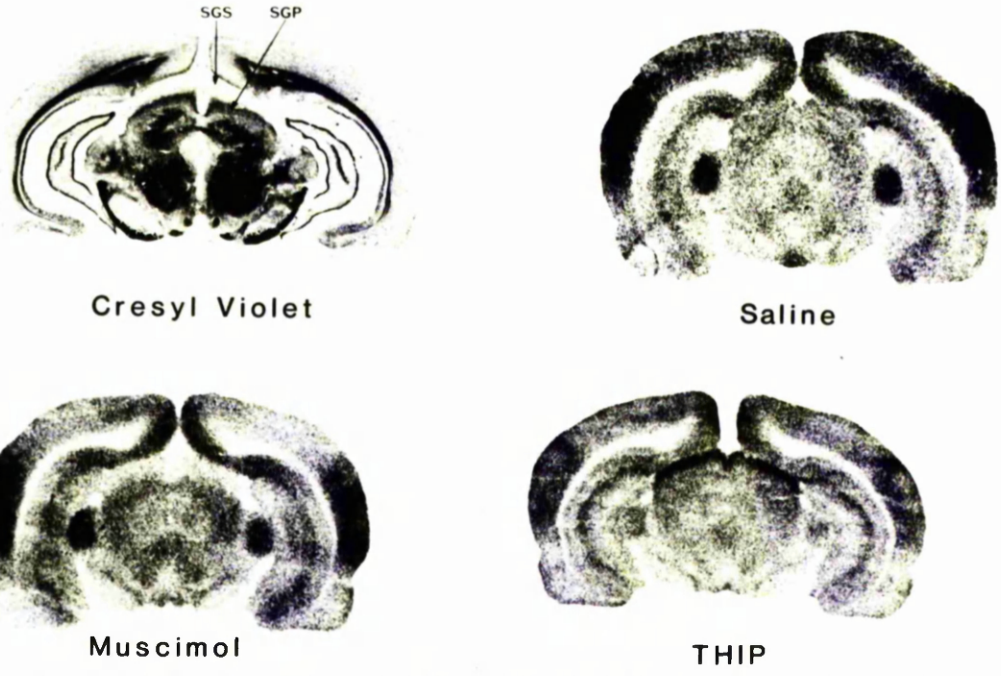
Lower: Muscimol 1.5 mg/kg. Marked medial-lateral heterogeneity of O.D. in caudate nucleus.

other, more responsive neocortical areas was apparent from the autoradiographic pattern (as seen in Fig. 14). The largest single group of structures were those in which glucose utilisation was relatively insensitive to the action of muscimol. Large concentrations of muscimol (0.5 mg/kg) were necessary to elicit quite modest reductions in the measured rates of glucose use. Included in this group were the cerebellum and related motor relay nuclei, such as red nuclei and inferior olivary bodies, white matter, the lateral habenula (see Fig. 14), pontine reticular formation, amygdala, hypothalamus and some brain stem auditory relay nuclei (inferior colliculi and superior olivary bodies).

The overall pattern of responsiveness to THIP was essentially similar to that described in detail following muscimol (Figs. 11-15; Tables 2 and 4), notwithstanding the five to six-fold difference in potency of muscimol over THIP. One notable exception to the parallelism of sensitivity of local glucose utilisation to the action of both agonists was observed in the superficial layers of the superior colliculi. The qualitative difference in the response of this area following muscimol and THIP was immediately apparent upon visual inspection of the autoradiograms (Fig. 17). Quantification confirmed that glucose use in the superficial layers was markedly depressed by muscimol, but was unaffected by THIP (Fig. 18). Thus, whilst this area was amongst those most sensitive to the action of

Figure 17

Autoradiograms prepared from coronal sections of rat brain at the level of the medial geniculate bodies.



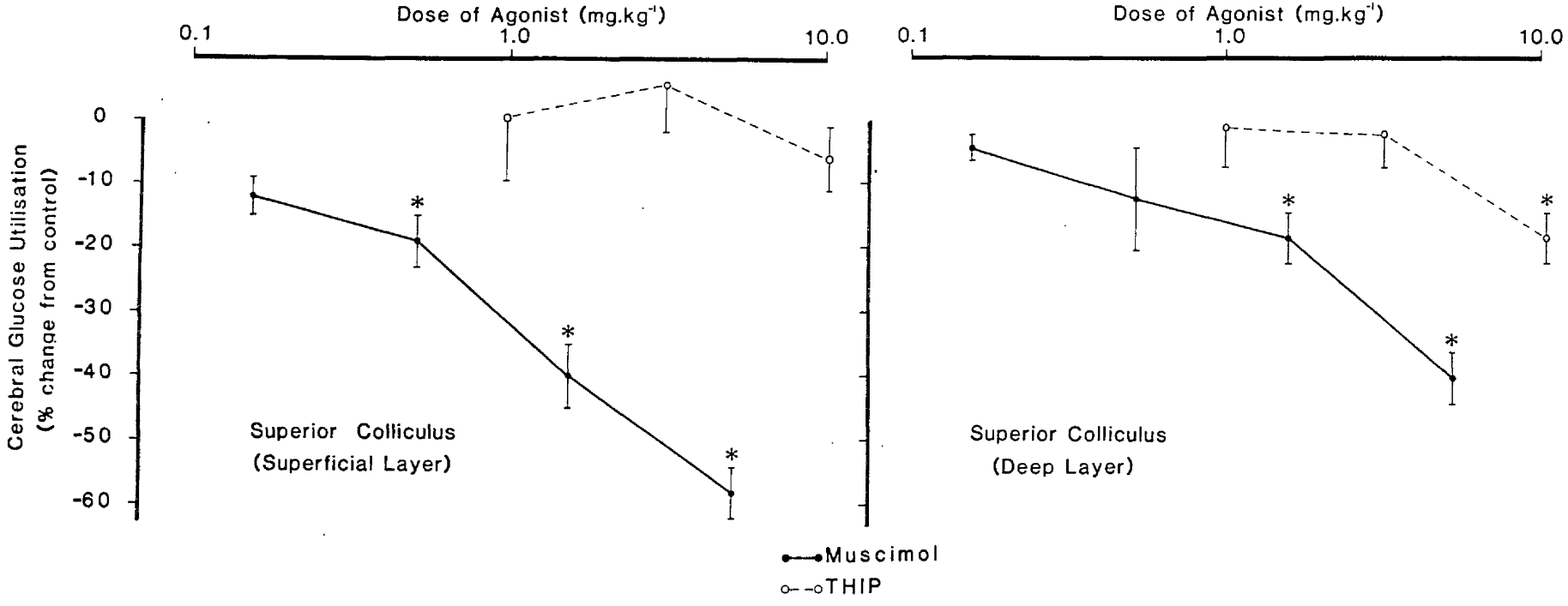
Upper right: Differential histological staining of superficial (SGS) and deep (SGP) layers of the superior colliculi.

Upper left: Control animal. The superior colliculi appear relatively homogeneous in optical density.

Lower left: Muscimol (1.5 $mg.kg^{-1}$) treated animal. The more superficial layers of the superior colliculi appear to be less dense than do the deeper layers of the same structure.

Lower right: THIP (10 $mg.kg^{-1}$) treated animal. The superficial layers of the superior colliculi appear more dense than do the deeper layers.

Figure 18



Differential sensitivity of glucose utilisation to muscimol and THIP in superior colliculus; superficial layer (left) and deep layer (right). Log-dose response curves of glucose utilisation following the intravenous administration of GABA agonists.

Data are presented as mean percentage change from control ± SEM. * $p < 0.05$.

muscimol, it was one of the areas least sensitive to THIP. Within the deeper layers of the superior colliculi the responsiveness was once again essentially similar with both agents (Fig. 18), and could be ranked as minimally sensitive.

2.3 Local cerebral blood flow following GABA agonist administration: In none of the 38 areas analysed were any significant increases in local cerebral blood flow measured following either 0.5 mg/kg or 1.5 mg/kg (see Appendix III). At 0.5 mg/kg muscimol, only minor, non-significant decreases in blood flow were observed in some brain areas, but the majority showed no apparent response.

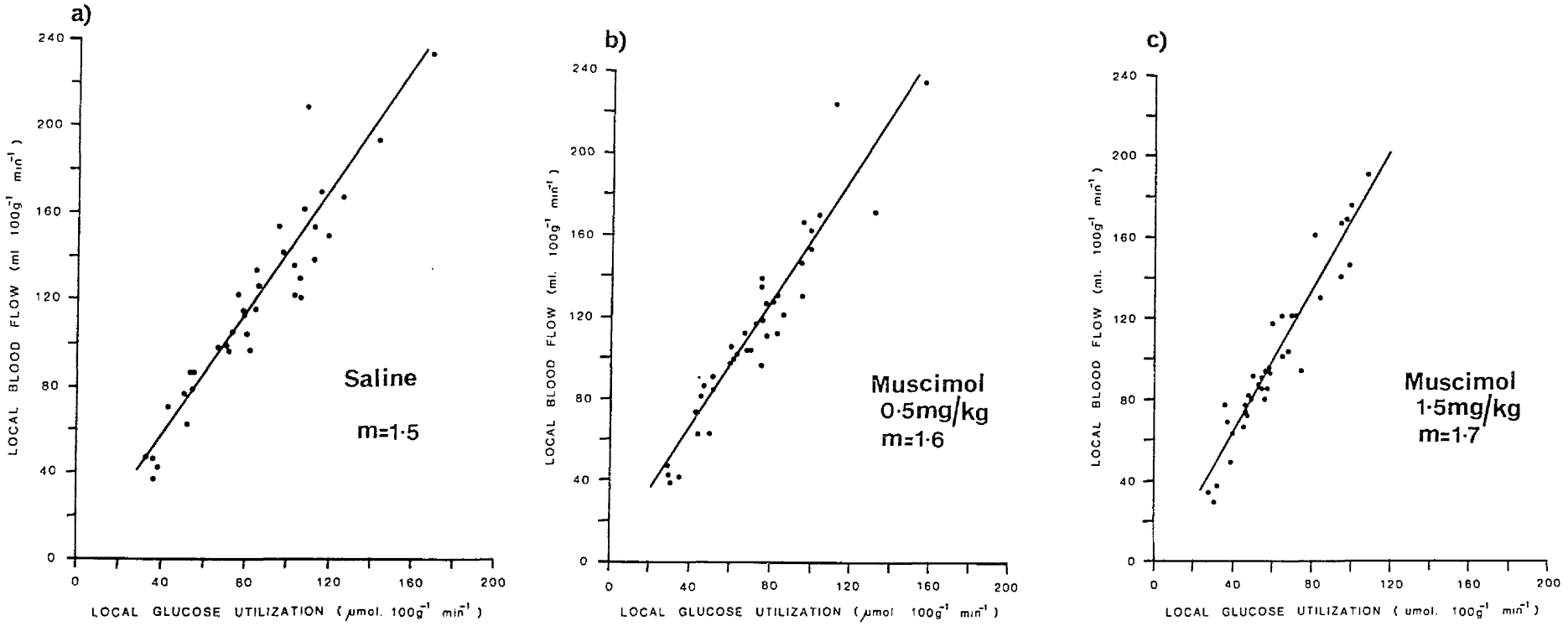
Following 1.5 mg/kg muscimol, most regions were subject to modest decreases in local cerebral blood flow, although these changes reached significant levels ($p < 0.05$) in only 10 of the areas measured (Appendix III). Most of the significant changes were found in neocortex, but decreases were also observed in sensory relay nuclei of the thalamus, elements of the extrapyramidal system in the telencephalon and the superior colliculus (Appendix III). Of the regions to which perfusion appeared almost completely unaffected by muscimol, many form part of what may be termed the limbic system (lateral habenula, amygdala and hippocampus, for example), but also mesencephalic areas involved in motor function (red nucleus, cerebellum), prethalamic nuclei of the auditory pathway (inferior colliculus and cochlear nucleus, amongst others) as

well as grey matter areas of diverse functional involvement (pontine reticular formation and hypothalamus) all displayed little, if any, response at either dose of muscimol (Appendix III). No correlation was evident between the responsiveness of local cerebral blood flow to alteration by muscimol and either neuroanatomy or vascular anatomy.

2.4 Relationship between local cerebral blood flow and glucose utilisation: Without further analysis of the data it is apparent that, in saline treated control groups, a gross relationship exists between local blood flow and glucose use within brain regions. Thus, areas of highest measured blood flow, primary auditory regions, for example, also display the highest rates of glucose utilisation, whilst areas of lowest measured blood flow, white matter tracts, for example, consistently show lowest rates of glucose use (Appendix III).

From a plot of mean control blood flow versus mean control glucose use for each area, and taking all areas together, the ratio of the two parameters, which may be identified from the gradient of the best fitting straight line for the data points, is 1.45 (Fig. 19). A similar manoeuvre performed on data from muscimol treated groups reveals that the ratio increases to 1.55 and 1.75 at 0.5 and 1.5 mg/kg muscimol, respectively (Fig. 19). However, simple regression analysis is inappropriate in this instance to test for significant changes in either the overall relationship or the relationship for any given structure.

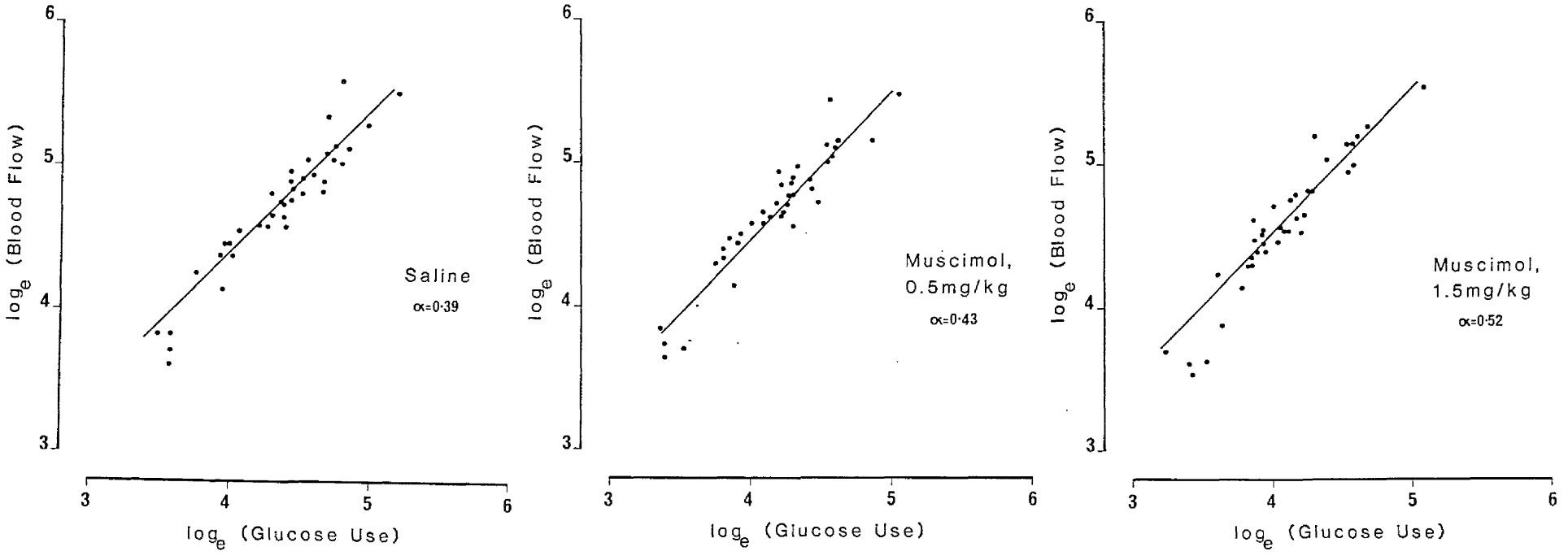
Figure 19



Relationship between mean local cerebral glucose utilisation and mean local cerebral blood flow in 38 anatomically discrete regions of brain of conscious rats, following a) saline, b) 0.5 mg/kg muscimol, and c) 1.5 mg/kg muscimol. The gradient of the best fitting straight line (m) is given for each plot.

The application of a statistical model specifically developed for this form of data, and fully described and validated by us previously (McCulloch et al. 1982a), allowed further appropriate statistical analyses of the relationship between blood flow and glucose use to be undertaken. The transformation of data to natural logarithms of the mean values (Fig. 20) alleviates problems associated with the obvious differences in variability between observations of high and low numerical value (cf. SEM of $\pm$ 13 in control inferior colliculus flow with SEM of $\pm$ 1 in white matter). Transformation of data in this way reveals a linear relationship between the two parameters, which is characterised by unit slope and intercept, $\overline{\alpha}$ (Fig. 20). It becomes a relatively simple matter in comparing treatments to analyse the difference between $\overline{\alpha}$ values (McCulloch et al. 1982a). Within the confidence intervals set, the relationship defined by the derived line was appropriate for all structures within each treatment group - that is, no structure showed a significant change in only one parameter and not in the other. Furthermore, the interaction remained relatively constant between treatment groups. Although $\overline{\alpha}$ changed from 0.39 in saline controls, through 0.43 at 0.5 mg/kg muscimol to 0.52 at 1.5 mg/kg, these changes did not approach significant levels (Fig. 20), the F-ratio being considerably below the critical value. Thus, the relationship which exists between local cerebral blood flow and glucose utilisation in conscious control rats remains intact and unaltered following muscimol treatment.

Figure 20



Relationship between local cerebral glucose utilisation and local cerebral blood flow presented as a logarithmic transformation of mean values for each of 38 discrete regions of conscious rat brain, following a) saline, b) 0.5 mg/kg muscimol, and c) 1.5 mg/kg muscimol. The relationship is described by a line with gradient unity and point of intercept, , given for each plot (see text).

2.5 Local cerebral glucose utilisation following benzodiazepine administration: The altered rates of local cerebral glucose utilisation measured following the intravenous administration of diazepam are presented in Appendix IV. Significant reductions in glucose use were evident throughout the brain and, although no significant response was observed in any region following a dose of 0.1 mg/kg diazepam, none of the areas measured failed to display a statistically significant reduction at 0.3 mg/kg. No increases in glucose use were observed at any dose of diazepam (Appendix IV).

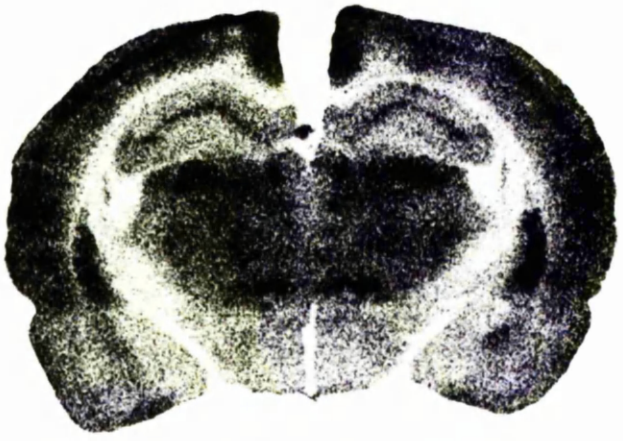
Not only were the observed responses qualitatively similar in all of the brain regions investigated (i.e., generalised reductions in glucose use), but analysis of the dose-response relationships (Appendix IV) also revealed that, with few exceptions, the responses were remarkably similar in quantitative terms. Thus, the autoradiographic pattern of isotope accumulation was essentially similar for both control and drug treated animals (Fig. 21), with relativities between areas maintained. Equal degress of responsiveness to diazepam treatment were apparent in areas of the brain with diverse functional roles (Fig. 22). Glucose use was decreased by similar extents in area of neocortex at all laminar levels, in areas of sensory and motor thalamus, most extrapyramidal motor areas, hippocampus and brain stem nuclei (Appendix IV).

In contrast to the homogeneity of response observed in the majority of brain regions following diazepam

Figure 21

Autoradiograms prepared from coronal sections of rat brain at the level of the habenular nuclei.

Saline

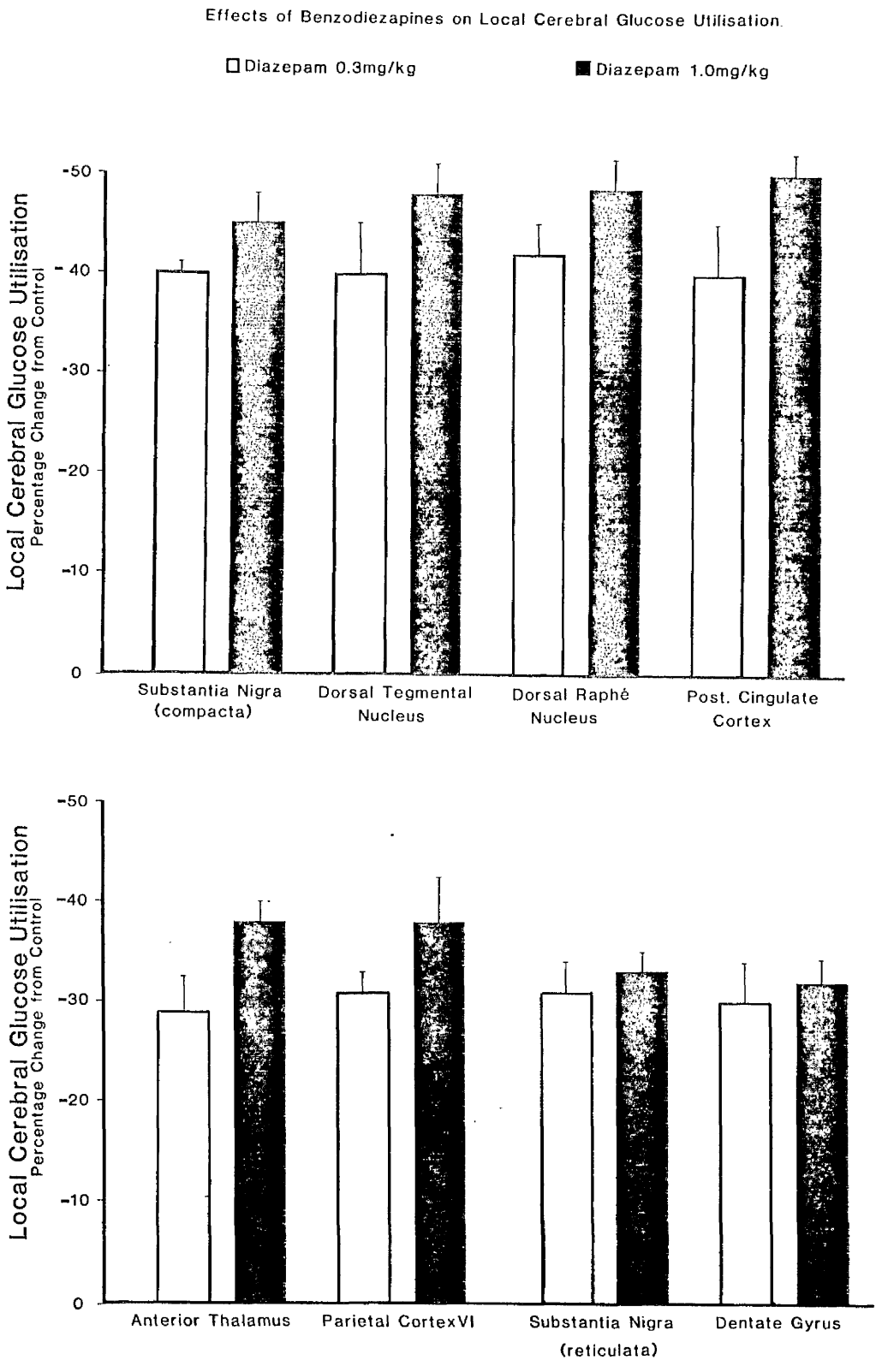


Diazepam(0.3mg/kg)

Upper: Control animal. Cerebral cortex, thalamus and lateral habenula display similar levels of density.

Lower: Diazepam treated animal. Relative densities are maintained, although reset at a lower level.

Figure 22



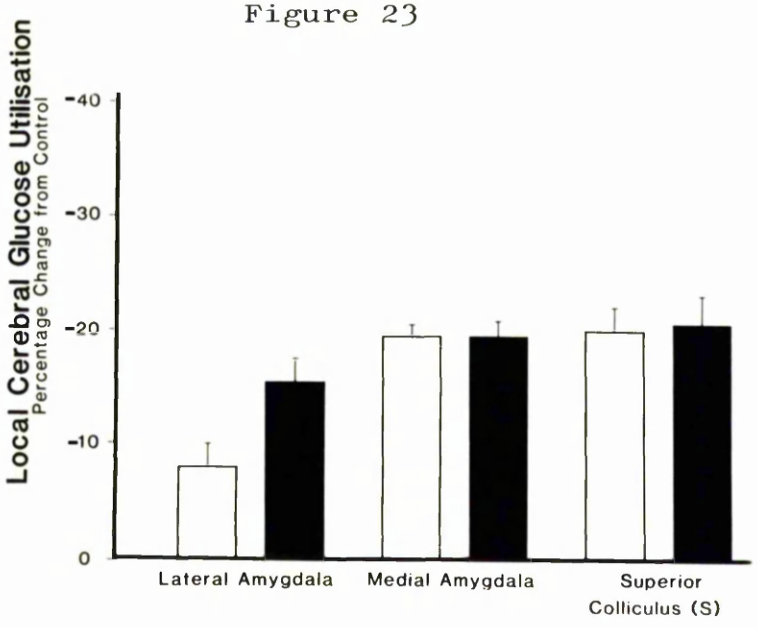
Upper: Areas of diverse functional involvement which are very sensitive to diazepam.

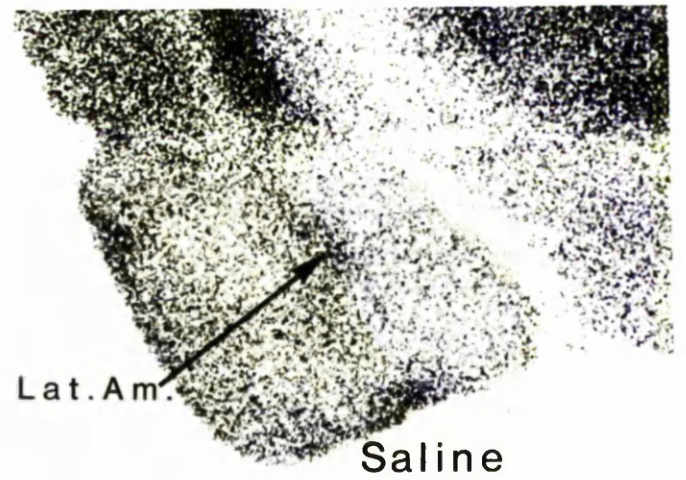
Lower: Areas of diverse functional involvement which are moderately sensitive to diazepam.

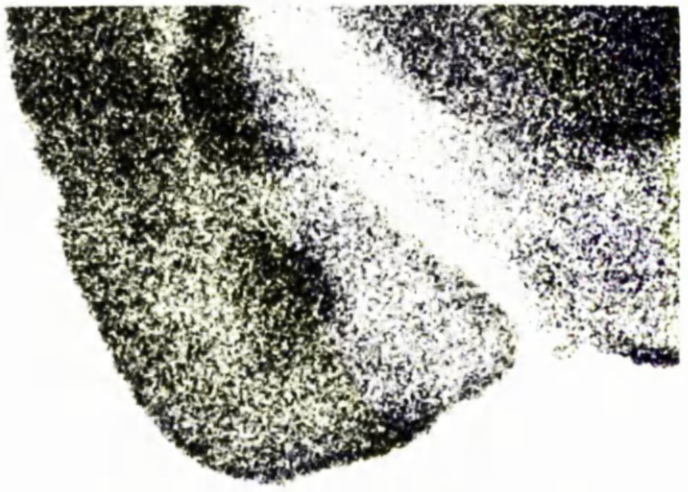
administration, the mamillary body, the dorsal tegmental nuclei, raphé nuclei, pars compacta of the substantia nigra, and posterior cingulate cortex all appeared to be very responsive to diazepam (Fig. 22). However, the lateral amygdala and, to a lesser extent, the medial amygdala, superficial layer of the superior colliculus and frontal cortex were less sensitive to diazepam (Fig. 23) than the majority of brain regions. White matter areas were also relatively unaffected by diazepam.

Ranking the 60 studies analysed according to absolute magnitude of response at 0.3 mg/kg (i.e., percentage change from control at this dose), and also according to the calculated index of deviation across the dose range (i.e., F-ratio), confirmed the original analysis of the data (Tables 5 and 6) above. Both methods revealed a similar grouping of structures with definite discontinuities in the continuum of responsiveness marking the very sensitive and minimally sensitive groups, and allowing only four groupings to be separated across the range of responses. The majority of regions analysed fell within the sensitive and moderately sensitive groups.

Figure 23







Upper: Areas of diverse function which are insensitive to diazepam.

Lower: Preservation of glucose use in lateral amygdala, apparent from autoradiograms.

Table 5

Hierarchy of Responsiveness to Diazepam.
Magnitude of Depression from Control (see legend).

Extremely Sensitive	Sensitive	Moderately Sensitive	Minimally Sensitive
Dorsal Raphe N.	Ventral Septal N.	Lateral Lemniscus	Superior Colliculus (S)
Posterior Cingulate Cortex	Ventral Tegmental Area	Nucleus Accumbens	Corpus Callosum
Dorsal Tegmental N.	Mediodorsal Thalamus	Frontal Cortex IV	Medial Amygdala
Substantia Nigra (Compacta)	Auditory Cortex IV	Inferior Olive	Genu
	Parietal Cortex IV	Anterior Cingulate Cortex	Cerebellar White
	Red Nucleus	Visual Cortex IV	Internal Capsule
	Subthalamic Nucleus	Lateral Caudate	Lateral Amygdala
	Ventrolateral Thalamus	Pons	
	Lateral Habenula	Cerebellar Vermis	
	Medial Geniculate	Superior Olive	
	Superior Colliculus (P)	Visual Cortex II	
	Vestibular Nucleus	Dorsal Septal N.	
	Auditory Cortex VI	Cerebellar Hemisphere	
	Auditory Cortex II	Frontal Cortex II	
	Interpeduncular N.	Cochlear Nucleus	
	Medial Habenula	Inferior Colliculus	
	Lateral Geniculate	Globus Pallidus	
	Parietal Cortex VI	Sensory-motor Cortex VI	
	Parietal Cortex II	Sensory-motor Cortex II	
	Cerebellar Nuclei	Frontal Cortex VI	
	Anterior Thalamus	Prefrontal Cortex	
	Sensory-motor Cortex IV		
	Substantia Nigra (Reticulata)		
	Hippocampus		
	Dentate Gyrus		
	Hypothalamus		
	Medial Caudate		

Table 6

Hierarchy of Responsiveness to Diazepam.
Distribution of F-Values (see legend).

Extremely Sensitive	Sensitive	Moderately Sensitive	Minimally Sensitive
Dorsal Raphe Nucleus	Medial Geniculate	Medial Habenula	Corpus Callosum
Posterior Cingulate Cortex	Superior Colliculus (S)	Lateral Caudate	Prefrontal Cortex
Mediodorsal Thalamus	Cerebellar Nuclei	Parietal Cortex VI	Lateral Amygdala
Dorsal Tegmental N.	Ventrolateral Thalamus	Parietal Cortex II	
Vestibular Nucleus	Substantia Nigra (Compacta)	Frontal Cortex II	
		Pons	
	Anterior Thalamus	Frontal Cortex IV	
	Lateral Geniculate	Auditory Cortex VI	
	Subthalamic Nucleus	Cerebellar Hemisphere	
	Medial Caudate	Anterior Cingulate Cortex	
	Red Nucleus	Visual Cortex IV	
	Interpeduncular N.	Superior Olive	
	Parietal Cortex IV	Visual Cortex II	
	Ventral Tegmental Area	Lateral Lemniscus	
	Auditory Cortex IV	Visual Cortex VI	
	Lateral Habenula	Dorsal Septal N.	
	Ventral Septum	Frontal Cortex II	
	Cerebellar Vermis	Ventral Septal N.	
	Inferior Olive	Globus Pallidus	
	Sensory-motor Cortex IV	Sensory-motor Cortex VI	
	Dentate Gyrus	Sensory-motor Cortex II	
	Nucleus Accumbens	Genu	
	Hypothalamus	Medial Amygdala	
	Substantia Nigra (Reticulata)	Superior Colliculus (S)	
		Cochlear Nucleus	
	Inferior Colliculus	Cerebellar White	

LEGENDS TO TABLES 5 AND 6

Criteria for establishing hierarchies of responsiveness to diazepam

TABLE 5

Extremely sensitive:	decreases > 40% at 0.3 mg/kg diazepam
Sensitive:	decreases > 30% at 0.3 mg/kg diazepam
Moderately sensitive:	decreases > 30% at 1.0 mg/kg diazepam
Minimally sensitive:	decreases < 20% at 1.0 mg/kg diazepam

TABLE 6

F values generated by analysis of dose response curves to diazepam (range 6 to 32.0) divided into 5 equal interval groups. No structures fell within the interval between moderately and minimally sensitive groups.

2.6 Commentary: In keeping with the importance increasingly being attached to GABA as the pre-eminent inhibitory neurotransmitter substance within the mammalian CNS (Krnjevic, 1974; Curtis, 1978), major GABA specific projection pathways have been identified which are integral to the normal functioning of the brain and, in particular, the activity of the extra-pyramidal and limbic systems (Fonnum et al. 1978; Ribak et al. 1980; Jones and Mogenson, 1980; Walaas and Fonnum, 1980). Moreover, in a number of brain areas with a diversity of functional involvement GABA neurons have been identified which remain intrinsic to their sites of origin. Thus, GABA interneurons have been localised within elements of the motor systems (McGeer and McGeer, 1975; Storm-Mathisen, 1976), limbic systems (Storm-Mathisen, 1976; Hökfelt et al. 1977) and visual systems (Okada, 1974; Kayama et al. 1980), as well as being present within areas of thalamus (Heuser et al. 1980; Starr and Kilpatrick, 1981) and neocortex (Curtis and Felix, 1971; Iversen et al. 1971) which, with their widespread cerebral interconnections, allow GABAergic influences to be disseminated throughout the brain.

The localisation of GABA systems with the use of biochemical markers for GABA synthesis, release and re-uptake, together with the measurement of agonist-specific receptor binding, whilst indicating areas of the brain in which one or more of these parameters is elevated, presents a general picture of GABAergic

activity distributed ubiquitously (albeit heterogeneously) throughout the brain. The hierarchies of regional sensitivity of glucose utilisation to depression by the putative GABA agonists, muscimol and THIP, which are reported here, do not correlate in any simple relationship with neurochemical markers for GABAergic mechanisms. Thus, the alterations in regional glucose use are not related to the reported topography of GABA itself in the CNS (van der Heyden et al. 1979), to the distribution of GAD, the proposed rate-limiting enzyme in the synthesis of GABA (Tappaz et al. 1976; Roberts, 1979; Nieoullon and Dusticier, 1981), nor to the levels of GABA specific receptor sites which have been characterised on the basis of radioligand binding (Zukin et al. 1974; Guidotti et al. 1980; Young and Kuhar, 1980; Palacios et al. 1981). Although the substantia nigra pars reticulata, for example, contains amongst the highest levels of both GABA and GAD in the CNS (Tappaz et al. 1976; van der Heyden et al. 1979), only relatively moderate alterations in glucose use were measured in this region following large concentrations of muscimol and THIP. The distribution of GABA agonist-induced changes in glucose utilisation and the distribution of muscimol recognition sites (Palacios et al. 1981) are similar in essence, with high densities of muscimol receptors detected in neocortical and thalamic areas correlating with the marked reductions in glucose use measured in these regions following systemic muscimol administration. However,

the relationship is by no means absolute, with a number of important anomalies. The low levels of muscimol receptors in the caudate nucleus contrast sharply with the pronounced reductions in glucose utilisation in this region resulting from muscimol administration.

Unlike investigations of marker distributions which produce a relatively static measure of GABA system activity, the 2-deoxyglucose approach provides a more dynamic index of the potential importance of functional involvement of GABAergic mechanisms in integrated cerebral activity. It is of interest, therefore, that in the relatively few anatomical areas in which GABA turnover has been established (caudate nucleus, nucleus accumbens, globus pallidus and substantia nigra (Mao et al. 1978), there appears to be a good inverse correlation between GABA turnover and the magnitude of response, in terms of depression in glucose use, measured in these four areas. If this relationship were to hold good for all of the 60 anatomical structures measured, although there is no compelling evidence that it should, it would be tempting to conclude that those areas in which glucose use is most profoundly sensitive to GABAergic manipulation are also those in which, under normal circumstances, GABAergic activation is low, thus leaving a greater potential for extrinsic GABA mimetics to exert an influence. Conversely, those areas which are most insensitive to change following GABA agonist administration should, following this hypothesis, be those in which GABAergic activation is normally high, leaving

little or no excess capacity for additional, extrinsic agents to act upon. However, it is doubtful whether local turnover rates alone could explain the heterogeneity observed in the pattern of changes in glucose utilisation, which is more likely to be the net result of both regional responses to GABAergic manipulation as well as the effects of changed afferent activity from other affected areas.

Previous neuropharmacological investigations to which the 2-deoxyglucose technique has been applied have demonstrated that alterations in glucose utilisation are not restricted to those regions known to be rich in receptors specific for the agent being studied, but are also observed in areas with primary or secondary neuronal connections with the receptor-rich regions (Sokoloff, 1981). Alterations in glucose use may thus be associated with both the initiation of the pharmacological response as well as in the areas linked by neuronal pathways which are involved functionally in the expression of the response. The manipulation of dopaminergic systems (McCulloch et al. 1979, 1980, 1981), which has profound behavioural effects, amply demonstrates the complexity of responses spatially removed from the initial sites of action, and revealed with the 2-deoxyglucose method. In contrast to dopaminergic systems (and many other neurotransmitter systems) which display discrete patterns of distribution, the localisation of GABAergic receptors throughout the brain (Zukin et al. 1974; Palacios et al. 1981)

and the almost universal distribution of neuronal cells in which the firing rates can be reduced by GABAergic manipulation (Johnston, 1978) render difficult the elucidation of what are the primary and secondary actions of systemically administered muscimol and THIP upon regional glucose utilisation. However, the present studies provide a comprehensive examination and description of the regional hierarchies of involvement in response to the systemic administration of muscimol and THIP, two widely used GABAergic agonists.

Cortex and Thalamus: In all of the areas of neocortex examined, local glucose utilisation decreased following the administration of either muscimol or THIP. At the highest dose of muscimol the response represented a depression from normal, resting levels of up to 60%. The rate of glucose use in neocortex was thus reduced to levels observed only in the most metabolically inactive regions of white matter in control animals. This degree of cortical depression is remarkable, bearing in mind that the animals remained conscious, albeit apparently highly sedated, and were capable of displaying the appropriate startle response to mildly noxious auditory (sudden loud noise) or tactile stimuli (blast of air directed at the head). The rate of neocortical glucose utilisation was less than that measured during barbiturate anaesthesia (Siesjö, 1977).

The intracortical distributions of putative neurotransmitters display marked laminar as well as regional heterogeneity (Emson and Lindvall, 1979). In the rat,

for example, dopamine-containing neurons and dopaminergic receptor mechanisms are restricted to the deeper laminae (layers V and VI) of a few rostral cortical areas (Lindvall and Björklund, 1978; Emson and Lindvall, 1979; Murrin and Kuhar, 1979). In contrast, noradrenergic and serotoninergic fibres are present in all cortical layers throughout the entire neocortex, although both systems are particularly prevalent in the more superficial laminae (Beaudet and Descarries, 1978; Emson and Lindvall, 1979). Biochemical markers for cholinergic neurons (choline acetyltransferase and acetylcholine esterase) are concentrated in layer IV of neocortex and the deeper portion of layer III (Beesley and Emson, 1975). These highly specific intracortical patterns of neurotransmitter distributions are considered to be directly related to the functional role played by each agent within the various regions of neocortex.

GABAergic processes have been proposed as the major inhibitory neurotransmitter system in neocortex (Dichter, 1980), and intrinsic cortical GABAergic neurons have been identified (Ribak, 1978). In contrast to the laminar heterogeneity in distributions of other cortical transmitters, GABA has been found in all cortical laminae (Emson and Lindvall, 1979), and electrophysiological evidence confirms that GABAergic synapses are distributed in all cortical layers (Krnjevic, 1974). Moreover, glutamic acid decarboxylase (GAD), the enzyme required for GABA synthesis, can be detected throughout cortex,

although GAD-positive perikarya are more prominent in layers I, II and IV (Ribak, 1978). Conflicting with the electrophysiological evidence, high affinity uptake of ^{3}H-GABA, which may be indicative of GABAergic synapses, is associated mainly with cells of laminae II and III (Hökfelt and Ljungdahl, 1972), although autoradiographic localisation of GABA receptors suggests a relative uniformity of distribution through layers I to IV, with concentrations in layers V and VI being considerably less (Palacios et al. 1981).

Although in the present study all cortical laminae were affected by both GABA agonists, the reductions were proportionately greater in layer IV than at other depths of cortex. In control animals, glucose utilisation in cortical layer IV is approximately 25% greater than in other cortical laminae, possibly because of the high dendritic density of this region and its resultant relatively higher energy requirements (Schwartz et al. 1979). The greater glucose utilisation in lamina IV results in a readily identifiable, well defined band of increased optical density on the autoradiograms. However, following muscimol and THIP administration, glucose use was more homogeneous throughout the different laminae, reflecting the quantitatively greater reduction in glucose use in layer IV (see Fig. 15).

The importance of cortical layer IV in the response to GABA agonist activity may be of wider significance in the understanding of the complexities of both normal cortical function and pathological dysfunction. Many

neocortical afferents terminate in layer IV, and the dense dendritic ramification allows widespread dissemination of this input throughout a large area of cortex (Cajal, 1929; Lorente de No, 1949). Cortical layer IV could thus be considered as a prime site at which intrinsic inhibitory processes could modulate excitatory input and thereby act to gate the levels of excitation within cortex. It is of interest in this context that seizure activity, which may be regarded as a state of cortical hyperexcitation, is associated with an apparent dysfunction of GABA systems (Ribak, 1979), and the epileptogenic focus in penicillin-induced models of seizure is primarily in the region of lamina IV (Lockton and Holmes, 1980). Furthermore, the two GABA agonists which this study shows to have such marked effects on cortical layer IV are also noted for their potent anticonvulsant properties (Enna, 1980). This distinct cell population of neocortex, therefore, is clearly of major importance in the maintenance of normal, co-ordinated cortical function.

Changes in the rates of glucose utilisation as measured using the 2-deoxyglucose technique need not, however, be a direct reflection of local neurochemical events. The actions of the GABA agonists may manifest themselves at the dendrites and terminals of post synaptic cells, but may also have presynaptic effects, or may change the functional activity of distant sites through the integrative activity of complex neuronal pathways (McCulloch, 1982). Thus, the drug-induced

decreases in glucose use in cortical layer IV may be mimicking the activity of intrinsic GABA neurones, or the activity of GABA neurones within areas which send projection fibres to cortex. The two possibilities are not, of course, mutually exclusive, and the effects which we have measured may be the net result of both mechanisms acting together. The thalamus is one of the main sources of cortical afferent fibres and, whilst the non-specific thalamic nuclei project to all cortical layers, the afferents from the specific nuclei of the thalamus terminate primarily in cortical layer IV (Lorente de No, 1943). Thus, changes in sensory input to the cortex mediated via thalamic nuclei are manifest more in layer IV (Kennedy et al. 1976).

Following GABAergic manipulation, a loose correlation became apparent between thalamic nuclei and the cortical areas to which they project. For example, glucose utilisation in both the anterior thalamic nucleus and cingulate cortex, which have extensive reciprocal neuronal connections (Domesick, 1969; Beckstead, 1976), was reduced to a lesser extent than in the thalamo-cortical systems involved in the processing of visual (i.e., lateral geniculate - occipital cortex), auditory (medial geniculate - auditory cortex) and somatosensory information (ventral thalamus - rostral, dorsolateral cortex). Furthermore, the greater reduction in cortical layer IV, which is the major site of termination of input from specific thalamic nuclei (Herkenham, 1980) when compared against the response

in other cortical laminae, points to a possible integrated cortico-thalamic response. The very high levels of GABA and of muscimol binding observed in thalamic nuclei (Palacios et al. 1981; Starr and Kilpatrick, 1981), and the marked behavioural alterations which can be induced by direct intrathalamic injections of muscimol (Di Chiara et al. 1979), suggest a possible primary interaction of the GABAergic agonists with receptors in the thalamus to which changes in cortical glucose use are a secondary response. However, whilst the acute depression in glucose use observed in areas of neocortex may be due to reduced input from a depressed thalamus, a possible role for intrinsic cortical GABA systems cannot be discounted.

Extrapyramidal and Motor Areas: Intensive investigation of the extrapyramidal system has revealed that the organisation of GABAergic systems within the basal ganglia play an important part in the regulation of motor behaviour (Okada et al. 1971), and that dysfunction of these neuronal elements is primarily responsible for pathological syndromes of dyskinetic motor behaviour (Marsden, 1978). GABAergic neurones have been identified projecting from the striatum to the globus pallidus and pars reticulata of the substantia nigra (Fonnum et al. 1978; Ribak et al. 1980). Inhibitory GABAergic interneurones have also been described in both the caudate and substantia nigra (Ribak et al. 1979; Ribak et al. 1980). From the basal ganglia pallidal GABAergic efferents are directed

to ventrolateral thalamus (Penney and Young, 1981) and subthalamic nucleus where terminal release of GABA invokes an inhibitory post-synaptic response (Rouzaire-Dubois et al. 1980), whilst nigral GABAergic projections proceed to the ventromedial thalamus and tectum (Di Chiara et al. 1979).

The regions of the extrapyramidal system which were analysed displayed only a moderate to minimal responsiveness to both muscimol and THIP, with the exception of the caudate nucleus in which glucose use proved to be amongst the most sensitive to GABAergic manipulation. The degree of sensitivity within these structures is closely mirrored by the levels of high affinity GABA receptors (Palacios et al. 1981), i.e., the caudate nucleus, being most sensitive to the agonists, also has greater concentrations of GABA receptors compared to pallidus and nigra, although this could possibly be fortuitous in view of the lack of correlation between these two parameters found elsewhere in the brain. Perhaps a more interesting inverse correlation is that, described earlier, which exists between the response in terms of depression in glucose use and GABA turnover rates, but the lack of data for other brain areas leaves an element of doubt as to whether, again, this correlation is merely fortuitous.

Although the caudate nucleus in toto was found to be the most sensitive extrapyramidal area to GABAergic manipulation, there was a marked quantitative heterogeneity in the response between the medial and lateral

portions of this nucleus (see Fig. 16). Previous investigations have emphasised the rostro-caudal distributions of striatal GABA systems, GAD levels in the tail of the caudate being found to be almost twice those measured in the most rostral portions (Scally et al. 1973). Similar non-uniform distributions of markers for other striatal neurotransmitter systems, most notably acetylcholine, dopamine, 5-hydroxytryptamine, glutamate, substance P and met-enkephalin, have also been reported (Fonnum and Walaas, 1978). The topographical organisation of these neurotransmitter systems may be a reflection of heterogeneous functional involvement of different parts of this nucleus (Tassin et al. 1976; Fonnum and Walaas, 1978; Scally et al. 1978), which contrasts markedly with the homogeneity of anatomically definable cell types. However, interactions of neurotransmitter systems in the functional activity of the caudate nucleus are possibly much more complex than has hitherto been suspected from the gross topography of transmitter markers. The distribution of acetylcholine systems, which are thought to be the main interneuron transmitter systems (Divac and Oberg, 1979), shows a microscopic medial-lateral difference (Rea and Simon, 1981), whilst at a higher level of resolution the distribution pattern appears to be more of a mosaic within these regions of elevated biochemical markers for acetylcholine (Graybiel and Ragsdale, 1978). Similarly, striatal projection neurons and cells postsynaptic to afferent terminals also appear

to form clusters within the general cellular matrix (Graybiel and Ragsdale, 1978; Wright and Arbuthnott, 1981).

The medial-lateral functional heterogeneity suggested by the present investigation cannot be explained simply from anatomical or neurobiochemical data available on striatal GABA systems, and the development of high resolution techniques with which to map the distribution of specific GABA receptors has so far failed to identify a heterogeneity similar to that of the pattern of altered glucose utilisation (Palacios et al. 1981). Although GABA is known to be of primary importance to striatal function, complex interaction with other neurotransmitter systems - for example, cholinergic neurones (Scatton and Bartholini, 1979, 1980) - makes the possibility of observing a purely GABAergic effect upon glucose utilisation unlikely. The profound changes induced by GABAergic manipulation in areas sending projection fibres to the caudate nucleus could, together with possible action upon intrinsic neurones and collaterals from striatal efferents (Park et al. 1980), summate to produce the high degree of responsiveness of this nucleus above all other elements of the extrapyramidal system. It is interesting in view of the medial-lateral differences in caudate response that whilst cingulate cortex, which was more resistant to the action of the agonists, has been shown to be functionally linked to the less responsive medial portion, sensory-motor cortex, which was extremely

sensitive, has been shown to be similarly linked to the more responsive lateral portion (Divac and Diemer, 1980). The significance of this possible functional interdependence in integrated striatal activity awaits further elucidation.

In the major sites receiving almost exclusively GABAergic projections from the caudate nucleus, the globus pallidus and pars reticulata of the substantia nigra (Ribak et al. 1976), administration of the GABA agonists produced only moderate to minimal alterations in glucose utilisation. The decreases in glucose use observed in the present study in the pars reticulata are in excellent agreement with previous electrophysiological measurements of reduced cell firing rates in this area (Grace and Bunney, 1979; Waszczak et al. 1980b), both in the magnitude of the changes and the concentration percentage response curves for both GABA agonists. However, in the pars compacta of the substantia nigra, systemic administration of muscimol is followed by an increase in firing from putatively dopaminergic cells (MacNiel et al. 1978; Grace and Bunney, 1979; Waszczak et al. 1980a) which contrasts with the decreases observed in the measured rates of glucose utilisation. It remains to be determined whether these apparently contradictory results are a reflection of differences in response between the conscious animals employed in this study and electrophysiological preparations in which chloral hydrate was used, in a similar way to that described following

dopaminergic manipulation (Grome and McCulloch, 1981). Furthermore, general anaesthetics, most notably barbiturates, exert a profound influence upon GABAergic neurotransmission (Lodge and Curtis, 1978; Skolnick et al. 1981). The use of conscious animals, which the 2-deoxyglucose approach facilitates, presents a major advance over other techniques where anaesthesia is a prerequisite in the investigation of CNS responses to GABAergic agonists.

In most motor areas outwith the basal ganglia, glucose utilisation was only minimally reduced by muscimol or THIP. In contrast to a recent report (Palacios et al. 1981) describing the effects of these two agonists upon glucose use in a limited number of brain structures, no increase in glucose use was observed in the present study with any dose of either agent, although in other brain regions - auditory cortex, medial geniculate and lateral habenula - the responses observed in both studies were in general agreement. The difference in the response of the red nucleus may result from pharmacological and/or methodological differences between the studies, such as the apparent lack of restraint to allow plasma sampling, the use of ether anaesthesia during surgical preparation or the starvation of the rats prior to the experiments, with the attendant possibility of cerebral tissue switching to the use of β-hydroxybutyrate and aceto-acetate to meet its energy requirements (see Ruderman et al. 1974, for the consequences of 24-hour starvation on energy

substrate use). The very low levels of glucose use (43 ± 2 umol/100g/min) observed in the red nucleus of control animals (Palacios et al. 1981) compared to that noted by ourselves (73 ± 3 umol/100g/min) and others (76 ± 5 umol/100g/min; Wechsler et al. 1979) could in itself be directly responsible for the apparent discrepancy in the two investigations.

Of the areas involved in motor function, the cerebellum was the region in which glucose utilisation was least responsive to GABAergic manipulation. In keeping with the observation that the cerebellum contains the greatest density of GABAergic receptors in the CNS (Beaumont et al. 1978), neuronal inhibition within cerebellar cortex appears to be mediated almost entirely via GABA mechanisms (Roberts, 1978). Moreover, the Purkinje cells, which are GABAergic in nature (Obata et al. 1967), provide the sole efferent connections from cerebellar hemisphere to the underlying nuclei (Eccles et al. 1967). Thus, the known primacy of GABA in the cerebellum, taken together with the minimal reductions in glucose use following administration of GABA agonists in intact, conscious rats, would appear to indicate that, in vivo, cerebellar GABA mechanisms of neurotransmission are near maximally active.

A growing number of discrepancies are being identified between the regional distribution of receptor sites and the topography of the relevant neurotransmitter systems (Palacios et al. 1979, 1980; Simantov et al. 1977). Whilst ligand binding studies provide evidence

for possible sites of drug action, the Vmax for receptor affinity upon which these approaches are based supplies little indication of the circumstances under which receptor populations will be activated in the intact, conscious animal. The response of cerebellar glucose use to GABAergic agonist administration provides an interesting insight into the interaction between endogenous and exogenous GABA agents, and stresses that the outcome of this interplay is complex and not easily predictable.

Primary Visual System: GABAergic activity has been found at all levels of the primary visual system. Within the retina GABA is restricted to a discrete population of cells (Nakumara et al. 1980), whilst in the visual cortex, although GAD activity may be localised to a specific, histologically definable cell type, these cells are distributed throughout all cortical layers (Ribak, 1978). The visual relay nuclei, the lateral geniculate bodies and superior colliculi, both exhibit relatively high GAD activity (Tappaz et al. 1976), and the inhibitory input to occipital cortex from the lateral geniculate utilises GABA as the synaptic neurotransmitter substance (Armstrong, 1968; Iversen et al. 1971). In the present study, local rates of glucose utilisation in cortex and lateral geniculate were at least moderately sensitive to both muscimol and THIP. In contrast, glucose use in the deep layers of the superior colliculus was relatively insensitive to the action of the two agonists, perhaps

reflecting the lesser functional involvement of this region in primary visual processing.

The superficial layer of the superior colliculus was the only area of the CNS measured in this study in which the response, in terms of altered glucose utilisation following muscimol, was qualitatively different from the response following THIP. The basis for this disparity in the actions of the two GABAergic agonists upon the functional activity of the superior colliculus is not immediately apparent, especially as the responses observed within the other, functionally related, primary visual areas were essentially similar in their patterns of depression. The laminated structure of the superior colliculus reflects a heterogeneity of functional activity at different depths. Not only is afferent input segregated into different layers (Lund, 1964; Casagrande et al. 1972; Sterling, 1973; Grofova et al. 1978), but neurotransmitter systems are also distributed unevenly through the layers. Although it may be surmised from similarities in response elsewhere in the brain that input to the area is the same following both agonist treatments, there is a possibility that differential interaction of the GABAergic agents with other neurotransmitter systems could account for the dichotomy in the effects of muscimol and THIP within this region. Although in vivo experiments have so far failed to show any differences in the effects of these agents upon neuronal function (Waszczak et al. 1980), their pharmacological profiles, characterised

in vitro, do show marked dissimilarities (Krøgsgaard-Larsen et al. 1979). The potential for differences in the pharmacological actions of muscimol and THIP is therefore available, although the present study represents the first observation of any such divergence of activity in vivo. The exact neuroanatomical and neurochemical conditions which are absent from any other region of the CNS but which prevail in the superficial layer of the superior colliculus, allowing differences in the pharmacological characteristics of muscimol and THIP to be manifest, remain to be determined.

Cerebrovascular System: Although investigations into the functional role of GABA in the brain have, in the main, focussed upon neuronal systems, recently a novel GABAergic system has been identified in association with the cerebral vasculature, indicating a possible role of GABA in cerebrovascular regulation. Unlike vessels taken from peripheral sources, cerebral arteries, when studied in vitro, displayed a vasodilatatory response to GABA and putative GABA agonists (Fujiwara et al. 1975; Edvinsson and Krause, 1979) which was subject to a parallel shift to higher agonist concentrations in the presence of antagonists (Edvinsson and Krause, 1979). The contrast between peripheral and cerebral arteries was further emphasised by the localisation of glutamic acid decarboxylase (GAD), the rate-limiting enzyme in the synthesis of GABA, and GABA transaminase (GABA-T), the principal enzyme in GABA

degradation, in large concentrations in brain vessels, but not in those from the periphery (von Gelder, 1968; Krause et al. 1980a; Hamel et al. 1981). The endothelial location of these enzymes in the cerebral vasculature has gained added importance with the recent demonstrations of the crucial role played by endothelial cells in the relaxation of arterial smooth muscle (Furchgott and Zawadzki, 1980). The demonstration of GABA specific binding sites and the correlation between binding affinity and potency displayed by pharmacological agents in eliciting a dilatatory response (Edvinsson and Krause, 1979; Krause et al. 1980a,b) further indicated a possible role for intrinsic GABA systems in the control of cerebral vasomotor activity, and thereby participating in the regulation of cerebral tissue perfusion.

The importance of maintaining cerebral homeostasis to protect normal brain function in the face of dramatically changing physiological conditions is widely thought to provide the driving force behind control of cerebral blood flow (Roy and Sherrington, 1980). Homeostatic conditions may, however, vary widely in different parts of the CNS as increases or decreases in activation create localised changes in the cellular milieu, by virtue of the altered requirements for energy substrates necessitated by neuronal function and the production of metabolites which these processes entail (Des Rosiers et al. 1974; Sokoloff, 1978). The development of quantitative autoradiographic techniques allows

functional activity and, therefore, energy demand (using 2-deoxyglucose), and cerebral blood flow (using iodoantipyrine) to be measured with a similar degree of spatial resolution. However, one major limitation of this approach is that the individual measurements of blood flow and glucose use cannot be performed on the same animals. The problems associated with statistical analysis of data in this form have hitherto been largely ignored (Kuschinsky et al. 1981; Ohata et al. 1981), but a mathematical model recently developed to accommodate the interdependence of multiple regional measures within an individual animal, as well as the inter-animal variability which is concealed with the use of regression analysis, has allowed valid analysis of the results from the present study (McCulloch et al. 1982a).

The vascular response to GABA receptor activation described in vitro would, if transposed intact to the whole animal, result in an increase in cerebral blood flow through the dilated vessels. However, if this mechanism did indeed have a physiological role to play in the control of cerebral tissue perfusion, then manipulation of GABA systems with systemically administered agonists could elicit opposing forces upon the vasculature to increase flow (direct vascular effect) or reduce flow (mediated via reduced metabolic demand in the brain). From the evidence of the present investigation it is clear that the relationship which exists between local blood flow and glucose utilisation in each region of the CNS in normal animals was unaltered

by muscimol administration. It would thus appear likely that the alterations in tissue blood flow ~~which were observed following~~ muscimol administration are a secondary consequence of the reductions in metabolic activity which result from muscimol administration. Moreover, the regions of the brain where the greatest reductions in functional activity were observed, e.g., neocortex and thalamus, are not necessarily the primary sites at which muscimol acts, and the observation that these areas were also the regions to which blood flow was most significantly reduced provides further evidence for a close link between metabolic activity and blood flow. However, a direct effect of muscimol upon the cerebral blood vessels in this animal model cannot be wholly discounted. A slight resetting of the blood flow/glucose utilisation function, which appears to some extent to be dose-dependent, may reflect a vasodilatatory effect of muscimol tempering the vasoconstrictory effects of reduced metabolism. The lack of statistical significance which may be attached to this effect may be a reflection of the degree of variability intrinsic in all blood flow measurements together with the relative conservatism of the rigorous statistical model which was used.

Contrary to a previous investigation in which cortical blood flow was reportedly <u>increased</u> by up to 90% by muscimol (Edvinsson et al. 1980), it was in the cerebral cortex that the most consistent, significant <u>reductions</u> in blood flow (and glucose utilisation)

were observed. The reason such discrepancies arise is difficult to define with certainty. However, the use of anaesthesia in the previous study may be of crucial importance, especially as GABAergic mechanisms in general are particularly sensitive to modification by anaesthetic agents (Curtis and Lodge, 1977). Similarly, the absence of any data in the previous report concerning metabolic alterations resulting from muscimol administration renders the comparison between the studies more difficult. The present studies, by eliminating some of the sources of uncertainty (anaesthesia, metabolic influence, heterogeneity of response), and by the choice of measurement techniques which we have employed (i.e., quantitative autoradiography with ^{14}C-iodoantipyrine and ^{14}C-2-deoxyglucose), provides a more accurate and complete description of the cerebral circulatory consequences of administering muscimol intravenously than does the previous report.

The exact physiological role of cerebrovascular GABA mechanisms remains to be determined. Clearly, under non-pathological conditions they are unlikely to be linked to neuronal GABAergic activity with the dichotomous effects upon blood flow which the two enzymes induce. Moreover, pharmacological manipulation of vascular GABA systems in the intact animal without concomitant, opposite effects elicited via metabolic influences would appear not to be feasible. Recently, however, a relationship has been suggested between GABA release and cerebrovascular disease on

the basis of increased levels of the neurotransmitter measured in CSF (Welch et al. 1976), although whether this is of vascular or neuronal origin, or whether it is a cause or effect of the condition, is uncertain. Under pathological conditions it is possible that the relationship between the perfusion and metabolic requirements of brain tissue may no longer prevail, and manipulation of both vascular GABA mechanisms to promote increases in blood flow whilst neuronal GABA systems are reducing metabolic demand could possibly be used as a prophylactic manoeuvre to reduce ischaemic cell damage in the management of cerebrovascular insult. Such a possibility could be worthy of further investigation.

GABAergic Interactions with Benzodiazepine: Alterations in cerebral glucose utilisation following GABAergic manipulation with muscimol or THIP, when compared with the pattern of changes following administration of the benzodiazepine substance, diazepam, revealed a general similarity of response. With increasing doses of the three agents, rates of glucose utilisation decreased throughout the brain, all functional systems being affected. This observation is in keeping with the common actions of GABA agonists and benzodiazepines in promoting hypnotic and ataxic (i.e., sedative) effects. Closer analysis of the results revealed that within the general depressant response the hierarchy of responsiveness to GABA agonists was markedly different from that following diazepam, this

despite the fact that muscimol (but not THIP) promotes benzodiazepine binding (Karobath et al. 1979) and should therefore enhance the activity of any endogenous benzodiazepine (Braestrup et al. 1981). The hierarchies differed in two major respects. In general, the range in magnitude of response to GABA agonists was greater than that observed following diazepam administration. Although a few regions of the brain were immediately apparent as being areas of low or high sensitivity, the great majority of regions appeared to be equally sensitive to the benzodiazepine. Moreover, in this majority of areas little or no further decrease in glucose use was observed between 0.3 and 1.0 mg/kg diazepam, contrasting with the generally steady, dose-dependent decreases observed following muscimol or THIP. In this instance, hierarchies based upon the F-ratio reveal the extent to which the depression in glucose use in response to diazepam is considerably more homogeneous than that to muscimol or THIP.

The particulars of the hierarchies of sensitivity were also quite different. Whilst the GABA agonists were most effective in reducing functional activity in thalamus and cortex, the areas most sensitive to diazepam were much more diffuse, including elements of the extrapyramidal motor system, the raphé nucleus and the mamillo-tegmental axis. Interestingly, the cortical area least affected by GABA agonists, namely cingulate cortex, was amongst the areas most sensitive to diazepam. No correlation was evident between the

sensitivity of glucose utilisation in any region to change by diazepam and the distribution of benzodiazepine binding sites. For example, the amygdaloid nuclei, which appeared to be very sensitive to the action of diazepam in terms of altered functional activity, have been reported to possess a dense receptor population (Young and Kuhar, 1980). This apparent discrepancy has previously been discussed in full in relation to GABAergic manipulation.

The differences in the hierarchies of sensitivity to GABAergic and benzodiazepine manipulation confirm that the changes observed following GABAergic manipulation were not explicable as being merely the product of generalised cerebral depression. Rather, the altered functional activity was a direct function of specific GABA-mimetic activity, a specificity not shared by diazepam (Gallager, 1978; Geller et al. 1978). Conversely, the differences in the hierarchies may elucidate, to some extent, the possible mode and site of action of benzodiazepines in producing anxiolytic effects specific to those agents.

The GABA receptor has been proposed to be part of a greater protein complex which also includes benzodiazepine and barbiturate recognition sites (Olsen, 1981). However, considerable uncertainty remains over the extent to which these related central nervous system depressants rely upon modulation of the postsynaptic response to GABA in the expression of their actions (Costa and Guidotti, 1979; Haefely, 1981).

Electrophysiological investigation has indicated that benzodiazepines enhance post-synaptic, GABA-mediated inhibition, both in neuronal cell culture (MacDonald and Barker, 1978) and in areas of the intact brain (Polc and Haefely, 1976). In contrast, several studies have indicated that GABA binding to membrane receptors in vitro could not be significantly enhanced by the addition of benzodiazepines (Zukin et al. 1974; Mohler and Okada, 1978; Andrews and Johnston, 1979; Olsen, 1979), although the protocol for preparation of the membranes appears to be quite critical, and may produce opposite results (Guidotti et al. 1978). The reverse interaction of GABA receptor agonists in promoting benzodiazepine binding could be demonstrated (Briley and Langer, 1978; Tallman et al. 1978; Chiu and Rosenberg, 1979; Karobath and Spark, 1979), this inverse effect being found in all brain regions (Wastek et al. 1978) and reversed by GABA antagonists (Tallmann et al. 1978). The regional distribution of benzodiazepine receptor sites in the brain, although similar to that of GABA receptors, is not exactly identical (Placheta and Karobath, 1979; Young and Kuhar, 1980), an observation which reflects the finding that not all GABA receptors are coupled to benzodiazepine receptors. Whether the converse is valid and benzodiazepine receptors are to be found in any cells which are devoid of GABA receptors remains an open question.

The effects of diazepam upon glucose utilisation in many areas of the brain analysed, presented a pattern

of response remarkable for the apparent lack of dose-dependence. This may be a reflection of poor choice of doses, but such changes over a semilog dose increase would be difficult to accommodate within any experimental design. Alternatively, the alterations in functional activity at low doses of diazepam (0.1 mg/kg) may be beyond the power of the method to resolve. Again, this appears to be unlikely in view of the fact that relatively small, 8% decreases in glucose use were not only detected, but were found to be statistically significant, such was the limited variance of the measurement. Such an apparent 'all or none' response to diazepam would be difficult to justify, given the range of different actions reported for benzodiazepine, but it is probable that, for example, only in stressed animals would the altered functional activity of selected brain regions truly reflect the anxiolytic properties of the agent.

On the basis of receptor distributions, a tentative map of brain areas involved in the different pharmacological effects of benzodiazepines has been established (Young and Kuhar, 1980). On this basis, the amygdala and frontal cortex were proposed to be possible sites of anxiolytic action, both areas being of dense benzodiazepine receptor population. Using a behavioural model of anxiety in the intact animal, local injections of benzodiazepine into the amygdala (Shibata et al. 1982a) or the mamillary body (Shibata et al. 1982b) have indicated these areas as potential

sites of anxiolytic action. From the present investigation it is apparent that functional activity within amygdaloid nuclei and the deeper laminae of the frontal cortex is only minimally affected by diazepam administration. Although these regions may be the site of primary action of the drug, in terms of receptor binding, these are patently not the areas of the brain where the actions are manifest. In contrast, the mamillary body and the dorsal tegmental nucleus, which share reciprocal innervation (Briggs and Kaebler, 1971), were amongst the areas which proved to be most sensitive to the action of diazepam in terms of reduced glucose utilisation. These two areas also display moderate to dense occurrence of benzodiazepine receptors (Young and Kuhar, 1980). It is apparent, therefore, that the functional responsiveness to diazepam in different areas of the brain does not parallel receptor distributions and, indeed, areas possessing similar levels of receptor activity are capable of displaying widely divergent responses.

Although these studies may present an interesting adjoiner to the search for the site of action of benzodiazepines in their capacity as antianxiety agents, the deoxyglucose technique (when linked with systemic administration) is perhaps not the most appropriate tool with which to analyse specific drug/receptor interaction. While such studies will continue to generate interest, providing some insight into the wider effects of possible therapeutic agents in the

brain, the limitations of combining 2-deoxyglucose technology with systemic administration as an approach to further understanding of basic neuronal mechanisms are becoming increasingly recognised (McCulloch, 1982).

3. Striatal Lesioning with Kainic Acid

3.1 General results and observations: During the first 48 hours after the injection of kainic acid, lesioned animals displayed spontaneous, predominantly contraversive turning, and were both aphagic and adipsic over the same time period. The disruption of feeding behaviour together with increased motor activity resulted in the rats suffering a substantial weight loss (up to 50g in 48 hours) accompanied by a marked reduction in plasma glucose levels. By seven days, weight loss had been almost restored, but plasma glucose concentrations remained depressed. Ten days after the injection of kainic acid the weights of all the animals were greater than the pre-injection values, and plasma glucose levels were similar to those observed in normal, well nourished rats. Animals which had received intrastriatal injections of CSF displayed no overt abnormalities of motor or of feeding behaviour at any time over the 10 days.

Following pharmacological challenge with muscimol (1.5 mg/kg) the behavioural changes were exactly those described previously, and were the same for both sham-lesioned and lesioned groups of animals. The stereotyped behaviour observed following apomorphine (1.0 mg/kg) conformed with that described in the literature for this particular, partly restrained, animal model (McCulloch et al. 1982c). No consistent asymmetry of posture or orientation of responses was observed from the lesioned animals following either drug treatment.

3.2 Histology: Histological examination of the tissue sections from the group of animals which had been perfusion-fixed on sacrifice, revealed a pattern of damage which was generally restricted to the caudate nucleus (approximately two thirds of total volume affected) and the overlying cortex through which the needle had passed. However, in two of the animals, kainic acid-associated damage was observed in the ipsilateral hippocampus corresponding to terminal field CA3. No damage was observed in any other area spatially removed from the injection site, or in the contralateral hemisphere. In animals subjected to intrastriatal injections of CSF, damage was restricted to the needle tract.

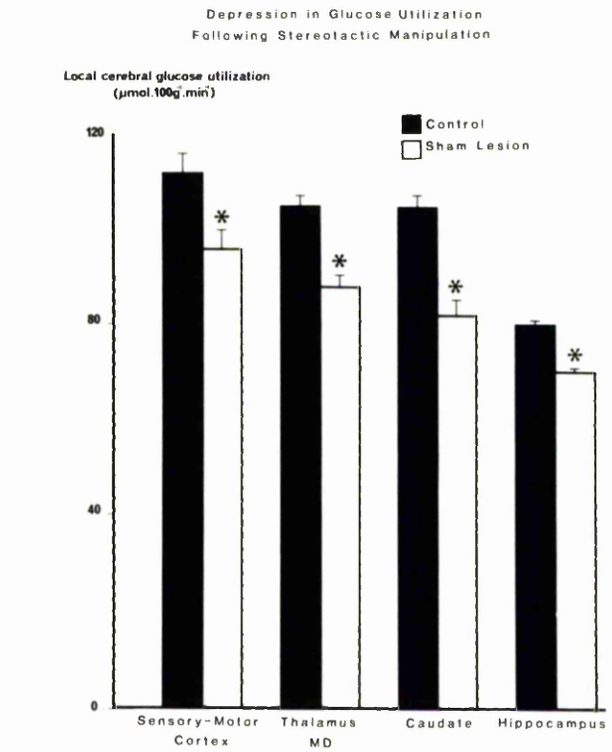
Although the quality of frozen sections precludes the detection of subtle neuropathological changes in cellular morphology, the pattern of damage observed in sections taken from the 2-DG experimental animals, both short-term (10 days) and long-term (30 days) survival, and those subjected to subsequent pharmacological challenges, closely resembled that found in the parallel histological study. The greatest damage observed was in the caudate nucleus and overlying cortex but, in three of the animals, a limited degree of damage was found in ipsilateral hippocampus. In CSF-injected animals, mechanical damage was evident around the needle tract. Frozen sections from the septal lesioned animal revealed extensive damage in the injected septum and bilaterally in hippocampus,

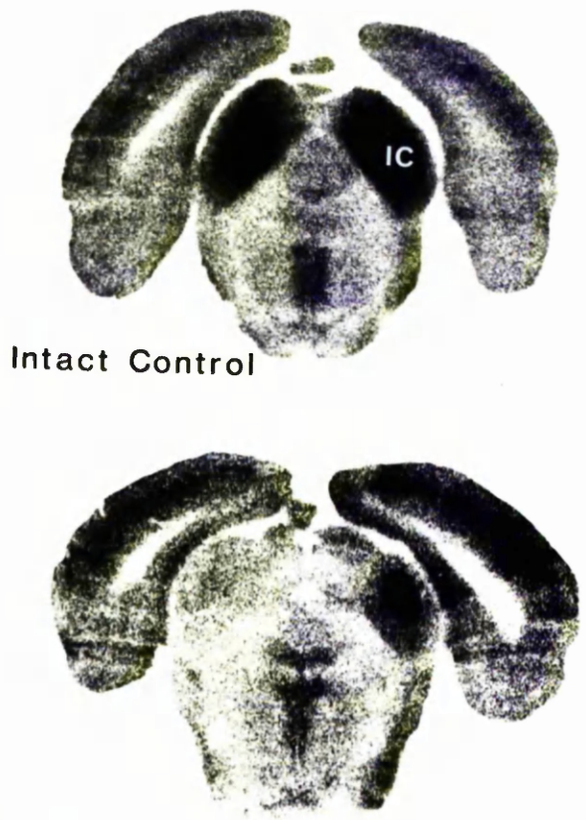
but with limited involvement of ipsilateral caudate or contralateral septal nuclei.

3.3 Local cerebral glucose utilisation: In animals which had been subjected to the stereotactic injection of CSF, glucose utilisation was significantly reduced bilaterally in a large number of functionally diverse structures when compared to intact, saline-injected control animals (Fig. 24). In some sham-lesioned animals, the pattern of glucose utilisation in primary auditory structures was very different from intact controls (Fig. 24), suggested an altered sensory input, possibly accounting for generalised, lowered arousal.

Alterations in local cerebral glucose utilisation were measured in structures both ipsi- and contralateral to the striatal lesion site when compared with the corresponding side of CSF-injected controls. The most pronounced changes, which were immediately apparent from a visual inspection of the autoradiograms, were observed within the histologically defined limits of the lesion site in the caudate nucleus and in areas which correspond to the primary sites of striatal efferent termination, in the ipsilateral globus pallidus (GP), entopeduncular nucleus (EP) and substantia nigra pars reticulata (SNR) (Fig. 25). Although diminished somewhat in intensity, these areas of change remained discernible 30 days after induction of the lesion. Within the histologically defined limits of the lesion site, ^{14}C-labelled leucine incorporation was reduced by around 35% when compared to the intact striatum (Fig. 26).

Figure 24





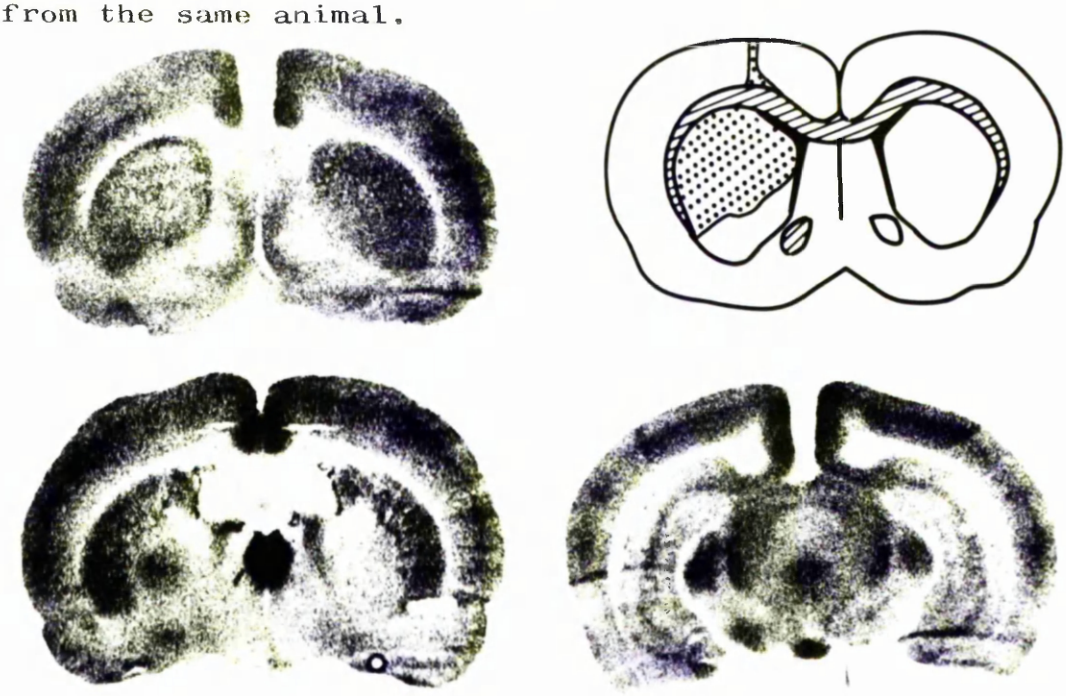
Upper: Examples of reduced glucose use in functionally diverse areas following sham striatal lesion ($*p < 0.05$).
Lower: Autoradiograms showing asymmetrical reductions in OD of inferior colliculi (IC) following sham striatal lesion, on left of image.

Figure 25

Autoradiograms taken from coronal sections of rat brain following unilateral kainic acid-induced striatal lesions.

Upper left: Autoradiogram at the level of the caudate nucleus. Marked reductions in OD are clearly visible in lesioned caudate (l.h.s.).

Upper right: Schematic diagram of extent of striatal damage mapped from cresyl violet stained sections taken from the same animal.



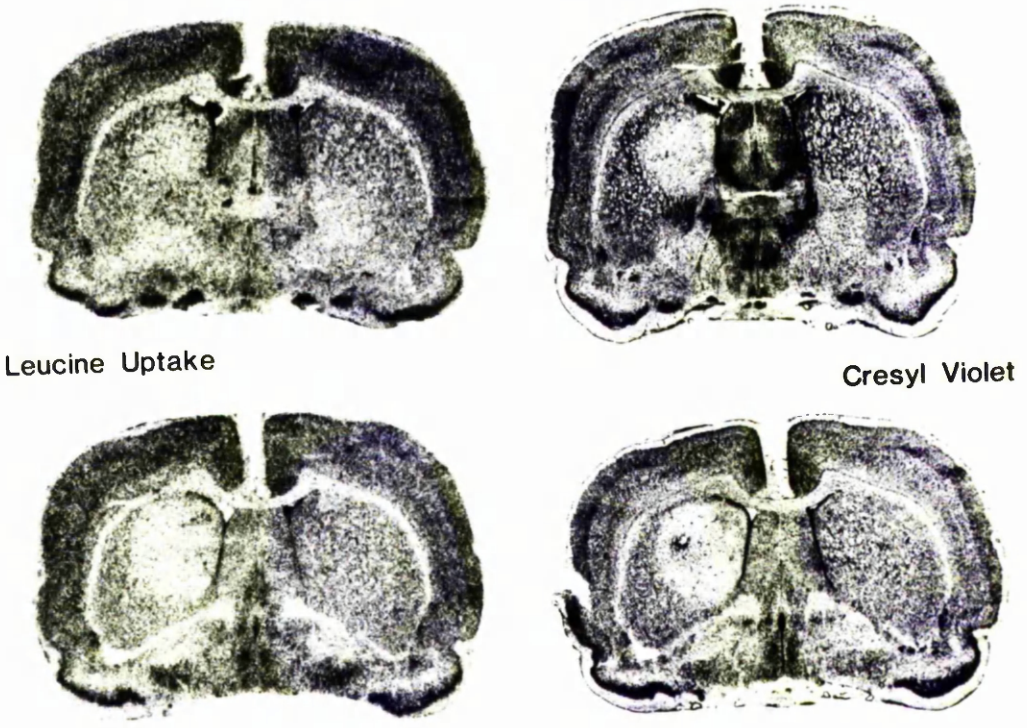
Lower left: Autoradiogram at the level of the globus pallidus. Marked increases in OD are clearly visible in globus pallidus ipsilateral to lesion site.

Lower right: Autoradiogram at the level of the substantia nigra. Marked increases in OD are clearly visible in SNR ipsilateral to lesion site.

The increases in OD, in GP and SNR represented increases in glucose use in these areas (see Fig. 28).

Figure 26

Coronal sections of rat brain at two levels of caudate nucleus following unilateral kainic acid-induced striatal lesions.



Left (upper and lower): Subtle reductions in ^{14}C-leucine accumulation in lesioned caudate nucleus (l.h.s.).

Right (upper and lower): Sections consecutive to those used for autoradiograms showing reduced Nissl substance in lesioned caudate nucleus (l.h.s.).

The limits of the areas of reduced OD and reduced Nissl substance are remarkably coincident.

The uptake of leucine ipsilateral to the lesion site increased in GP and SNR by 10% and 8%, respectively. No other side-to-side differences were observed.

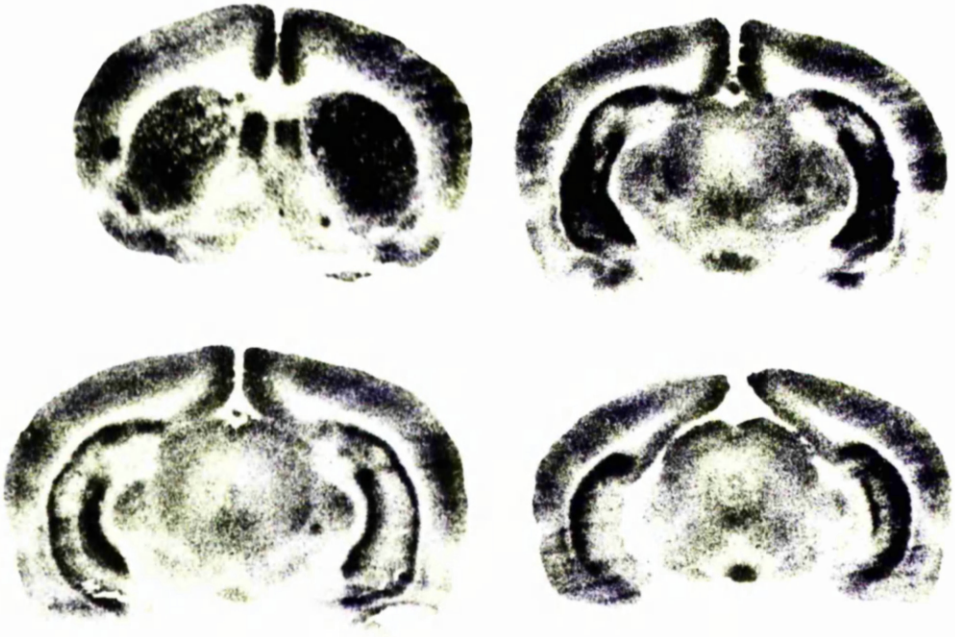
The pattern of response to intraseptal injection of kainic acid, which was also visually striking, involved an increase in OD in the lesioned septal nucleus and also bilaterally throughout all levels of the hippocampal formation (Fig. 27). Within both of these areas, microscopic analysis of the frozen sections revealed marked morphological changes associated with damage. However, around the injection site in ipsilateral caudate and contralateral septal nucleus, where damage was apparently minimal, OD changes were also observed (Fig. 27). In caudate OD was decreased, but was increased in septum.

Quantification of the optical density changes in primary striatal projection areas, ipsilateral to the lesioned striatum, revealed large increases in glucose utilisation in GP and SNR (82% and 74%, respectively; see Fig. 28), but a numerically smaller (36%) increase in the EP (Fig. 28) when compared to the appropriate side of the sham-lesioned brains. These increases were significantly less when measured 30 days after the placement of the lesion, but glucose use was still elevated by between 20% (EP) and 50% (SNR and GP).

Despite exerting little or no effect in sham-lesioned rat brains, muscimol administered at 1.5 mg/kg, i.v., significantly attenuated the increases in glucose utilisation which followed removal of striatal

Figure 27

Autoradiograms from coronal sections of rat brain following injection of kainic acid into the septal nucleus.



Upper left: Autoradiogram taken at the level of the caudate nucleus. Bilateral increases in OD in septal nuclei and small unilateral (l.h.s.) decreases in OD in mediodorsal caudate.

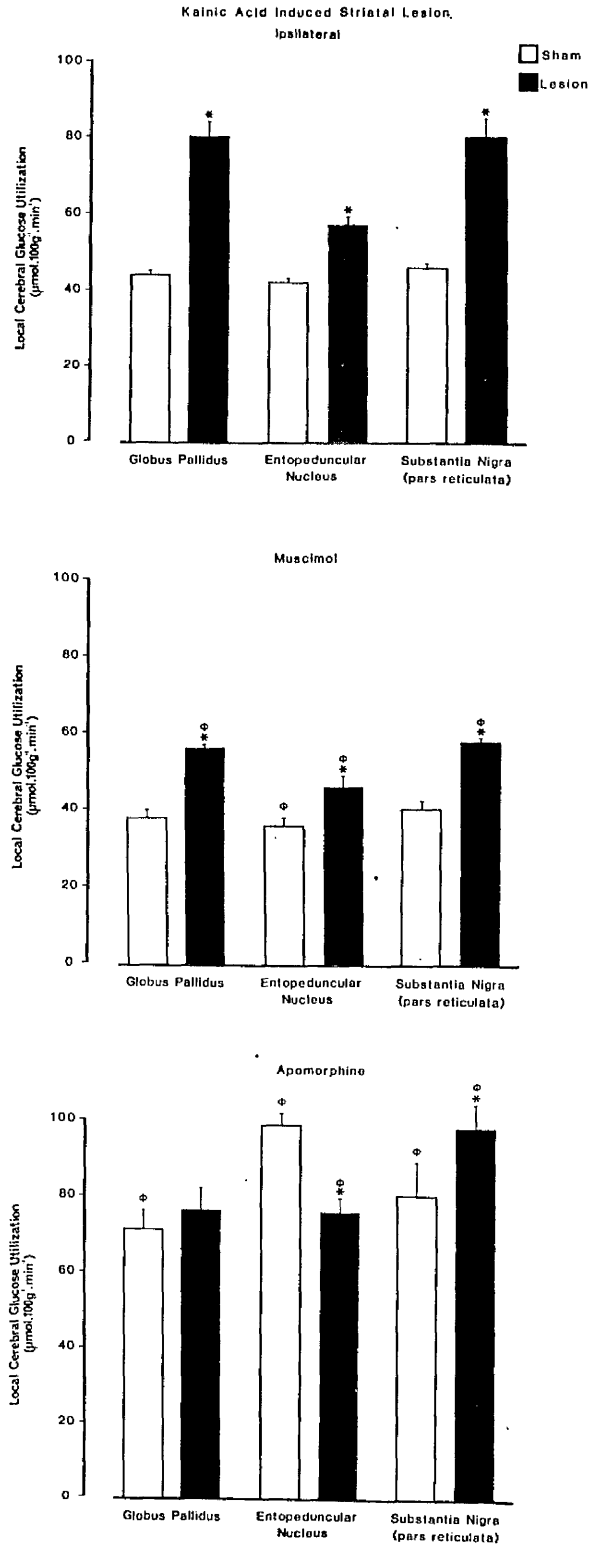
Upper right, lower left and right: Autoradiograms taken at different levels of the hippocampal formation showing marked bilateral increases in OD. These areas of deranged isotopic tracer accumulation were coincident with areas of pathological changes in cellular morphology observed in cresyl violet stained sections.

input to these three ipsilateral areas (Fig. 28). However, at this dose, rates of glucose use did not return completely to basal levels, except in the EP which appeared to be more sensitive to the action of muscimol, being alone in displaying a small decrease at the limits of significance when the agent was delivered to sham-lesioned animals.

In contrast to the limited effects of muscimol in the three areas of sham-lesioned brains, apomorphine administration (1.0 mg/kg) resulted in large, significant increases in glucose use of 136% in EP, 117% in SNR, and 73% in GP, which approached, or exceeded, the changes observed following striatal lesions alone (Fig. 28). The two treatments together did not, however, produce a summation of the two responses. Although significant increases in the kainic acid lesion responses were observed in EP (33%) and SNR (23%), only in SNR did the two treatments (striatal lesion and apomorphine challenge) produce a level of glucose utilisation which was greater than with either treatment alone (Fig. 28), whilst in the EP the presence of the striatal lesion in fact significantly attenuated the apomorphine-induced increase in glucose use (Fig. 28). In the GP of lesioned brains, apomorphine treatment resulted in a small, non-significant decrease in glucose use when compared to saline-treated lesioned animals (Fig. 28).

Despite the absence of any measurable change in functional activity in the caudate nucleus contralateral

Figure 28



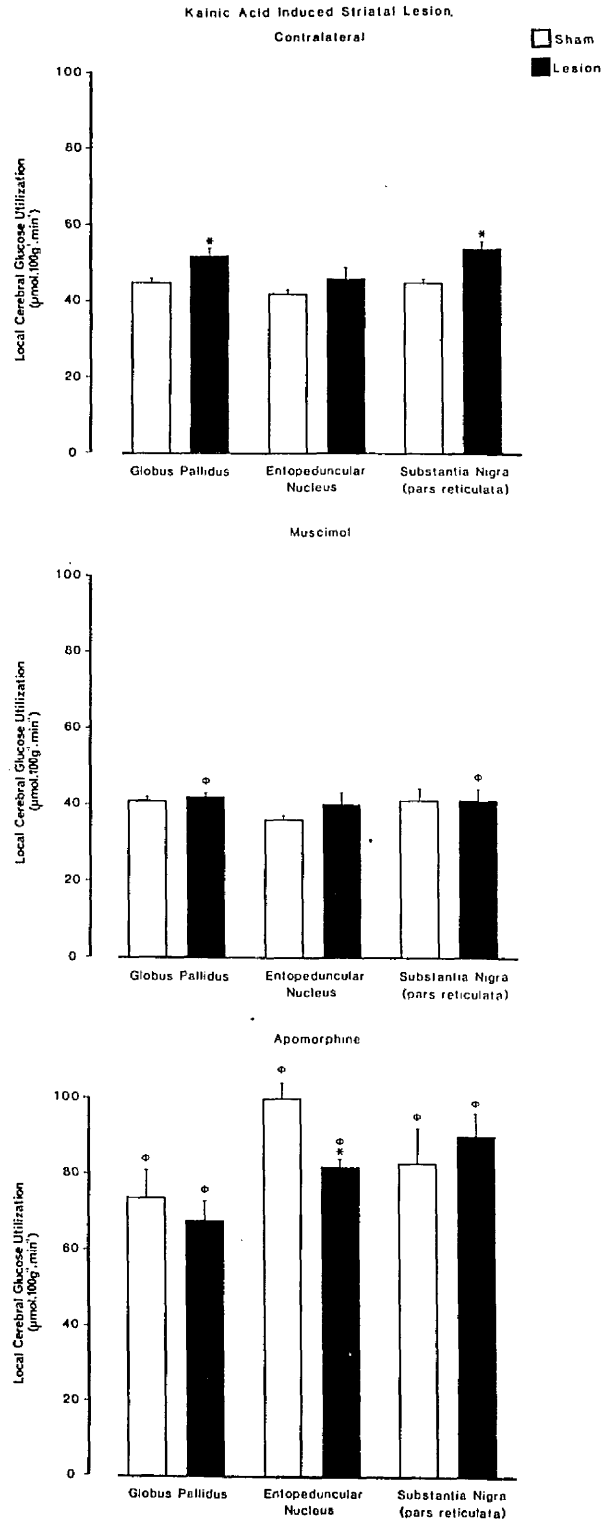
Rates of glucose use in ipsilateral area of primary striatal projection following unilateral striatal lesioning (upper) and subsequent challenge with (middle) muscimol, 1.5 mg/kg, or (lower) apomorphine 1.0 mg/kg. Comparisons between sham and lesioned treatments for each area ($*p < 0.05$) and between saline and drug challenges for each area ($\phi p < 0.05$).

to the lesion site, increased glucose use was also measured in the contralateral GP and SNR (Fig. 29), but although these metabolic alterations were highly significant, they were quantitatively less marked (16% and 20% in GP and SNR, respectively) than those in the same regions of the ipsilateral hemisphere. No significant change was measured in the contralateral EP in which mean glucose use increased by less than 10%.

Whilst muscimol at the dose used merely attenuated the effect of striatal lesions upon ipsilateral GP and SNR, the contralateral increases in glucose use were completely eliminated by muscimol challenge (Fig. 29), to the extent that no significant differences could be found between the sham and lesioned groups which had received muscimol (cf. a maintenance of a 45-50% differential ipsilaterally). As was observed on the ipsilateral side, the contralateral EP of sham-lesioned brains displayed a decrease in glucose use in response to muscimol which just reached over the limits of significance, but in brains of lesioned animals muscimol did not reduce glucose utilisation to the same low levels, although there was no significant difference between the two means (Fig. 29).

In animals subjected to sham-lesioning procedures, the large increases in glucose use which were measured in the ipsilateral areas of striatal projection following apomorphine administration were also observed in the corresponding areas in the contralateral hemisphere (Fig. 29). Administration of apomorphine to lesioned

Figure 29



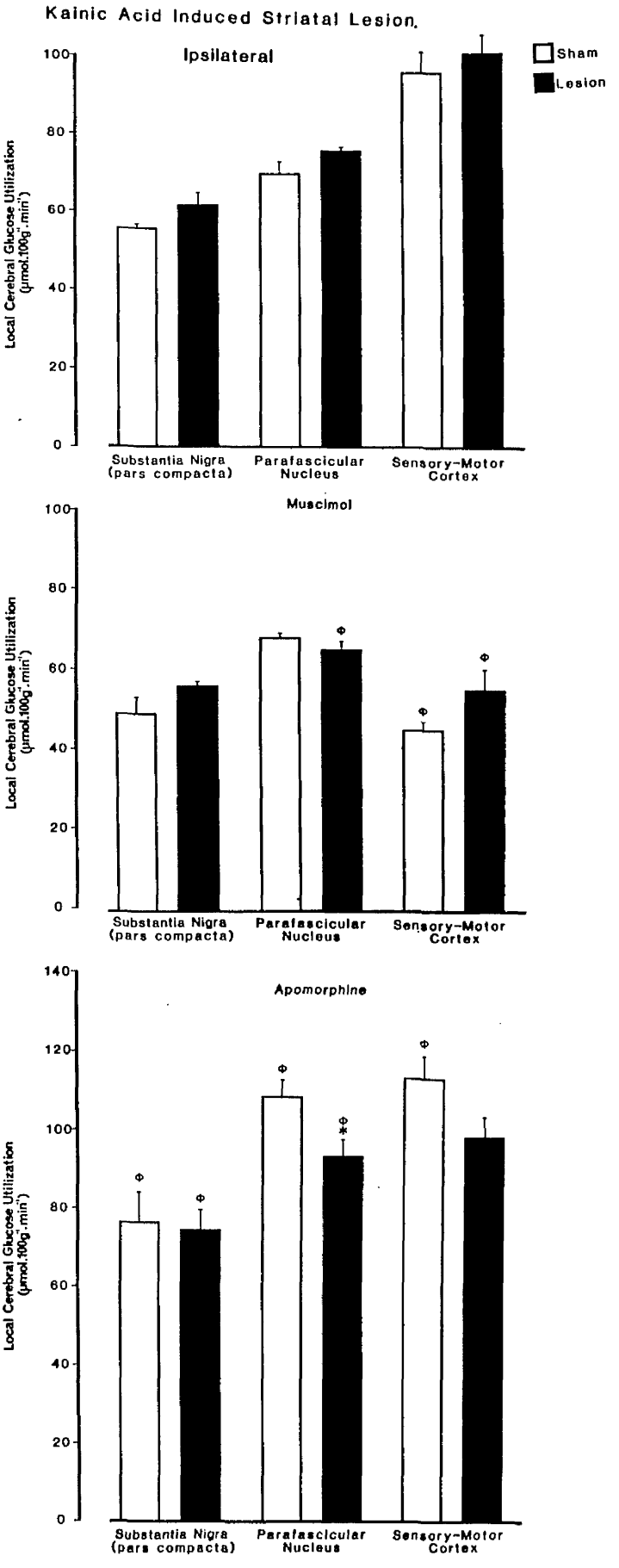
Rates of glucose use in area of contralateral primary striatal projection following unilateral striatal lesioning (upper) and subsequent challenge with (middle) muscimol 1.5 mg/kg or (lower) apomorphine 1.0 mg/kg. Comparisons were made between sham and lesioned treatment for each area ($*p < 0.05$) and between saline and drug challenges for each area ($\phi p < 0.05$).

animals increased glucose use in the contralateral GP, EP and SNR (Fig. 29) to levels above those observed following lesions alone, although again no summation of the two effects was evident. Indeed, in both GP and SNR no significant difference was apparent between sham-lesioned and lesioned brains which had been treated with apomorphine. Only in EP, where the effects of the lesion alone were least marked, was the apomorphine response significantly different in sham and lesioned groups (Fig. 29). The presence of a contralateral striatal lesion attenuated the apomorphine response in EP by 18%, a quantitatively similar response to the attenuation observed in the ipsilateral EP.

In contrast to the asymmetric, more marked ipsilateral changes in functional activity measured in striatal outflow areas, the source of striatal afferent connections from primarily motor areas, the pars compacta of the substantia nigra (SNC), parafascicular nucleus of the thalamus (PFN) and sensory-motor cortex (SMCx) displayed significant contralateral increases in glucose utilisation of 14%, 21% and 15%, respectively (Fig. 31). No significant changes were observed in these areas ipsilateral to striatal lesions (Fig. 30).

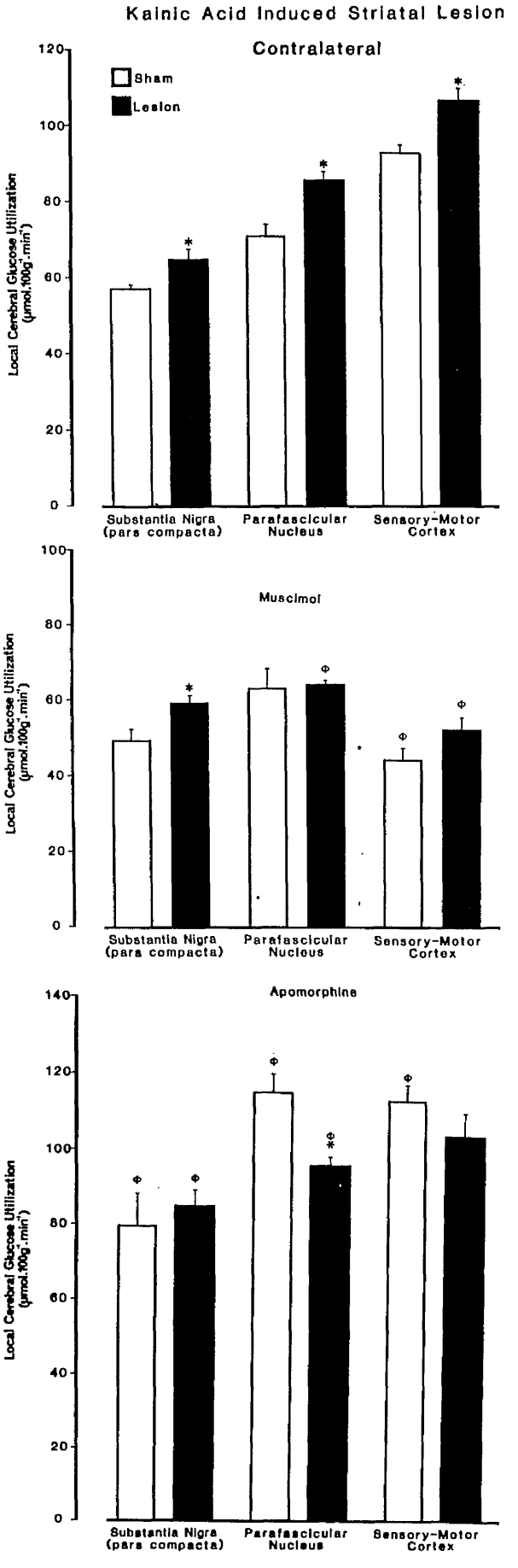
Muscimol at the dose used did not result in significantly altered glucose use in contralateral SNC of either sham-lesioned or lesioned animals, and thus a significant 20% difference (Fig. 31) was maintained between the mean values of the two groups. Significant attenuation of the lesion-induced increases in

Figure 30



Rates of glucose use in areas projecting to ipsilateral striatum (see legend to Fig. 28).

Figure 31



Rates of glucose use in areas projecting to contralateral striatum (see legend to Fig. 28).

functional activity was evident in both PFN and SMCx following muscimol administration, such that a significant difference was no longer found between sham and lesioned groups (Fig. 31). In view of the general sensitivity of thalamic areas in intact animals to the actions of systemically administered muscimol (see previuously reported data), it is of interest that no significant response to the drug was measured in the PFN of sham animals, either contralaterally or ipsilaterally to the site of intervention. However, a significant decrease in glucose use from saline-injected control levels was found when muscimol was injected into lesioned animals both ipsilaterally, where the lesion itself had little effect, as well as contralaterally, where a significant lesion-induced increase in functional activity was apparent (Figs. 30 and 31).

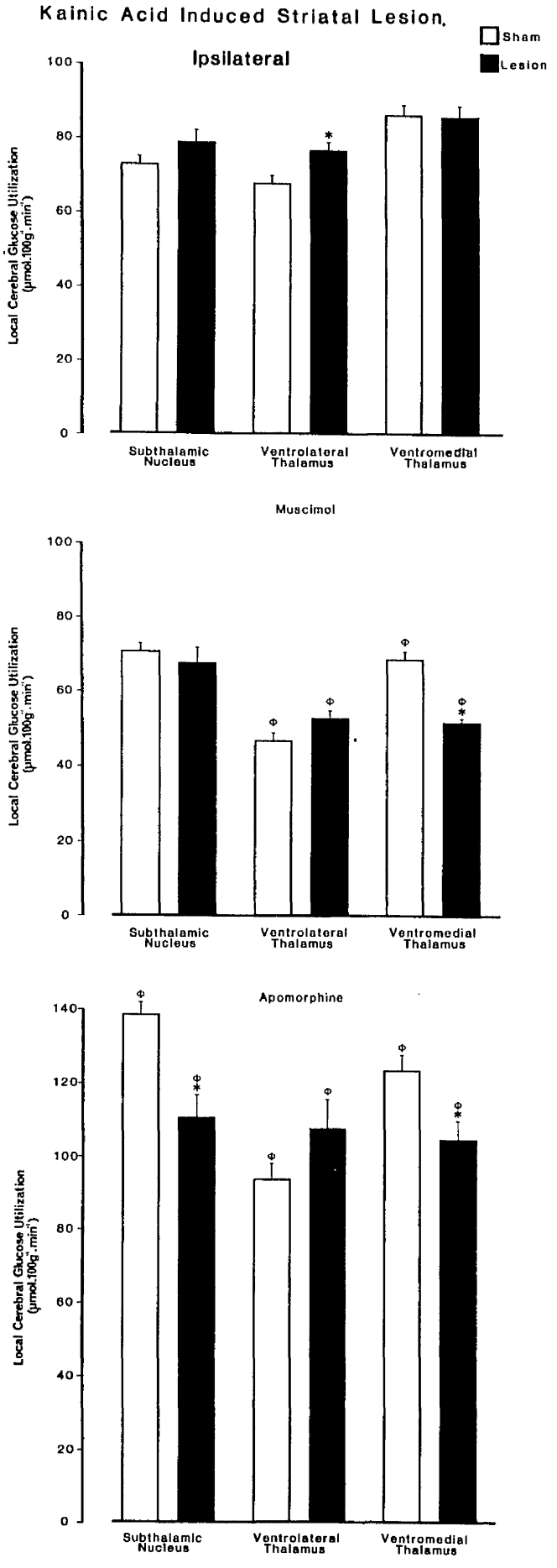
Apomorphine administration produced significant increases in glucose use in SNC, PFN and SMCx of sham-lesioned animals to a similar degree both ipsi- and contralateral to the striatal intervention site (Figs. 30 and 31). Although significant bilateral increases were also measured in SNC and PFN of lesioned animals, in PFN the apomorphine response was significantly less than that found in sham-operated rats (Figs. 30 and 31). Again, however, there was no evidence for summation of lesion-induced and drug-induced increases in glucose utilisation and, indeed, in PFN the response to apomorphine was severely attenuated from around 60% in the sham group to 23% ipsilateral and 12% contralateral

to the striatal lesion (Figs. 30 and 31). The rate of glucose use in the SMCx of lesioned animals was not significantly different from that observed in the sham-lesioned rats following apomorphine, but the level of functional activation was no greater than that measured following lesion alone. Thus, bilaterally in this area of cortex, no response to apomorphine was found following unilateral striatal lesion (Figs. 30 and 31).

Regions of the diencephalon, described in functional terms as subserving motor activity and which receive projections from the striato-pallidal-nigral system, showed no consistency in their responses to unilateral striatal lesions, despite the fact that increases in functional activation were apparent at the sources of afferent fibres in the extrapyramidal system. In the subthalamic nucleus (afferent connections with GP) and ventromedial thalamus (afferent connections with SNR) no significant response was observed ipsilateral to the lesioned striatum (Fig. 32). However, within the ventrolateral thalamic complex (afferent connections with GP) a small (13%), but significant increase in the rate of glucose utilisation was measured ipsilaterally (Fig. 32).

As observed previously, thalamic nuclei appear to be very sensitive to the actions of systemically administered muscimol. In sham-operated animals a significant decrease in glucose utilisation was measured in both ipsilateral ventrolateral and ventromedial thalamus following 1.5 mg/kg muscimol delivered intravenously

Figure 32



Rates of glucose use in areas of secondary ipsilateral striatal projection (see Fig. 28).

(Fig. 32). The lesion-induced increase in glucose utilisation in the ventrolateral complex was attenuated (Δ - 31%) with muscimol at this dose, such that no significant difference was discernible between sham and lesioned groups following drug treatment (Fig. 32). Despite the lack of any apparent change in glucose use in the ventromedial thalamus in response to striatal lesion, the decrease in functional activity following muscimol was significantly greater in the lesioned animals (Δ - 45%) than in those subjected to sham intervention (Δ - 22%). No alteration in the rate of glucose use in subthalamic nucleus was noted in response to muscimol at the dose used (Fig. 32).

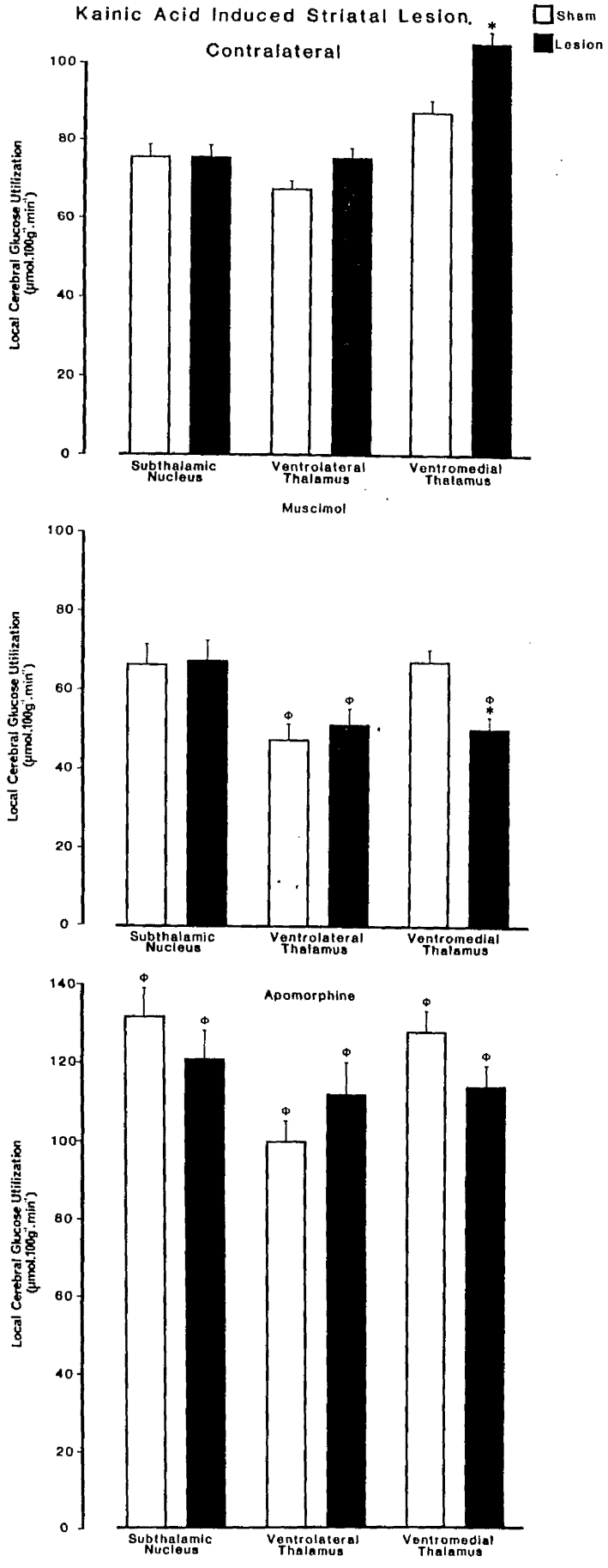
In all three ipsilateral diencephalic motor areas, glucose use was significantly increased in sham-operated animals following apomorphine administration (1.0 mg/kg). The most pronounced changes were observed in the subthalamic nucleus where an increase of 90% was observed over saline-injected animals, but relatively large increases in glucose use (around 40%) were also measured in the thalamic nuclei. The response to apomorphine in the subthalamic nucleus was radically attenuated in the absence of striatal efferent fibres, a much smaller (40%) increase in glucose utilisation being measured in lesioned animals (Fig. 32). Similarly, the increased functional activity of the ventromedial thalamus associated with apomorphine treatment was significantly reduced (again by about half) in the presence of striatal lesions (Fig. 32). In marked

contrast to these attenuating effects of the lesions upon responsiveness to apomorphine in the ventrolateral thalamus, which alone displayed any significant alteration in glucose use following removal of striatal perikarya, the differential between the sham and lesioned groups (13%) was maintained following apomorphine (15%), although the greater degree of variability associated with the two treatments together rendered the difference no longer statistically significant (Fig. 32).

In these diencephalic regions contralateral to the striatal lesions, only within the ventromedial thalamus was any significant change in glucose use observed (Δ + 20%) (Fig. 33). Although the ventrolateral thalamic complex displayed an increase in glucose use of a magnitude (11%) similar to that observed ipsilaterally (13%), the contralateral effects did not achieve statistical significance (Fig. 33).

Following systemic administration of muscimol, no significant difference was observed in the response between sham and lesioned animals in either the contralateral subthalamic nucleus (Δ - 10%) or the ventrolateral thalamus (Δ - 30%) (Fig. 33). However, muscimol not only attenuated the kainic acid lesion-induced increases in the rate of glucose use in the ventromedial thalamus (Δ - 52%) but, as was the case ipsilateral to the lesioned striatum, functional activity was reduced to a level significantly less than that of sham-lesioned animals following muscimol treatment

Figure 33



Rates of glucose use in areas of secondary contralateral striatal projection (see Fig. 28).

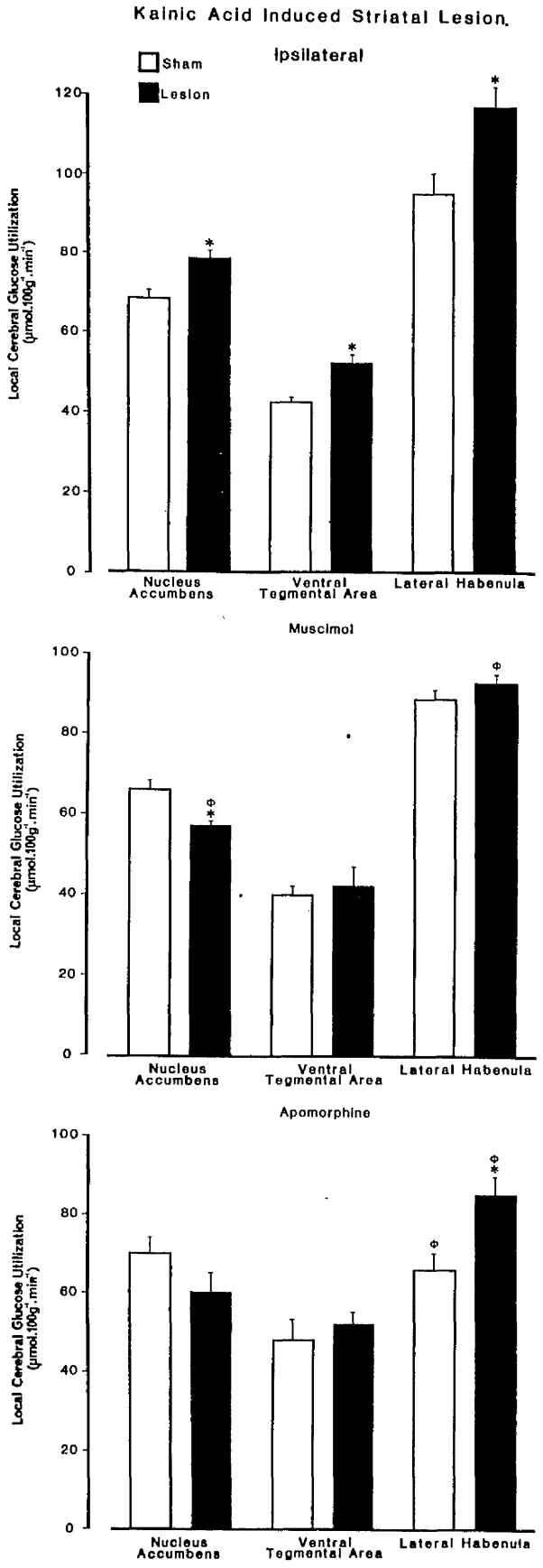
(Fig. 33).

A similar consistency of response between sham and lesioned groups was found in contralateral subthalamic nucleus and ventrolateral thalamus where, as in the ipsilateral hemisphere, large increases in functional activity resulted from apomorphine administration. Whilst glucose utilisation in the ventromedial thalamus of sham-operated animals was significantly increased by apomorphine (Δ + 45%), a minimal (Δ + 7%), non-significant increase was found above that following lesion alone. The responsiveness of the ventromedial thalamus to apomorphine thus appears to be limited by contralateral striatal lesions.

Within the elements of the limbic system, which possess direct afferent or efferent neuronal connections with the extrapyramidal system, the nucleus accumbens (NAc), ventral tegmental area (VTA) and lateral habenula (LH), significant ipsilateral increases in glucose utilisation were measured following striatal lesions (Fig. 34). The changes ranged from +15% in NAc to +23% in VTA and LH where the associated increase in tracer accumulation was clearly discernible from the autoradiograms (Fig. 35).

No significant response to muscimol treatment was observed in these limbic areas of sham-lesioned animals. However, the ipsilateral lesion-induced increases in glucose use were attenuated to levels equivalent to or, in NAc, significantly less than in the sham group (Fig. 34), revealing a sensitivity to the GABA agonist not in

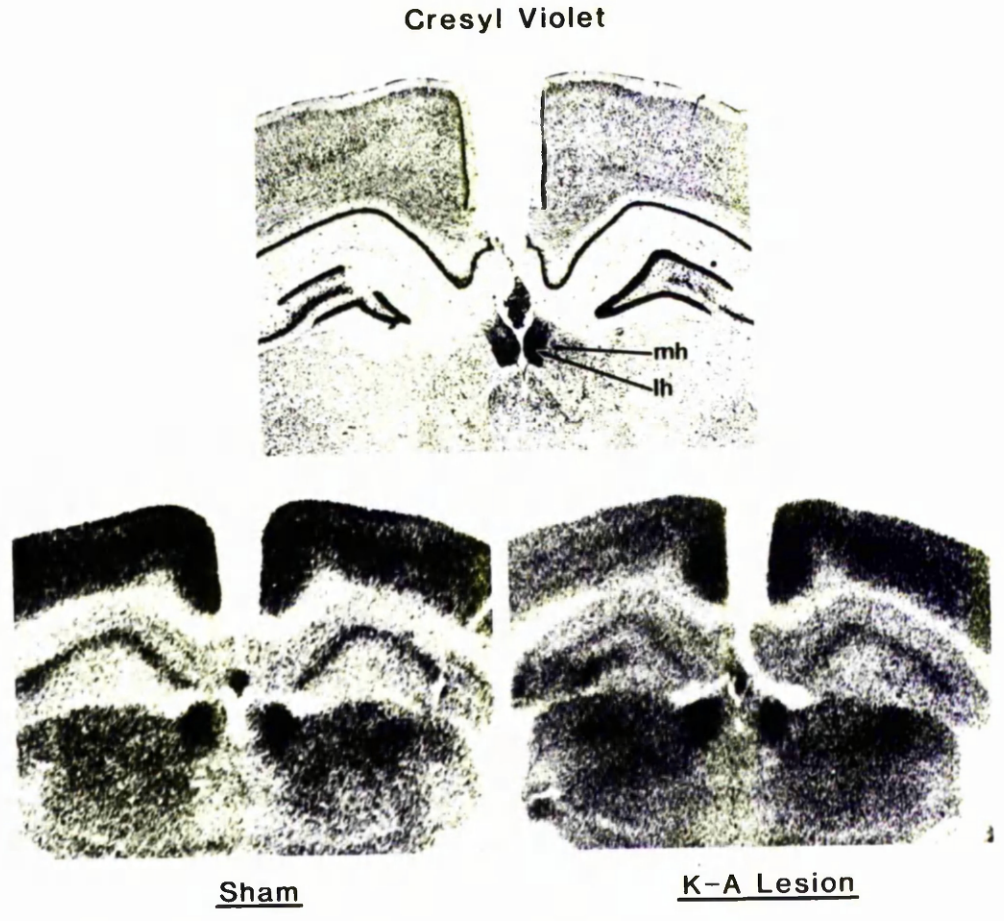
Figure 34



Rates of glucose use in non-motor areas associated with ipsilateral extrapyramidal system (see legend to Fig. 28).

Figure 35

Autoradiograms from coronal sections of rat brain at the level of the lateral habenula.



Upper: Cresyl violet stained section showing medial (mh) and bilateral (lh) habenula.

Lower left: Autoradiogram of the lateral habenular nuclei in a sham striatal lesioned rat. Symmetrical OD in both nuclei (see Figs. 34 and 36).

Lower right: Autoradiogram of the lateral habenular nuclei in a rat with unilateral striatal lesion. A marked increase in OD in ipsilateral habenula (l.h.s.) results in an asymmetrical pattern (see Figs. 34 and 36).

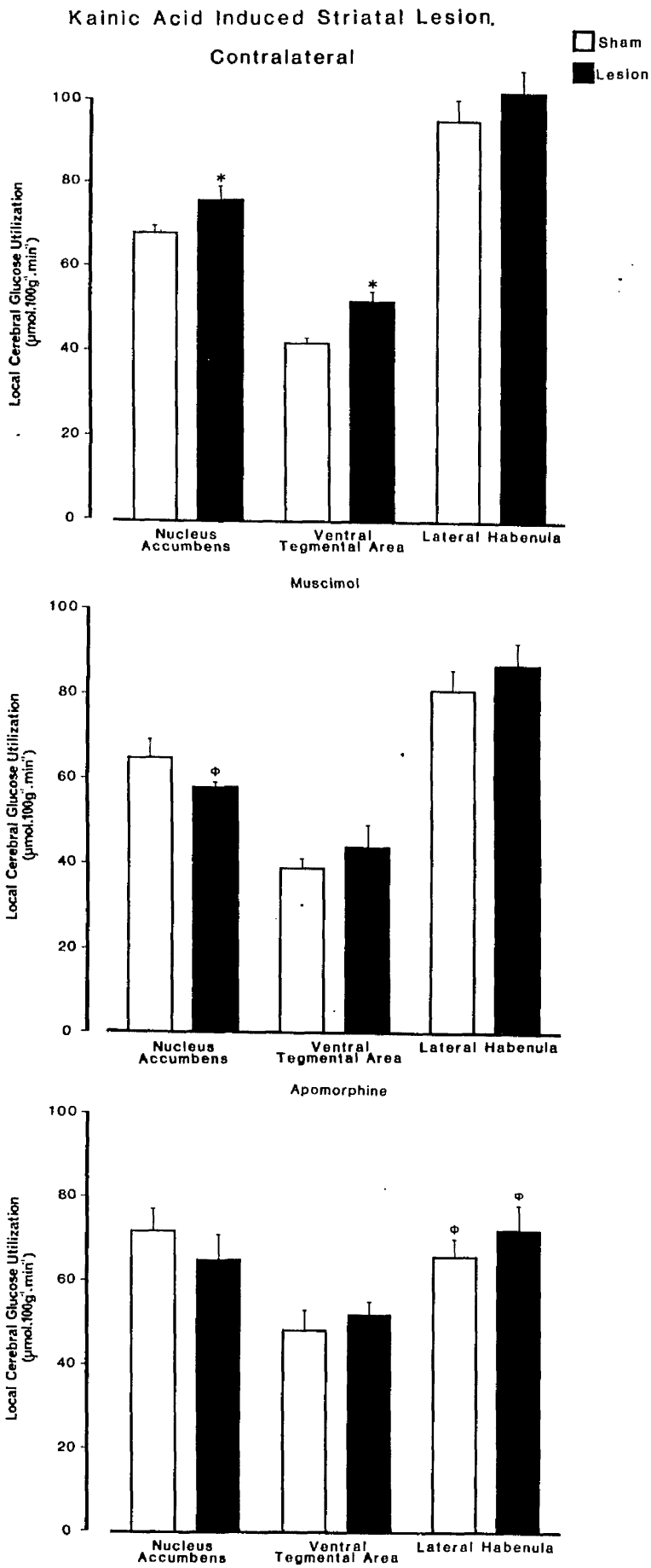
evidence in intact animals.

Only in LH was any significant alteration in glucose utilisation in response to apomorphine treatment observed ipsilaterally (Fig. 34). A large (30%) reduction in functional activity was measured in this region of sham-lesioned animals. A similar magnitude (-27%) of change was also found in response to apomorphine in lesioned animals, but this was sufficient to reduce glucose utilisation to levels significantly higher than in sham, drug-treated animals, and which were equivalent to those observed in saline-treated, sham-lesioned animals.

The altered glucose utilisation in evidence ipsilaterally was paralleled in magnitude by significant contralateral changes in NCa and VTA, but not LH (Fig. 36). The responses to pharmacological challenge were also similar to those measured on the ipsilateral side, with attenuation of lesion-induced increases by muscimol and no significant response to apomorphine in either NAc or VTA. However, the contralateral LH displayed an equal degree of responsiveness to apomorphine in both sham (-30%) and lesioned (-29%) animals in contrast to the significant difference found in the ipsilateral structure.

Alterations in glucose use following kainic acid-induced striatal lesions were also observed in some other brain regions, most notably the red nuclei where bilateral, 16% increases were measured. These results, as well as those from other brain areas, are shown in Appendix V.

Figure 36



Rates of glucose use in non-motor areas associated with contralateral extrapyramidal system (see legend to Fig. 28).

3.4 Commentary

3.4.1 Kainic acid in conjunction with 2-DG autoradiography: The development of the 2-deoxyglucose technique to map function-related glucose use (Sokoloff, 1977) and the introduction of kainic acid to effect selective lesions of neuronal perikarya (Coyle et al. 1978) represent two major advances for neuroscience research in the recent past. The stereotactic injection of kainic acid, a neurotoxic analogue of glutamate, results in selective destruction of nerve cell bodies within the injected region, whilst afferents and fibres of passage are left largely intact (Coyle et al. 1978). Although the selectivity of action of kainic acid constitutes a major advantage over other lesioning techniques, such as electrolytic lesioning, the frequency with which structural damage occurs in areas outwith the injection site (Coyle et al. 1978; Schwob et al. 1980) emphasises the need for careful histological monitoring whenever this method is employed.

The combination of the two experimental approaches, whether with systemic (Collins et al. 1980) or intracerebral administration of kainic acid (Kimura et al. 1980; Wooten and Collins, 1980), results in visually spectacular alterations in the appearance of the autoradiographic pattern of deoxyglucose uptake. However, meaningful interpretation of these observations can present considerable difficulties. Strong doubt exists as to whether deoxyglucose accumulation in damaged CNS

tissue truly represents the rate of glucose phosphorylation, particularly in view of the large, dynamic alterations in the value of the "lumped constant" (a crucial determinant of deoxyglucose uptake) which have been reported to occur in damaged tissue (Ginsberg and Reivich, 1979). It is tempting to conclude from the present study that the measured reduction (18%) of glucose utilisation in the striatum into which kainic acid was injected 10 days previously represents a loss of contribution from interneurons and efferent perikarya to the overall energy consumption of the striatum. However, it is doubtful whether the measured rate of glucose phosphorylation is at all accurate. Even if the measurement was correct, it is unlikely that the rate of glucose utilisation in damaged tissue would bear a direct relationship to energy production, as it does in intact CNS tissue, in view of the accumulation of lactate which almost invariably occurs in damaged tissue (Folbegrova et al. 1974).

In a recent report (Wooten and Collins, 1980) widespread, pronounced alterations in deoxyglucose uptake were reported in the acute and sub-acute period following intrastriatal injections of kainic acid. However, widespread neuronal necrosis and glial reaction paralleled the disordered uptake of deoxyglucose. Although others have reported neuropathological changes in distant regions following intrastriatal application of kainic acid (Coyle et al. 1978; Schwob et al. 1980; Zaczek et al. 1980), they have rarely been as extensive

as those reported in that previous study with 2-deoxy-glucose, where damage involved hippocampus, nucleus accumbens, globus pallidus, amygdala, frontal and entorhinal cortices, etc. In view of the widespread occurrence of structural damage, interpretation of the measured deoxyglucose uptake in terms of glucose utilisation is fraught with difficulties. In the present studies, discrete lesions restricted to the caudate nucleus resulted from intrastriatal injections of kainic acid. Damage was absent from adjacent areas such as the septal nucleus, globus pallidus and nucleus accumbens. Within a discrete region of the ipsilateral hippocampus, the dorsal aspect of terminal field CA3, a limited degree of structural damage was evident in three of the six lesioned animals. As with the caudate nucleus where damage was present, it is uncertain whether the measured alteration in deoxyglucose uptake accurately reflects altered glucose utilisation. The limited extent of damage to the hippocampus and the consistency in the pattern of altered glucose use in the extrapyramidal system, irrespective of the presence or absence of hippocampal damage (half of the experimental group in each category), allow a greater credibility to be attached to the measured changes in glucose use found in these animals. However, the marked differences which have been reported previously in the distribution of neuropathological alterations following intrastriatal injections of kainic acid emphasise the necessity for careful histological

verification of the extent of the lesion and involvement of areas outwith the injection site. Differences in the extent of the lesion produced are likely to result from technical considerations such as the volume and rate of injection, dose, period during which the needle remains in situ, and the choice of anaesthesia. However, unexpected variables - impurities in the kainic acid itself, for example - may also contribute to the degree of resultant damage.

A major difference between the present study and previous investigations into the consequences of local kainic acid injection upon glucose use and neuronal damage lies in the minimal, unilateral changes observed in this series, and the marked bilateral increase in deoxyglucose uptake observed formerly in the hippocampus. In one additional animal the kainic acid injection was aimed at the septal nucleus, which resulted in a discrete area of damage with minimal involvement of either the contralateral septum or the ipsilateral striatum. In this animal, 10 days later, the bilateral pattern of increased deoxyglucose uptake and structural damage observed in hippocampus was similar to those changes reported previously following putatively local intrastriatal injections of kainic acid.

Two additional features should be emphasised in relation to the investigation of glucose utilisation using stereotactic lesions within the extrapyramidal system. Whilst glucose utilisation constitutes, almost exclusively, the energy generating pathway in

cerebral tissues of well-nourished animals, kainic acid lesions of the striatum disrupt feeding behaviour (Sanberg and Fibiger, 1979), resulting probably in substrates other than glucose being used to meet the functional demands of brain cells (Hawkins et al. 1971). The aphagia and the metabolic demands of increased motor activity, both associated with intrastriatal kainic acid injections (Sanberg and Fibiger, 1979), resulted in severe weight loss and hypoglycaemia for up to seven days afterward. Functional activity reflects meaningfully upon glucose utilisation only when normal feeding has returned and normal glycaemic conditions prevail, generally about 9-10 days after the striatal lesion. In addition to the nutritional problems following kainic acid, the sham intervention (i.e., the injection of mock cerebrospinal fluid into the striatum) was associated with subtle functional alterations in the CNS, and consequently altered glucose utilisation. Glucose use was reduced in all primary auditory areas of the CNS (inferior colliculus, medial geniculate body, auditory cortex, etc.), possibly as a direct result of mechanical damage during placement of the ear bars for stereotactic surgery. Moreover, small (10-15%) reductions in glucose use in sham animals were observed in regions of the CNS not primarily concerned with processing auditory information (e.g., mediodorsal thalamus, subthalamic nucleus and hippocampus) when compared with unoperated animals used in this laboratory. Irrespective of whether this

subtle depression in local cerebral glucose utilisation is a consequence of the neurosurgical intervention (craniotomy, needle tract damage, etc.), or to the extensive connections auditory areas have with non-auditory regions in the CNS, they emphasise the importance of performing rigorous control experiments, particularly when an investigative tool as powerful as 2-deoxyglucose autoradiographic mapping is employed.

3.4.2 Alterations in functional activity following striatal lesions: Both the afferent and efferent connections of the caudate nucleus have been characterised in some considerable detail. In the rat, the caudate nucleus receives fibres from the cerebral cortex, the posterior thalamus, the raphe nucleus and pars compacta of the substantia nigra (Kemp and Powell, 1971; Beckstead et al. 1979; van der Kooy, 1979; Veening et al. 1980). In contrast, the efferent fibres have a restricted distribution projecting to only two ipsilateral areas, the pallidal complex (both globus pallidus and entopeduncular nucleus) and pars reticulata of the substantia nigra (Kemp and Powell, 1971; Fonnum et al. 1978; Nagy et al. 1978; Nauta and Domesick, 1979; Veening et al. 1980). In the present study, marked increases in glucose utilisation were observed in these two areas 10 days after the selective destruction of striatal efferents with kainic acid. Striatal efferents appear to exert an inhibitory influence upon pallidal and nigral neurons (Feltz, 1971; Crossman et al. 1973) and, therefore,

the increased glucose use in these two areas is consistent with the effective removal of an inhibitory influence upon metabolic activity within these regions.

Previous observations in a limited qualitative investigation failed to reveal any increases seven days after striatal lesions with kainic acid; indeed, the tendency was toward decreases in glucose use (Wooten and Collins, 1980). The reasons for this obvious dichotomy are difficult to ascertain, other than as a result of the difficulties associated with the use of kainic acid and deoxyglucose in conjunction, and the necessity for appropriate controls (see previous discussion). However, a recent preliminary report, using a qualitative approach to deoxyglucose autoradiography (Kimura et al. 1980), demonstrated relative increases in deoxyglucose uptake in the pallidus and substantia nigra at approximately the same time after striatal kainic acid injection.

The limited studies in which leucine incorporation into protein was monitored suggest that the increased glucose utilisation observed in the ipsilateral pallidus and substantia nigra of lesioned animals may not be due exclusively to altered neuronal firing rates. The requirement for high energy phosphates, and the use which may be made of intermediate substrates from the glycolytic pathway in the increased synthesis of protein in these areas, could result in an increased demand for glucose in the absence of alterations in cell firing. The increased incorporation of

labelled leucine, however, amounted to only 10% over that of the unlesioned side, whilst changes in glucose utilisation were of the order of 50%. As glucose is unlikely to provide the major source of primary substrates for amino acid synthesis, 0.3% of entire cerebral glucose flux under normal circumstances (Siesjö, 1978), and as only a portion of the high energy phosphates produced from glycolysis will be used in the production of protein, the demand for glucose made by this moderate increase in protein synthesis would constitute only a very small fraction of the large increases measured in glucose utilisation. However, the large decreases in leucine incorporation evident in the lesioned caudate nucleus (around 30%) add further to the necessity for circumspection in the interpretation of altered glucose utilisation in this area.

The considerable derangements in measured glucose utilisation at the sites of primary striatal efferent termination are persistent, a qualitatively similar pattern remaining evident to visual inspection of the autoradiograms 30 days after the lesion. The contribution to this dramatic and long-lasting response which may be attributable to the loss of substance P- (to substantia nigra; Kanazawa et al. 1977) and enkephalin- (to globus pallidus; Cuello and Paxinos, 1978) containing striatal efferents remains unclear. It is not possible to speculate on the basis of the evidence presented here whether the effects of removal

of inhibitory GABAergic influences would be potentiated if the excitatory substance P neurons (Pasik et al. 1979) were left intact. The action of enkephalin in the globus pallidus is complicated by its putative role as a modulator of GABA induced inhibition (Nicoll et al. 1980), although a direct postsynaptic inhibitory action has also been suggested (Nicoll et al. 1977). If the latter action was predominant in the globus pallidus, then some at least of the post-lesion activation could result from loss of inhibitory enkephalin innervation.

The large scale alterations in local rates of glucose use observed in the ipsilateral pallidal complex and SNR proved to be highly susceptible to pharmacological manipulation with the GABA agonist, muscimol. The dramatic attenuation of the lesion-induced increases in glucose utilisation within these areas, at a dose of muscimol which had only minimal effects in sham operated animals, adds further weight to the conclusion that the alterations in functional activity which follow removal of input from the striatum result, in the main, from the loss of GABA-ergic inhibitory influences, with the ensuing dis-inhibition of postsynaptic neurons increasing energy demand. The contrast between the limited effects of systemically administered muscimol upon functional activity in pallidus and nigra of sham lesioned animals (also animals subjected to no intracranial intervention as shown in a previous section), and the

large reductions evident in lesioned animals, suggests that striatal GABAergic output fibres are near maximally activated in the intact animal. Only when competing endogenous GABAergic influences have been largely removed from the pallidal complex and SNR, is the full potential of muscimol in directly reducing functional activity revealed. In view of the data presented in a previous section it must, however, be stressed that, following intravenous administration of muscimol, the effects observed in these areas may be a secondary expression of a primary action elsewhere.

The effects of dopaminergic manipulation upon functional activity within the striato-nigral system have been reported in extensive detail (McCulloch et al. 1982c,d), and the results observed in sham lesioned animals (with the exception of the new finding in EP) were in close agreement with these previously published observations. However, the combination of striatal lesioning with subsequent apomorphine pharmacological challenge revealed a complex interaction of the drug with striatal efferent systems. The two factors combine to make interpretation of the results difficult. Firstly, whilst the effects of striatal lesions and muscimol treatment are opposite in direction, making analysis somewhat easier, both the lesion and apomorphine alone effect increases of a similar magnitude in the ipsilateral sites of striatal projection. Secondly, the technique does not allow the

basal levels of functional activation elicited by striatal lesions to be determined for each animal before the effects of apomorphine are superimposed. The variance of the two treatments cannot, therefore, be reduced by experimental design. Multiple comparisons between lesion and apomorphine treatment alone, and in combination, go some way to alleviating these problems.

Of the ipsilateral areas receiving direct striatal input, only in the GP was no significant difference found between the response to selective striatal lesion and apomorphine in combination, and either treatment alone. The quantitatively similar functional responses measured in GP under all three conditions are indicative of a mode of action common to both striatal lesion and apomorphine in influencing activation of GP. These results are suggestive of dopaminergic inhibition of GP afferent neurons from the striatum. Thus, removal of inhibitory striatal efferents to GP (by kainic acid lesioning) or the dopaminergic inhibition of inhibitory striatal efferents to GP (by apomorphine administration) both produce the same end result, i.e., disinhibition of pallidal neurons.

In both EP and SNR areas ipsilateral to the lesioned striatum, the combined effects with apomorphine administration were significantly greater than with lesion treatment alone. The integrity of striatal input to these areas does not, therefore, appear necessary for expression of at least part of the

responsiveness to apomorphine as measured by increased glucose use. However, in EP the increased functional activity observed in sham operated animals following apomorphine was significantly attenuated in the lesioned group, whilst in marked contrast the combined effects of lesion and pharmacological challenge in SNR resulted in a significant potentiation of the apomorphine response. In the intact animal the presence of striatal efferent, mainly GABAergic, neurons appears to increase the responsiveness to dopaminergic manipulation in EP, but to decrease the responsiveness of SNR. The exact mechanism of this dopamine - GABAergic interaction cannot be explained as readily, as could the response in GP, purely in terms of events within the striatum itself. A further complication is the proposed excitatory influence exerted over both EP and SNR projections by dopamine activation of striatal efferents (Scheel-Kruger et al. 1980). A direct action of apomorphine within the striatum should therefore attenuate the response in the projection areas in intact animals by virtue of increased GABAergic inhibitory activity. Whilst there is evidence in the data presented here for this being the mechanism which in SNR produces a potentiation of the apomorphine response when striatal efferent influences were removed, the underlying mechanism responsible for the opposite, attenuative response to striatal lesions in EP remains unresolved.

Unlike the increased glucose utilisation measured

in the ipsilateral pallidal complex and pars reticulata following striatal lesions, the neuroanatomical basis for the subtle, significant alterations in glucose utilisation in specific areas of the hemisphere contralateral to the lesion site is more difficult to identify, although there exists considerable biochemical evidence suggestive of a functional interplay between the two striato-nigral systems (Nieoullon et al. 1977 and 1979; Leviel et al. 1979; Andersson et al. 1980). Behavioural observations on the re-establishment of symmetrical motor activity, after the compulsive turning behaviour displayed acutely following striatal lesions (Coyle et al. 1979), suggest adaptive compensatory changes within the contralateral nigrostriatal system. In this context, the increased glucose utilisation in the contralateral pars compacta of the substantia nigra is of considerable interest in view of the compensatory contralateral alterations in dopaminergic systems following chronic, unilateral, kainic acid-induced striatal lesions (Andersson et al. 1980). Moreover, the observation of bilateral elevations in the pars reticulata of the substantia nigra, a region implicated in the regulation of activity within the pars compacta, highlights further the extent of the functional adjustment in the two nigrostriatal systems following a unilateral striatal lesion.

The bilateral attenuation of lesion-induced increases in glucose utilisation following muscimol

treatment emphasises the degree of functional interaction between the two hemispheres, as well as the part played by GABAergic influences in the maintenance of symmetry. In contrast, the subtle side-to-side differences in response to apomorphine, particularly in SNR, are indicative of the importance of the interplay between GABA and dopamine systems in the intact striato-nigral activity.

Although some evidence for a direct neuronal pathway linking the nigrostriatal systems has recently been published (Fass and Butcher, 1981; Gerfen et al. 1982), it is probable that the paucity of these connections reflects their limited functional importance in maintaining symmetry of action. A number of more complex neuroanatomical connections have been described which could provide the necessary circuitry to link the two systems (Kemp and Powell, 1971; Nauta and Domesick, 1979). From a knowledge of the known anatomical connections, the ventral nuclear complex of the thalamus and lateral habenular nucleus of the epithalamus constitute two important relays in the conveyance of information from one nigrostriatal system to the other. The ventral thalamus, which has extensive reciprocal connections with the neocortex (Deschenes and Hammond, 1980), receives input from EP (to ventrolateral nucleus) (Nauta, 1979) and from SNR (to ventromedial nucleus) (Di Chiara et al. 1979). The callosal connections of the neocortex and its extensive efferent projections (Glowinski et al. 1978) could provide

the final pathway via which functional processes in the two nigrostriatal systems could become integrated. The results of this study in which glucose use was elevated in the contralateral neocortex and ventromedial thalamus, and in the ventrolateral thalamus, provide some circumstantial evidence for the involvement of the thalamo-cortical route. Moreover, increased functional activity in the contralateral parafascicular-centrum medianum complex, which is innervated by collaterals from the pallido-thalamic pathway, may also act directly upon striatal function (Nauta and Domesick, 1979) or again via neocortical projections (Herkenham, 1980).

An alternative pathway allowing interhemispheric exchange of information between nigrostriatal systems could involve the lateral habenula in the role of intermediate relay nucleus, projecting bilaterally to SNC and receiving a major projection from the pallidal complex (Herkenham and Nauta, 1977 and 1979). Support for the possible involvement of this pathway is gained from the observation of increased glucose utilisation in the ipsilateral portion of the habenula in kainic acid injected rats. In view of the relative insensitivity of this area to systemic muscimol treatment in the intact rat (described previously), despite the known GABAergic innervation which it receives from EP (Nagy et al. 1978), it is of interest that lesion-induced increases in functional activation could be attenuated by muscimol. Irrespective of which

anatomical connections ultimately prove to be involved, the patterns of alteration in glucose utilisation, particularly within the contralateral hemisphere, emphasise the widespread extent of the functional changes which are present when the animal has adapted to the unilateral loss of a major portion of its striatal efferent fibres.

The most bilaterally consistent alterations of functional activity in lesioned rats were found within the mesolimbic system. Whilst striato-nigral activity is thought to maintain overall symmetry in motor behaviour, it is the mesolimbic system which promotes locomotion (Pycock and Marsden, 1978). The reciprocal neuronal connections which have been shown to exist between elements of these two systems (Beckstead et al. 1979; Nauta and Domesick, 1979) may provide the anatomical basis for circling behaviour in response to specific extrapyramidal lesions (Pycock and Marsden, 1978). Although at ten days after the induction of striatal lesions asymmetry of functional activity in the striato-nigral pathways was still apparent, the symmetrically increased mesolimbic activation could possibly explain why this residual side-to-side difference was no longer expressed in compulsive circling. Once again, the lateral habenula may be involved in integrating information from striatal and limbic forebrain areas (Herkenham and Nauta, 1977 and 1979; Wang and Aghajanian, 1977) and, together with its connections with VTA, may provide the route through

which the nucleus accumbens is functionally affected in response to unilateral lesioning of striatal efferents.

The unilateral loss of striatal post-synaptic dopamine receptors with any functional role could possibly raise an a priori expectation of asymmetrical motor activity following apomorphine treatment. The fact that no such asymmetry was observed may be due to the lack of expertise in behavioural monitoring, or to artefact in the restrained animal. However, the symmetry of functional activity maintained in the mesolimbic system, and also in the globus pallidus which has been implicated in circling behaviour (Slater, 1982), suggests that the behavioural observations are at least consistent with the measured cerebral functional activity. Elsewhere in the brain, the actions of apomorphine in lesioned animals proved to be very complex. The response to lesion alone could, in the main, be rationalised in terms of known anatomical connections. Subsequent challenge with muscimol, in attenuating lesion-induced increases in functional activity, provides support for the conclusion that the responses were due largely to loss of an endogenous GABA system. The effects of apomorphine in the absence of striatal efferent neurons reveals a complexity of interaction between GABA and dopamine which has hitherto not been appreciated in the description of dopamine agonist effects (McCulloch et al. 1982**b**).

4. Intrastriatal Injection of Muscimol

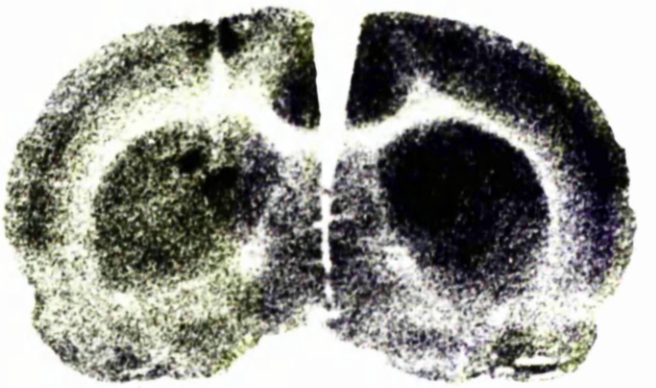
4.1 General results and observations: No changes in overt behaviour were observed following unilateral intrastriatal injections of 100 ng of muscimol. Following injections of 500 ng of muscimol, intermittent jaw movements were evident at random intervals throughout the period of measurement. The movements involved gaping, licking, and grinding of the teeth in no co-ordinated order. The animals showed no apparent stress, plasma glucose remaining stable at levels comparable with restrained animals with no intracranial intervention.

On sectioning of the brains for autoradiography, the spread of Evans blue was noted. Without exception, the inert dye was visibly evident only within the limits of the caudate nucleus. No marker was ever to be seen in the needle tract through overlying neocortex or subcortical white matter. Within the striatum the maximum spread of dye visible extended to a diameter of 1.8 mm from the epicentre of the injection as located by the needle tract. The injection needle fell within the dorsomedial quadrant of the caudate in all animals.

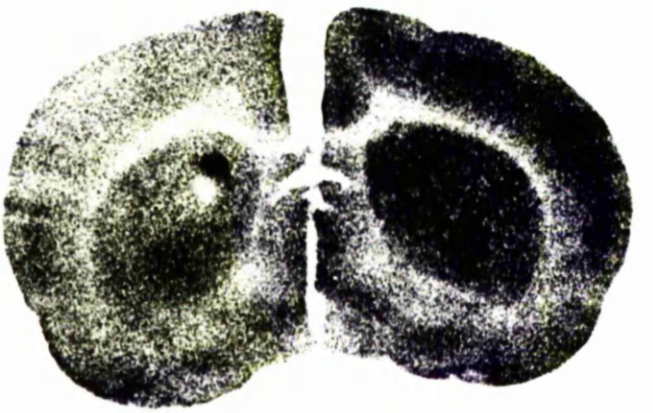
4.2 Local cerebral glucose utilisation: All animals subjected to intrastriatal intervention displayed a disordered pattern of 2-deoxyglucose uptake associated with the needle tract and injection site (Fig. 37). The tract itself appeared on the autoradiographs as a column of decreased optical density, penetrating through sensory-motor cortex and into the body of the

Figure 37

Autoradiograms from coronal sections of rat brain at the level of the caudate nucleus.



Intrastriatal CSF



Intrastriatal Muscimol

Upper: Punctate areas of increased optical density associated with the needle trace in cortex and caudate nucleus (l.h.s.). Note generalised reductions in OD in hemisphere subjected to intracranial intervention.

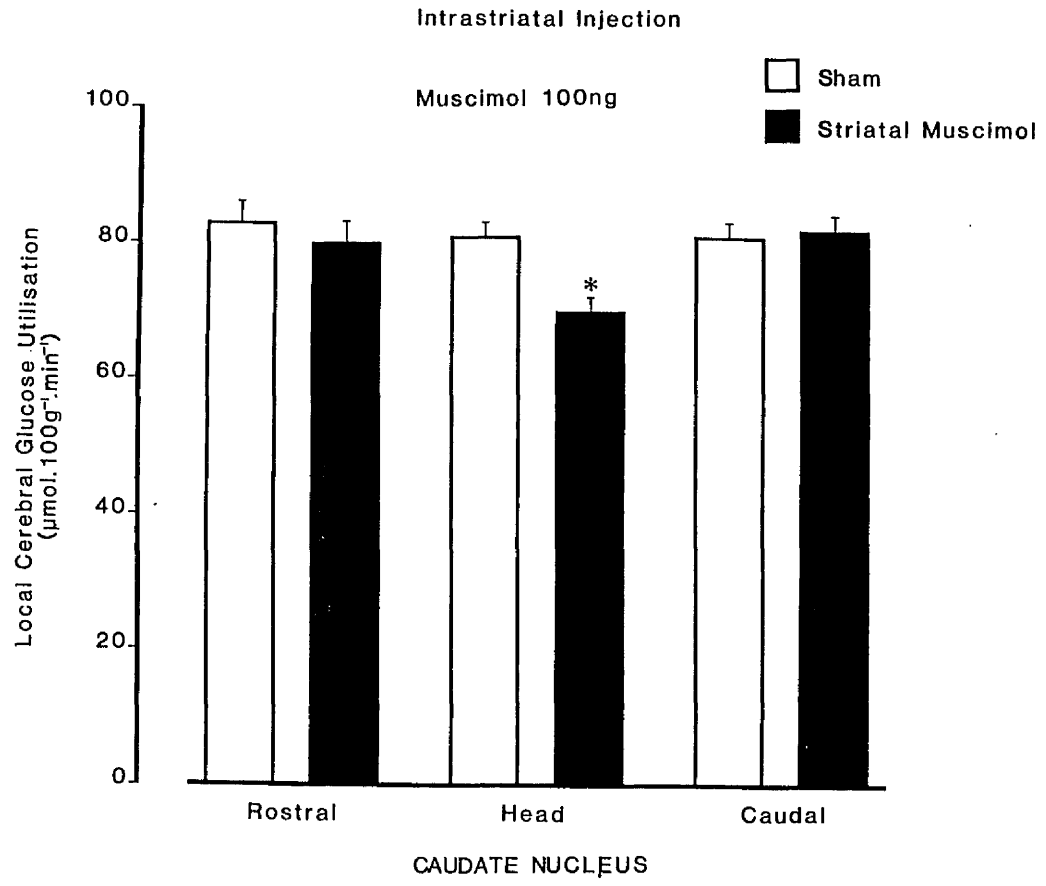
Lower: Decreased optical density in the caudate nucleus associated with intrastriatal injection of muscimol (500 ng).

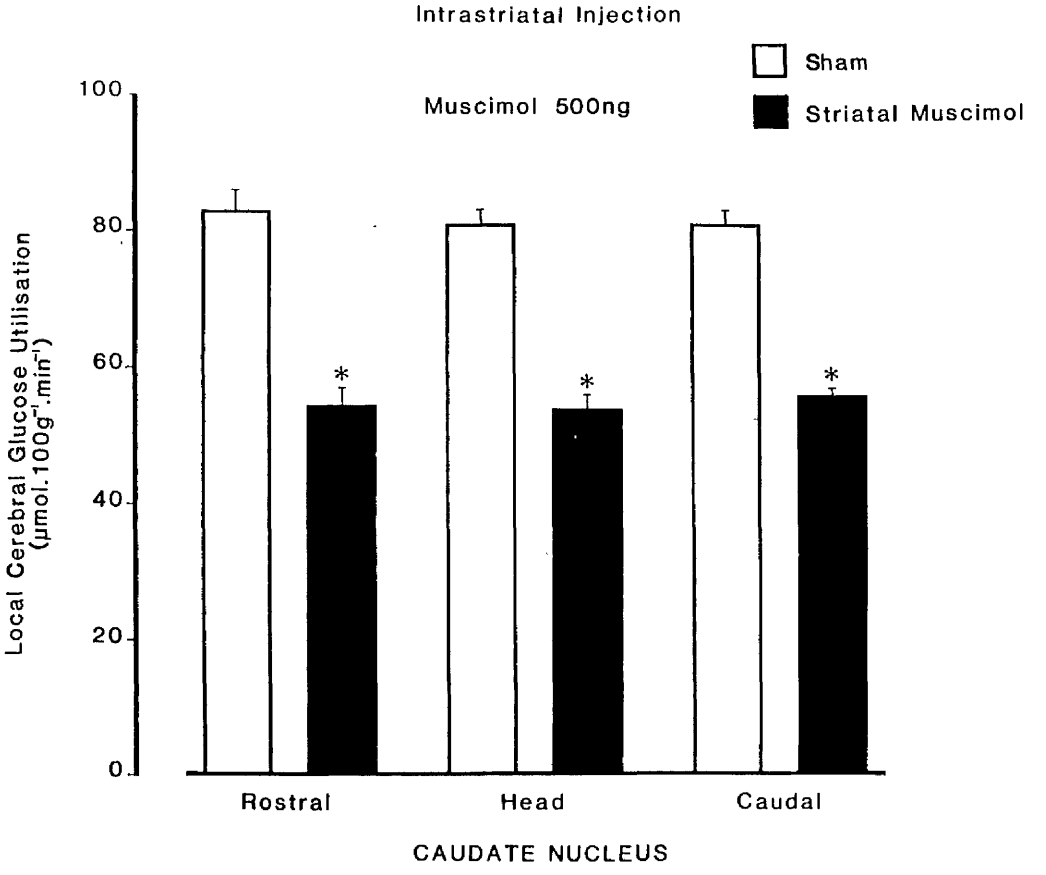
striatum. At the end of the tract, but also sometimes at discrete points along its length, small, punctate areas of increased optical density were observed. These areas were observed only in association with the tract, never at any distance from the injection site, and the frequency with which they corresponded to visible sites of localised haemorrhage make it highly likely that this autoradiographic pattern was due to structural damage caused by the insertion of the needle. In both CSF and muscimol injected animals a generalised depression in glucose utilisation was evident ipsilateral to the site of intervention, which resulted in a degree of asymmetry between the hemispheres. These non-specific effects were most marked in neocortex and thalamus.

Significant alterations in local cerebral glucose use were limited to a very few regions following intrastriatal injection of 100 ng of muscimol. Within the ipsilateral caudate nucleus itself, glucose use was decreased by around 14% in an area extending approximately 1.2 mm from the injection site to either side in the rostro-caudal axis. In areas of the nucleus removed from the injection site, no changes in glucose use were observed (Fig. 38). Contralateral to the injection, alterations in the rate of glucose use were only measured in an area roughly corresponding to the area of decrease found ipsilaterally. On this side, however, the smaller 8% decrease was not significant.

Although small in absolute magnitude, significant

Figure 38

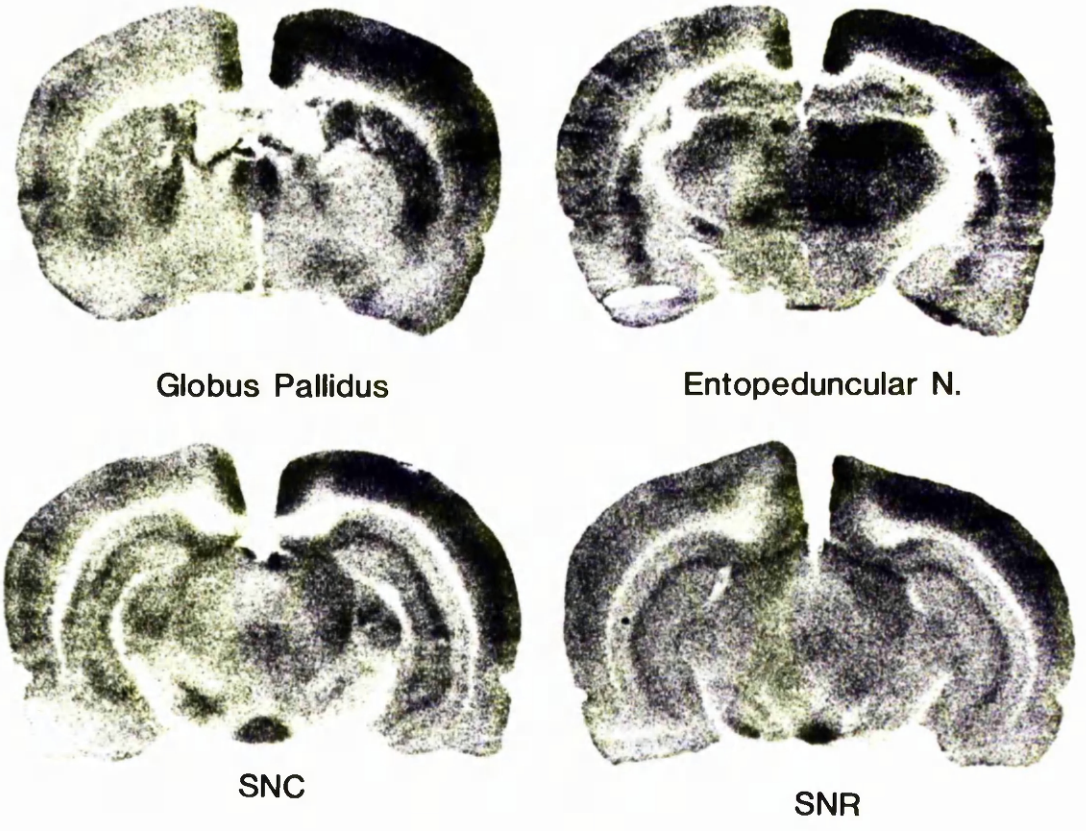




Rates of glucose use in ipsilateral caudate nucleus following intrastriatal injection of muscimol ($*p < 0.05$).

Figure 39

Autoradiograms from coronal sections of rat brain taken at different levels following intrastriatal injection of muscimol (500 ng).



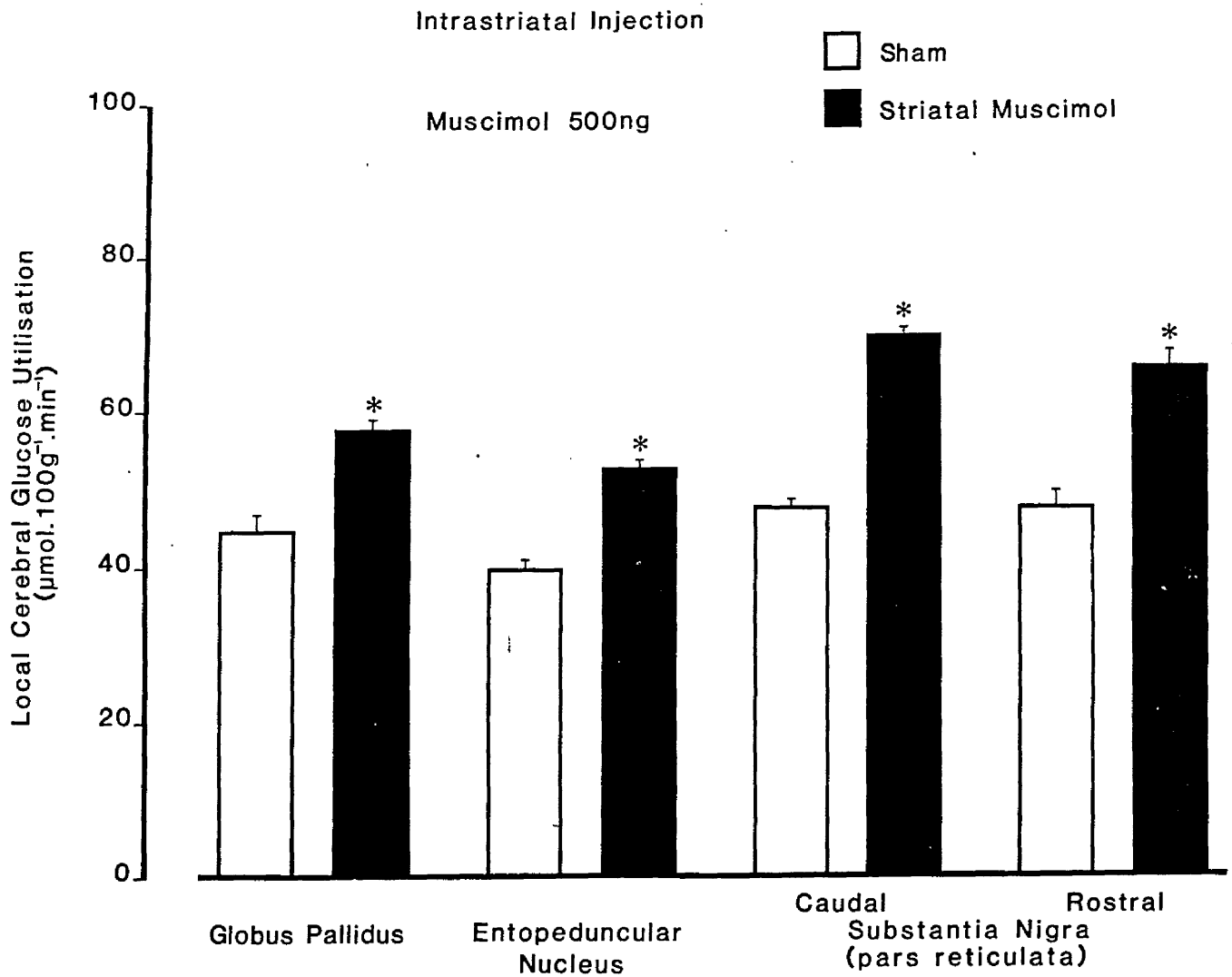
Upper left: Increased OD in globus pallidus ipsilateral to injection site (1.h.s.).
Upper right: Increased OD in entopeduncular nucleus, and decreased OD in ventrolateral thalamus ipsilateral to injection site (1.h.s.).
Lower left: Increased OD in substantia nigra pars compacta ipsilateral to injection site (1.h.s.).
Lower right: Increased OD in lateral portion of pars reticulata ipsilateral to injection site (1.h.s.).

20% increases in glucose use were measured in ipsilateral entopeduncular nucleus, the only primary striatal projection area to show any response at this dose. In no other area, either ipsilateral or contralateral to the injection site, were any significant changes in glucose utilisation observed following striatal injections of 100 ng of muscimol. In view of the entopeduncular changes in glucose use, it is interesting that in the ipsilateral lateral habenula a moderate, 10% increase in glucose use just failed to reach the critical value for significance in the statistical analysis.

Following unilateral injection of 500 ng muscimol into the striatum, widespread alterations in functional activity were observed, some of which were apparent from visual inspection of the autoradiograms (Figs. 37 and 39). Within the injected caudate nucleus large, 33% decreases in glucose use were observed at all levels of the nucleus (Fig. 38), in marked contrast to the effects of 100 ng muscimol which were much more localised around the injection site. Contralateral to the injection, mean glucose utilisation in the caudate nucleus was depressed by around 15%, but this did not achieve statistical significance.

In areas which receive the primary output of the striatum, both segments of the ipsilateral pallidal complex showed increased rates of glucose utilisation of around 30% following muscimol (Figs. 39 and 40). The ipsilateral pars reticulata of the substantia

Figure 40

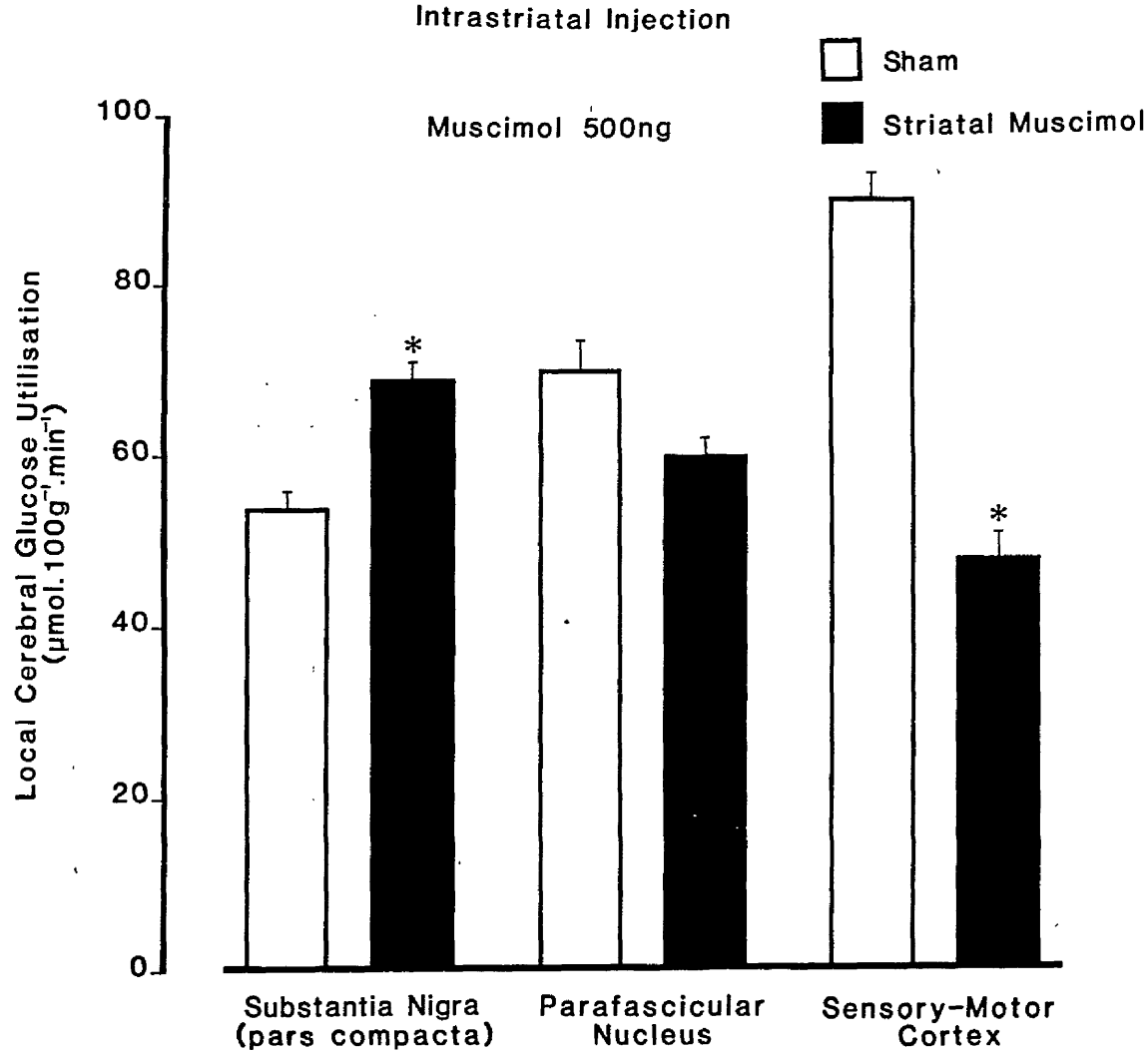


Rates of glucose use in areas receiving primary projections from ipsilateral striatum (*$p < 0.05$).

nigra was particularly responsive to striatal muscimol at the higher dose, with a measurable difference between the rostral (38% increase) and caudal (46% increase) parts of the region (Fig. 40). Moreover, the area of increase was highly localised and did not include the whole of the SNR visible in some sections (Fig. 39).

In motor areas of the brain sending projections to the striatum, a 20% increase in glucose utilisation was measured in the pars compacta of the substantia nigra (Figs. 39 and 41). From the autoradiograms it appeared that the changes were most pronounced in the more rostral parts of the SNC, but this impression was not sustained by the quantification. It is likely that the lesser response in SNR in its rostral parts provided greater contrast, allowing better visual discrimination than was possible in the caudal parts. The considerable 42% reductions in glucose use in sensory-motor cortex, measured at sites removed from the physical damage associated with the needle tract (Fig. 41), were suggestive of possible direct action of the GABA agonist by leakage back up the needle tract. However, in one preliminary animal in which the guide cannula had been misaligned, and in which excess volume (4 µl) had been injected into neocortex such that leakage had occurred to cover a portion of the cortical surface, the same degree of reduced glucose use was not in evidence. In particular, cortical layer IV was still clearly discernible on the autoradiograms, but was definitely not apparent when the injection was limited

Figure 41



Rates of glucose use in areas sending projections to ipsilateral striatum (*$p < 0.05$).

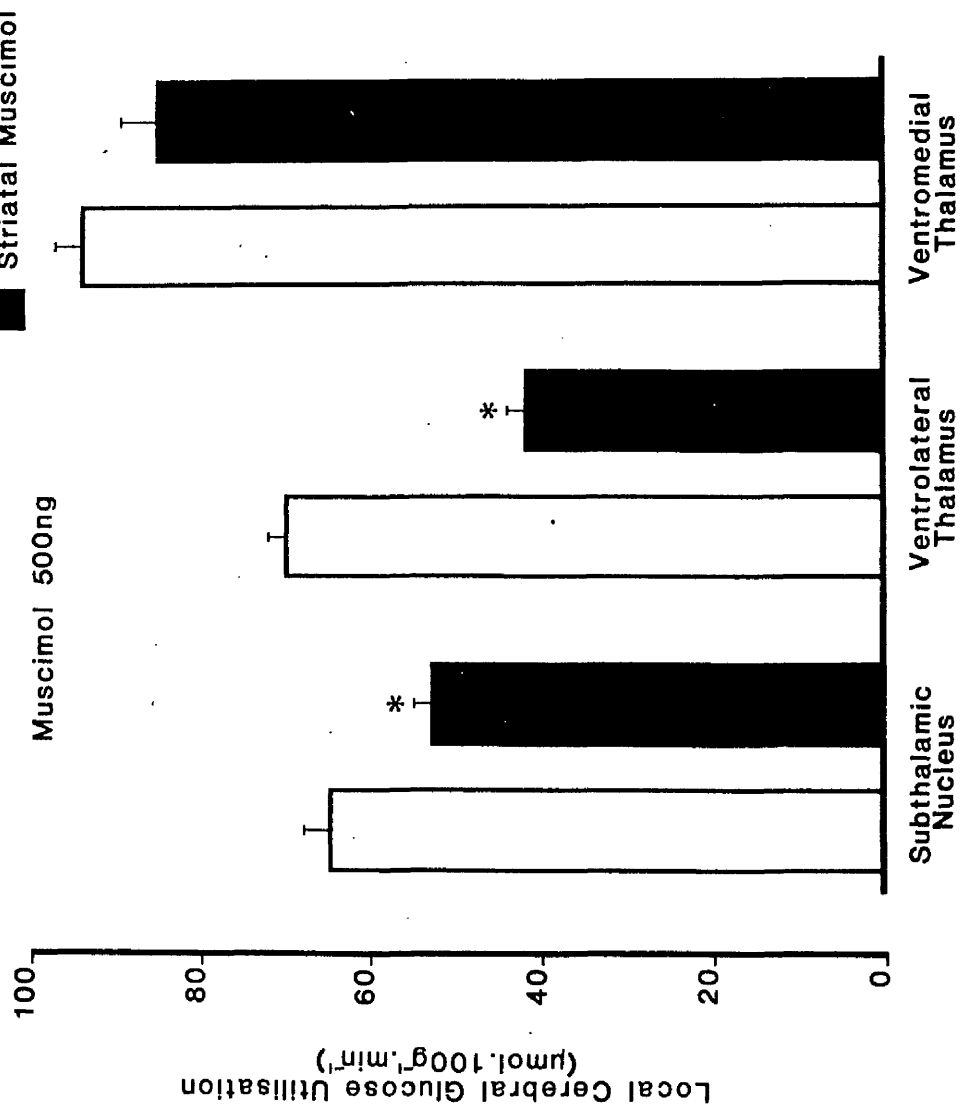
to the striatum alone (Fig. 37). Only minor, non-significant changes were measured in the parafascicular nucleus of the thalamus (Fig. 41) which sends projections to the striatum.

In areas of the diencephalon linked by efferent fibres to the pallidal complex, decreases in glucose use of 18% in subthalamic nucleus and 40% in ventrolateral thalamus were measured (Figs. 39 and 42). In contrast, the ventromedial nucleus of the thalamus, which is connected by afferent neurons with the substantia nigra, showed only non-significant decreases in functional activity (Fig. 42). Of the contralateral areas examined at 500 ng muscimol, throughout the brains only the ventrolateral thalamic nucleus displayed any significant alterations in glucose utilisation. However, the 17% decrease measured was less than half the response found in the corresponding ipsilateral nucleus.

Of the limbic areas closely associated with extrapyramidal function, no significant changes in functional activity were measured in the nucleus accumbens, but a small decrease was found in the ventral tegmental area following unilateral injection of muscimol into the caudate nucleus (Fig. 43). A moderate, though significant, 16% increase in glucose use was found in the lateral habenula (Fig. 43). This unilateral response was again easily discernible from a visual inspection of the autoradiogram (Fig. 44).

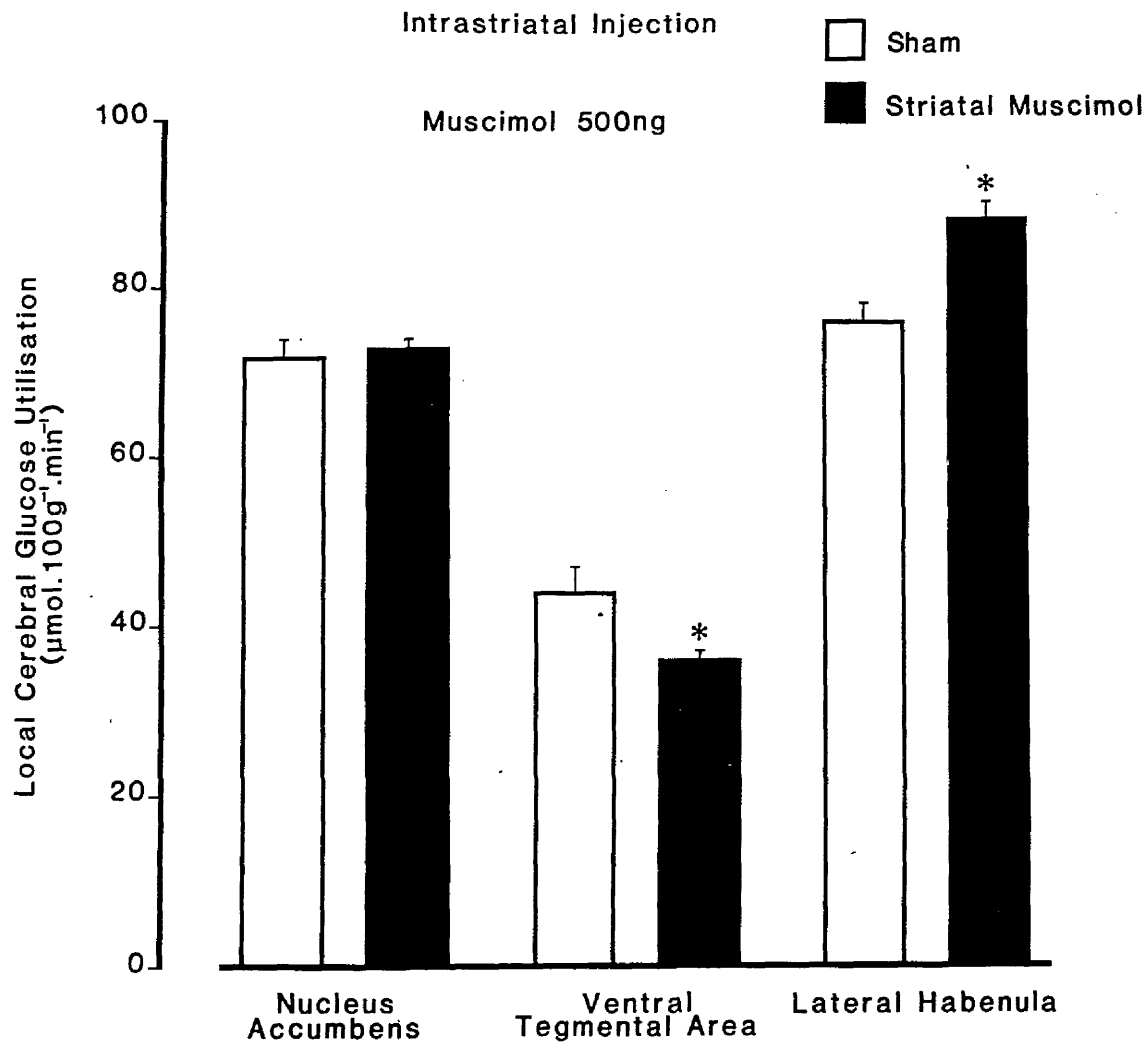
The only other alteration in glucose use resulting

Figure 42



Rates of glucose use in areas receiving secondary projections from ipsilateral striatum ($*p < 0.05$).

Figure 43

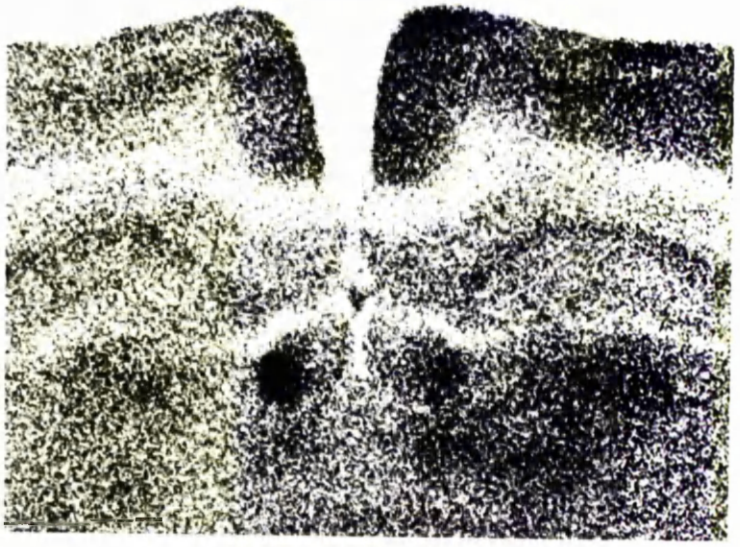


Rates of glucose use in non-motor areas associated with extrapyramidal system (*$p < 0.05$).

Figure 44

Autoradiograms from coronal sections of rat brain at the level of the habenula.

Intrastriatal CSF



Intrastriatal Muscimol

Upper: Similar OD in lateral habenula ipsi- and contralateral to intrastriatal injection of CSF giving symmetrical appearance.

Lower: Higher OD in lateral habenula ipsilateral to intrastriatal injection of muscimol (500 ng) giving asymmetrical appearance.

from striatal injection of muscimol was found in the deep layers of the superior colliculus, where a unilateral decrease of 18% was evident ipsilaterally. These results, and all those from other brain areas analysed, are presented in Appendix VI.

4.3 Commentary: Several putative neurotransmitters have been identified within the neostriatum of adult rats (Fonnum and Walaas, 1979), but the inhibitory neurotransmitter, GABA, participates at two levels in the functional operation of the striatum. Biochemical and morphological studies (McGeer and McGeer, 1975; Hassler et al. 1977; Ribak et al. 1979) have indicated the existence of GABAergic nerve terminals of both intrinsic striatal neurons and collateral fibres from neostriatal projection neurons to globus pallidus and substantia nigra (Ribak et al. 1979). Thus, GABA neurons may both control neuronal excitability within the caudate nucleus (Norcross and Spehlman, 1981) and carry messages initiated in the striatum to exert inhibitory influences at distant terminal sites.

The majority of neostriatal neurons are of the same morphological type, characterised by their medium size and high density of dendritic spines (Kemp and Powell, 1977). Previously, neuronal cell bodies of this type were considered to be local striatal circuit neurons (Kemp and Powell, 1971), but subsequent refinements in fibre tracing techniques indicated that these neurons are the origin of the bulk of neostriatal

efferents (Somogyi and Smith, 1979; Preston et al. 1980). Although the presence of striatal GABAergic aspinous interneurons has been confirmed, it now appears likely that much of the GABA release within the caudate is from terminals of an extensive network of axon collaterals (Wilson, 1979; Preston et al. 1980) projecting to a radius of 250 μm from the soma. The dense packing of medium spiney neurons suggests that cells brought into contact with collaterals will also be of the same morphological cell type. The resulting interaction of collaterals and dendrites may result in recurrent inhibition mediated by terminal GABA release, a possibility which has gained experimental support (Park et al. 1980). However, whether the effects of GABA in reducing caudate cell firing (Norcross and Spehlman, 1981) are due to the action of the transmitter at interneuron terminals or collateral terminals, the effect upon activity in striatal efferent neurons would be essentially similar.

The decreased rate of glucose utilisation observed in the caudate nucleus following intrastriatal injections of muscimol are compatible with the electrophysiologically measured depression of neuronal firing reported in response to GABA itself (Norcross and Spehlman, 1981; Misgeld et al. 1982). The direct application of muscimol into the brain does not suffer from the same potential limitation as does peripheral administration of this GABA agonist, in that metabolic breakdown of the drug is very much less

pronounced within the brain than is the case in the periphery (Baraldi et al. 1979) and, therefore, complicating effects of possibly active products of metabolism do not arise. Indeed, the pharmacology of muscimol, the avidity with which it binds to GABA receptors and its resistance to GABA-$_{\alpha}$-glutamate transaminase, makes it a more suitable pharmacological tool than GABA itself for injection into the striatum.

The area of decrease in striatal functional activity following injection of 2 µl containing 100 ng muscimol was highly localised, spreading no further than could be predicted on the basis of diffusion of this volume through brain tissue (Myers, 1966). Assuming a simplistic uniform distribution throughout the volume of diffusion, the effective concentration of muscimol would be 10^{-4}M over the first 10 to 15 minutes of the deoxyglucose experiment. The majority of the caudate, rostral and caudal to the centre of the injection, showed no change in glucose use following muscimol, and within the area of moderate metabolic depression glucose use was uniform. In marked contrast, the effects of injecting 500 ng of muscimol were both more marked and more widespread, despite the similar degree of spread noted in the diffusion of Evans blue dye. It is fair to assume that even if muscimol were to diffuse at a faster rate and over greater distances than the marker dye, it is unlikely that the whole rostro-caudal extent of the caudate covering some 5 mm would contain

sufficient agent to effect these changes. It is possible that this higher dose of muscimol is present in sufficient quantities over the diffusion area to produce inhibition of caudate neurons far removed from the injectate through the interconnections of interneurons and collateral fibres. The uniformity of the reductions in functional activity suggests that the responses observed close to the injection site, and at a distance, are similar in the underlying mechanisms responsible, involving mainly GABAergic neuron systems.

Intrinsic striatal GABA neurons would appear to be capable of exerting their effects throughout the caudate nucleus, with no apparent topographical organisation. This is in contrast to the heterogeneous distribution patterns of other neurotransmitters which form histochemically distinct compartments within a complex matrix (Goldman and Nauta, 1977; Graybiel and Ragsdale, 1978, 1981; Herkenham and Peart, 1981; Wright and Arbuthnott, 1981). The intricate organisation of patches has been suggested as reflecting the high degree of integration which is performed within the neostriatum, but to date there is no evidence that these histologically defined subunits of the caudate do indeed represent areas with discrete functional roles. Recently, however, we have provided evidence that the response within the caudate to local injections of the putative neurotransmitter/neuromodulator, VIP, in terms of alterations in functional activity, is organised in a punctate manner. The areas of

increased glucose use observed corresponded closely in their dimensions to those described for histochemical patches (Edvinsson et al. 1982). No such intricate organisation of responses to muscimol was noted in any part of the striatum at either of the doses used.

Although an injection of 100 ng of muscimol into caudate had no measurable effects outwith the nucleus, the acute local injection of 500 ng of muscimol into the caudate induced a response in striatal projection areas with marked similarities to the chronic pattern observed following kainic acid induced lesions of the caudate nucleus. Whilst the lesioning procedure removed striatal efferent, inhibitory effects in the pallidal complex and SNR, the injection of the GABA agonist effectively induced an inhibition of efferent inhibitory projection. In both cases the net result was an disinhibition of postsynaptic neurons at the terminal sites, which was manifest in an increase in functional activity.

Although the area of striatal involvement was very similar in both lesion and local injection studies, subtle differences were observed in the pattern of response in SNR. Following kainic acid lesions of the caudate, glucose utilisation was diffusely increased throughout the whole extent of SNR. In contrast, striatal muscimol injection resulted in an increased glucose use which was more marked caudally than in more rostral portions of the region, and was also more intense in the ventromedial parts. On this basis it

would appear that, although functional activity is reduced equally throughout the caudate nucleus by 500 ng of muscimol, not all efferent neurons are equally affected. From the known topographical arrangement of striatal efferents to SNR (Tulloch et al. 1978; Nauta and Domesick, 1979) it is apparent that the greatest effects of the muscimol were centred around the injection site, as it is from this mediodorsal area of the caudate that neurons originate which innervate that part of SNR displaying the largest increases in functional activity. It is not an unreasonable suggestion that as the injectate diffuses away from the centre and becomes more dilute, then the effects of muscimol upon efferent fibres becomes less intense. Injections of 100 ng muscimol had no discernible effect upon striatal efferent activity, and dilution of the higher dose to these levels by diffusion would presumably be similarly ineffective. Superimposed upon the direct effects of muscimol upon efferent neurons will be the effects mediated via intrinsic neurons disseminating the effects throughout striatum. No topographical organisation of response in either GP or EP was observed with respect to the positioning of the injection, in keeping with the generalised decrease in striatal function observed. The differences in degree of involvement in the pallidal complex response and that in SNR further reflects the complexity of integration of which the caudate nucleus is capable.

The functional response of the pars compacta of

the substantia nigra to altered striatal activation is of particular interest in view of the controversy which continues to surround the exact role of reciprocal striato-nigral innervation (Nauta and Domesick, 1979). Although a few striatal GABAergic efferent fibres do terminate in the pars compacta, the majority synapse within the pars reticulata (Fonnum et al. 1974; Ribak et al. 1976). Reciprocal innervation of the caudate originates in the pars compacta. These dopaminergic neurons form synapses with both intrinsic cholinergic and GABAergic neurons, as well as directly on to dendrites of striatal efferent neurons (Pasik et al. 1979). Activation of striatal afferents originating from SNC induces post-synaptic excitatory potentials (Wilson et al. 1982), whilst stimulation of the caudate nucleus produces GABA mediated inhibition of nigral cells (Precht and Yoshida, 1971; Yoshida and Precht, 1971). This inhibition has been shown to affect nigral dopaminergic cells (Feltz, 1971; Crossman et al. 1973), and on this basis an inhibitory feedback loop was postulated (Dray and Straughan, 1976). The functional interaction of GABAergic input to SNR with the dopaminergic outflow from SNC is, however, proving to be more complex than originally envisaged. A paradoxical increase in SNC cell activity has been measured behaviourally (Martin and Haubrich, 1978) and electrophysiologically (Grace and Bunney, 1979) in response to local injections of GABA or muscimol into SNR. This response has been explained by the proposed

presence of inhibitory neurons interposed between SNR and SNC (Grace and Bunney, 1979), although these intrinsic neurons have not been characterised histologically. In contrast, current evidence points to a monosynaptic link between GABAergic afferents and dendrites of dopamine neurons which penetrate the reticulata from cell bodies located in the pars compacta (Wassef et al, 1981). However, the interaction of GABAergic and dopaminergic neurons is further complicated by the dendritic release of dopamine in the SNR which can activate synaptic receptors coupled to a dopamine-sensitive adenyl cyclase. These receptors are localised on GABA (but not substance P) striato-nigral fibres (see Cheramy et al. 1981, for review).

The increase in glucose utilisation measured in SNC paralleled the changes observed in SNR following intrastriatal injection of muscimol. Thus it would appear that, in this conscious rat preparation, neurochemical inhibition of neuronal projections from striatum to nigra allows an increase in functional activity in both parts of the nigra. These results therefore support the idea that, under normal conditions in the intact animal, striatal efferents exert an inhibitory influence not only upon the SNR where the majority of fibres terminate, but also in SNC, possibly through direct synapses with dendrites of dopaminergic cells originating in the compacta. It should be stressed again that nigral activity would appear to be influenced to a high degree by anaesthetic agents

(Grome and McCulloch, 1981), so that the animal preparation used to study nigral function may influence the results and final conclusions arrived at. This is one factor which may be the underlying cause of so much contradictory experimental evidence on the interaction between SNR and SNC in extrapyramidal motor function.

Although the changes in glucose use observed within the basal ganglia following intrastriatal muscimol represented increases in functional activity which may be subject to credible interpretation in terms of known GABAergic afferent innervation, the decreased glucose use in neocortex and thalamus must be interpreted with some caution. Both the generalised neocortical depression invariably observed with this preparation (compare the CSF injected animals in this study with similar results reported for both intrastriatal and intracortical CSF reported previously) (Edvinsson et al. 1982) and the possibility of direct effects of muscimol through leakage back up the needle tract, which cannot be totally discounted, are sources of artefact which together could add to, if not account for, the decreases in measured glucose use. The problem of cortical depression by intracranial intervention may be alleviated in the experimental design by comparing ipsilateral sides in both CSF and muscimol injected animals. Attempts were made to prevent the leakage of injectate into overlying neocortex by constructing the guide cannula such that only the much thinner injection needle penetrated through cortex at

the time of injection. After the injection the needle was left in situ for the course of the whole measurement period to prevent the injectate being dragged back up the tract during withdrawl. However, due to the sensitivity of cortex to the action of GABA agonists and antagonists, possibly only very small amounts of muscimol would be necessary to cause large scale decreases in functional activity. One might expect, however, that leakage into cortex would result in a much more focal response than that observed here. Using a similar experimental protocol to examine the effects of leakage of picrotoxin from a striatal injection site into cortex, a very well defined behavioural syndrome was observed, and not the seizure activity which would accompany any wide diffusion of this potent GABA antagonist (Edvinsson et al. 1982). In contrast, the striatal injection of muscimol produced widespread reductions in functional activity in areas of sensory-motor cortex far removed from the injection site. It is possible, therefore, that a significant, but not necessarily greater part of the reductions in motor cortex, and areas of thalamus with which there are neuronal connections, was the result of functional changes in complex pathways originating from striatum, and was not due to direct cortical action alone.

Bearing in mind the limitations of the experimental approach as outlined above, the changes in functional activity observed within the diencephalon following intrastriatal muscimol nevertheless provide

an interesting insight into the interactive role of striatal output in the overall co-ordination of motor activity. GABAergic projections have been postulated from GP to the subthalamic nucleus (Fonnum et al.1978; Rouzaire-Dubois et al. 1980), from EP to the ventrolateral thalamic complex (Pennay and Young, 1981), and from SNR to the ventromedial thalamus (Di Chiara et al. 1979), and these pathways displayed a quantitative pattern of response which was common to all three. The extrapyramidal source of diencephalic afferents showed marked increases in functional activity (as described previously), whilst decreases were observed to a greater or lesser extent in the thalamic and subthalamic nuclei. The most pronounced alterations in functional activity were observed in the ventrolateral thalamus (Δ - 46%), which could account for at least part of the profound changes in glucose utilisation measured in sensory-motor cortex to which this area of thalamus projects (Nauta and Domesick, 1979). The neuronal connections between GABAergic input to pallidus and nigra from striatum and the efferent neurons from these areas to thalamus and subthalamus have not yet been clearly established. If, however, a direct synaptic contact is made between these two GABAergic systems, then activation of striatal efferents would result in disinhibition of the thalamic nuclei (Roberts, 1976). The net effect, therefore, would be to activate thalamic neurons. The results of the present studies do add support to this possible

mode of interaction. Muscimol-induced inhibition of striatum resulted in increased functional activity within pallidus and nigra and also, apparently, in the projection neurons to thalamus where decreased functional activity was observed. This observation is in keeping with the occurrence of the two GABAergic systems in series through which activation of striatal efferents can disinhibit the thalamus.

A similar pattern of innervation has been suggested as providing a pathway between striatum and lateral habenula via pallidus (EP), again with two GABAergic projections in series (Nagy et al. 1978; Gottesfeld et al. 1980). It is therefore interesting to observe that, in these studies, an increase in EP activity was accompanied not by depression of glucose use in the lateral habenula but, instead, by a moderate, but significant increase. On the basis of arguments advanced so far, it would appear unlikely that striatal inputs to pallidus synapse directly on to the efferent neurons which, in turn, project to habenula. Rather, it would seem that a degree of interaction occurs within EP, with intrinsic neurons interposed between afferents from striatum and efferents to habenula. The possibility of interaction of input from EP and from other sources (Herkenham and Nauta, 1977) to elicit the net response measured in habenula cannot be discounted, but the involvement of the lateral habenula in extrapyramidal function (Nauta, 1974), providing a relay between limbic and motor systems, is further supported

by these studies. In this context the observed decrease in ventral tegmental area, to which the habenula sends projections, perhaps provides evidence of the importance of this pathway in the further integration of activity in the two functional systems, although the apparent lack of involvement of the nucleus accumbens prevents development of proposed functional pathways beyond this stage.

The limited number of areas of the brain in which measurable alterations were observed following direct pharmacological manipulation of striatum with muscimol provides a novel insight into the integrated response of striatofugal pathways one, two, and possibly more synapses removed from the initial neuronal stimulation.

CHAPTER IV

DISCUSSION

The 2-deoxyglucose technique (Sokoloff et al. 1977) is proving to be an extremely potent tool in the neurosciences (for reviews see Sokoloff, 1980; McCulloch, 1982). The close coupling which has been demonstrated between rates of energy generation and functional activity within areas of the CNS (Sokoloff, 1977), taken together with the capacity to localise and quantify changes in metabolic demand autoradiographically, has provided the basis of a rigorous method with which to map the simultaneous involvement of all the anatomical components which take up any functionally integrated neuronal pathway (Kennedy et al. 1975; Sokoloff, 1977). Although widely used with experimental rodent models of cerebral function, the technique has also been used to provide an index of functional activity in single molluscan neurons (Sejnowski et al. 1980) and mouse neurons in culture (Ornberg et al. 1979), in the Drosophila visual system (Buchner et al. 1979) and teleost retina (Basinger et al. 1979). In higher order mammals, the 2-deoxyglucose autoradiographic method has been employed to measure rates of glucose utilisation in monkey brain (Kennedy et al. 1978), but perhaps the most exciting

recent development has been the fluorodeoxyglucose technique which, when combined with positron emission tomographic scanning, allows changes in cerebral functional activity of human subjects to be monitored (Reivich et al. 1979).

Functional mapping of integrated neuronal pathways in the intact mammalian brain has proved to be particularly successful in describing responses to clearly defined sensory stimuli which, by virtue of their limited access to the CNS via specific sensory receptors, may be easily controlled (see Sokoloff, 1980). Alterations in functional activity in elements of the primary visual system in response to specific visual stimulation have been extensively documented (Kennedy et al. 1976; Batipps et al. 1981; Toga and Collins, 1981). Although the activation of functional pathways involved in the processing of input from other sensory modalities has received less attention, responses to auditory (Wilson et al. 1980; Hungerbuhler et al. 1981; Ryan et al. 1982), olfactory (Lancet et al. 1982) and somataesthetic stimuli (Hand et al. 1978) have been mapped using 2-deoxyglucose autoradiography. Other stimuli which have been employed to elicit alterations in cerebral activity, from which functional pathways may be determined, have included passive (Sharp, 1976) and active locomotion (Schwartzman et al. 1981; Monamaur et al. 1982), acute hypotension (Savaki et al. 1982b), as well as direct electrical stimulation of cerebellum (Schim et al. 1981)

and motor cortex (Goldberg et al. 1980).

In the field of neuropharmacology, the pharmacological stimuli used in eliciting the functional changes to be subsequently mapped with 2-deoxyglucose autoradiography have, in general, been much less specific than those used in more physiological studies. The most widely used approach to date has been to deliver pharmacologically active agents systemically, and then to analyse the effects throughout the brain. In this manner, dopaminergic (McCulloch et al. 1982b) , cholinergic (Nelson et al. 1978; Weinberger et al. 1979), adrenergic (Savaki et al. 1982a) and serotonergic neurotransmitter systems (Grome and Harper, 1981, 1982) have been subjected to analysis. Although the 2-deoxyglucose technique does constitute an advance over classical electrophysiological methods of measuring drug action in that a larger number of brain regions may be studied simultaneously, the understanding of the primary sites at which a pharmacological agent may exert its influence has not been significantly advanced by this innovative technique in conjunction with such a diffuse manipulation as is systemic administration. If anything, these studies have served to emphasise that the normal brain functions in a completely interactive way with no clearly delineated or totally independent functional subunits.

A greater degree of specificity of pharmacological action has been introduced by either electrically stimulating cell populations of known neurotransmitter

functions (Schim et al. 1981), or by inducing lesions at the sites of origin or along the course of projection neurons (Schwartz, 1978; Steward and Smith, 1980; Wooten and Collins, 1980, 1981), thus removing neurotransmitter influences at the sites of termination. An added refinement to these 2-deoxyglucose studies was the subsequent pharmacological challenge of lesioned animals with appropriate agents (Kozlowski and Marshall, 1980; Sagar and Snodgrass, 1980) to allow greater insight into the relative importance of the lesion sites to the pattern of response observed in the intact animal. The most specific and direct method of pharmacological manipulation in the brain, i.e., localised injections of agents directly into areas of the brain, although extensively used in behavioural studies, has not been used in conjunction with 2-deoxyglucose technology to any great extent (Sakurada et al. 1978).

Despite the undoubted power of the 2-deoxyglucose technique, the approach has not as yet been widely adopted as a routine method in neuropharmacology. The similar degree of spatial resolution afforded by other autoradiographic approaches, in particular the visualisation of receptor distributions (Palacios et al. 1980; Young and Kuhar, 1980), appear to have been accepted as a genuine and worthwhile advance, despite a requirement for expertise and technological hardware no less than that demanded by the 2-deoxyglucose method. A survey of the literature on GABA systems, which remains

an ever expanding field, reveals the extent to which the 2-deoxyglucose technique has failed to a large extent to penetrate into neuropharmacology. Using the approaches to pharmacological manipulation of brain function outlined above, i.e., systemic administration of GABAergic agents, induction of specific lesions in a known GABAergic pathway (striato-nigral pathway) and local injection of a GABA agonist into an area of GABAergic activity, in conjunction with 2-deoxyglucose quantitative autoradiography, the studies which have been described in this thesis provide a comprehensive analysis of the role of GABAergic activity in influencing aspects of cerebral function. Such descriptions have hitherto not been possible using classical biochemical and physiological approaches of measuring cerebral function, many of which are static rather than dynamic measures, require the complicating factor of anaesthesia, lack the same degree of spatial resolution and are devoid of any understanding of events spatially removed from the primary site of interest.

Although the basic premise underlying functional mapping, that altered rates of glucose utilisation reflect dynamic changes in cerebral activity, has been validated under a number of conditions (Kennedy et al. 1975; Schwartz et al. 1979; Mata et al. 1980; Schoppmann and Stryker, 1981), it should be remembered that glucose is involved in the intermediary metabolism of the brain in pathways quite distinct from its

primary role in the generation of energy. Glucose provides a source of carbon for lipid and protein synthesis, although under normal steady-state conditions only a small fraction of the entire cerebral glucose flux is directed towards lipid and protein production (approximately 2% and 0.3%, respectively) (Maker et al. 1972; Siesjö, 1978). Of particular relevance to investigations which have been described is the involvement of glucose as a substrate in the synthesis of acetylcholine and GABA (Maker et al. 1972), especially in view of regional alterations in the rates of synthesis and turnover of these neurotransmitters reported to occur following muscimol administration (Zsilpa et al. 1976; Moroni et al. 1979; Scatton and Bartholini, 1979), and presumably any other form of GABAergic manipulation.

Acetylcholine synthesis, at its maximum possible rate, has been estimated to require only 1% of the total pyruvate generated from glucose and, as the normal rate of synthesis is only 10% of its maximum level (Gibson et al. 1975), even large changes in production would contribute minimally to the measured rates of glucose utilisation. A much larger fraction of the total pyruvate (approximately 8%) passes via the "GABA shunt pathway" under normal conditions, with a resultant small loss to the maximum total available energy generation from glucose (Maker et al. 1972; Siesjö, 1978). Dynamic alterations in GABA turnover following muscimol administration (Moroni et

el. 1979), occurring during the measurement of glucose utilisation, might affect the relationship between energy generation and total glucose phosphorylation. Under steady-state conditions, the importance of increased energy-generating capacity resulting from decreased flux through the GABA shunt is likely to be minimal, as the catabolic products of GABA (mainly succinate semialdehyde) would anyway be returned to the tricarboxylic acid cycle at the succinate level (Maker et al. 1972; Siesjö, 1978). However, GABA synthesis and catabolism may be physically located within different cells in the brain, and although the energetic cost of the GABA shunt to the brain taken as a whole may indeed be quite small, neuronal GABA synthesis and release could represent a net energy loss to the neurons with an almost equal energy gain by the glial elements. The neuronal cells and their processes have greater energetic requirements than other cell types (Schwartz et al. 1979), and so any decrease in GABA turnover elicited by muscimol administration, by reducing the loss of energy-generating substrates from the neuron, could decrease the measured rate of glucose phosphorylation independently of altered functional activity.

Two lines of evidence indicate against altered flux through the GABA shunt being a major contributory factor in the decreased rates of glucose utilisation measured following systemic muscimol administration. Firstly, one could justifiably assume that areas of

high GABA turnover would have a greater potential for energy conservation were the GABA shunt to be bypassed. As has been discussed in the appropriate commentary (p114), there appears to be an inverse correlation between regional rates of GABA turnover (Mao et al. 1978) and magnitude of decreased glucose use in response to muscimol. Secondly, only where glial cells are responsible for a large part of the re-uptake of synaptic GABA should neurons suffer anything other than a minimal loss of energy substrates to the glial element under normal conditions. This reduced flux through the GABA shunt, if this were the sole mechanism by which cerebral glucose utilisation decreased following muscimol, should result in greater reductions in glucose use in areas where glial re-uptake is most apparent. However, no direct correlation is evident between the cerebral regions which are most sensitive to muscimol in terms of reduced glucose utilisation and those in which glial cells contribute substantially to GABA re-uptake (Moroni et al. 1982). It can be concluded, therefore, that the decreases in glucose utilisation observed following systemic muscimol or THIP are a true reflection of altered functional activity with the concomitant effects upon the intermediary metabolism of glucose via the GABA shunt contributing only minimally to the overall effect.

Following the systemic administration of the putative GABA agonists, muscimol and THIP, the heterogeneous distribution of reduced rates of glucose

utilisation measured in the brain provides a novel insight into the relative sensitivities of functionally diverse regions to GABA influences. These results could not have been predicted on the basis of previously determined data. Although the effects of muscimol and THIP upon glucose use in the substantia nigra were in close agreement with the responses measured electrophysiologically (Waszcak et al. 1980), data of this nature is not available on all of the regions analysed simultaneously with the 2-deoxyglucose technique. A comparison with results from methods with a similar degree of spatial resolution and global analysis (e.g., GABA receptor distributions) (Palacios et al. 1981) reveals no good correlation between GABA binding potential and sensitivity of regional glucose use to the effects of the agonists. The dichotomy is particularly marked in a comparison of the alterations in glucose use observed in cerebral cortex and cerebellum, which both display similar distributions of biochemical and histological markers for GABA systems (Tappaz et al. 1976; van der Heyden and Korf, 1978), and yet which are at opposite extremes of sensitivity to the agonists.

Previous investigations of the functional role of GABA in the brain have concentrated in the main upon cerebellar, extrapyramidal and hippocampal functions (Krøgsgaard-Larsen et al. 1979). Although GABAergic synapses are known to constitute the principal inhibitory mechanism in cerebral cortex (Roberts, 1978), the extent to which the thalamo-cortical axis has the

potential to be influenced by GABAergic mechanisms has never before been fully appreciated. The particular susceptibility of functional activity in neocortical lamina IV may be indicative of a primary role of this region in the organisation of cortical function, with GABAergic neuronal mechanisms available to inhibit both cortical input and local circuit neurons.

The profound effects of exogenous GABAergic agents in cortex would tend to suggest that whilst there is a large potential for the expression of inhibitory influences in cortex, there is a relatively low activation of endogenous GABAergic neuronal systems under normal conditions. It is possible that cortical GABA systems are input stimulus-dependent, being activated to their full potential only in response to excitatory input into cortex. Such a scheme of functional organisation in cortex is not, however, compatible with the proposal of disinhibition as a general organising principle (Roberts, 1978). In contrast, in the extrapyramidal system where GABAergic neurons have been extensively characterised (Fonnum et al. 1978), the minimal effects of the GABA agonists on functional activity as reflected in rates of glucose utilisation suggest a higher steady-state activation of endogenous GABAergic neurons. In this instance, the possibility of disinhibition becoming a factor in the organisation of function becomes tenable.

The role of GABAergic neurons in the extrapyramidal system became apparent only when striatal efferents,

mainly GABAergic, were removed by kainic acid-induced lesions of the caudate nucleus. With the removal of inhibitory influences, functional activity in primary projection areas was markedly increased. The apparent disinhibition of those areas was reduced by systemically administered muscimol at a dose which had only moderate effects in intact animals. However, the relatively high dose used (1.5 mg/kg) was not sufficient to reduce the levels of functional activity to those in intact control animals. It would appear from this observation that endogenous GABA projections are both extremely potent and very active in the intact animal in their inhibitory influences.

In the extrapyramidal system contralateral to the site of striatal lesion, the significant alterations in functional activity which were apparent revealed the capacity of disinhibitory processes to provide an appropriate, organised response to altered functional needs; in this instance, the restoration of symmetry or posture and locomotion. That this response was indeed the result of subtle alterations in contralateral striatal GABAergic input into pallidus and nigra was further suggested by the action of muscimol in totally eliminating post-lesion increases in functional activity, presumably by compensating for reduced endogenous GABA-release. A lability of inhibitory influences of this nature would undoubtedly be necessary for disinhibition to be a viable means of organising extrapyramidal function.

Although the results described from lesion studies provide evidence for an important role for modulation of GABAergic neuronal activity as an organising principle, it is not clear what constitutes the necessary stimulus to promote attenuation of active GABAergic projections. Neither the anatomical pathways nor the neurotransmitter systems involved in induction of the contralateral responses to striatal lesion were immediately apparent, although some possibilities were discussed in the appropriate commentary. In the intact animal the striatum receives dopaminergic input from the pars compacta of the substantia nigra, serotonergic input from the raphe nucleus, and glutaminergic input from neocortex. GABAergic innervation of striatum arises in the thalamus, but GABA neurons are also found intrinsic to the caudate nucleus, as are cholinergic neurons (Walaas and Fonnum, 1979). Thus, a complex interaction may exist between striatal afferent systems and interneurons to affect the output of caudate nucleus and, ultimately, the disinhibition of globus pallidus and nigra. To determine the mechanisms and stimuli which alter striatal influences in the brain would present an extensive project in itself, but the data from this thesis does provide some insight into the mechanisms which promote disinhibition of striatal outflow areas.

The actions of the dopaminergic agonist, apomorphine, upon the extrapyramidal system of intact rats produced large increases in functional activity within

the globus pallidus and entopeduncular nucleus, and the substantia nigra. That these effects are a result of disinhibitory factors is clearly demonstrated by the attenuation of apomorphine effects in animals which lack striatal GABAergic efferents. Thus, it is possible to suggest that endogenous dopaminergic input into the caudate nucleus is integrated within the region, and results in decreased activity in striatal efferents which, in turn, allows areas receiving efferents to become disinhibited. A similar pattern of response ensued when a GABA agonist was injected directly into the striatum. Pallidus and nigra both displayed considerable increases in functional activation, presumably once again because of decreased activity in inhibitory efferents. It has been shown, therefore, that both dopaminergic striatal afferents and intrinsic GABAergic neurons have the potential to modulate disinhibitory influences. Although the net effects in the extrapyramidal system were essentially similar, the two treatments, systemic apomorphine and local injection of muscimol, produced opposite effects in the caudate nucleus itself. Whilst apomorphine increased striatal functional activity, as measured by the local rate of glucose utilisation, local application of muscimol reduced functional activity. If the caudate was examined without a knowledge of events elsewhere in the extrapyramidal system, it could be concluded that these two treatments may have opposite effects.

The 2-deoxyglucose technique should have an important role to play in future neuropharmacological studies. The data and discussions presented in this thesis have shown that the method is wholly applicable to studies of cerebral GABA systems, not only supplying novel insights into specific GABAergic functions in the brain, as discussed in the commentaries, but also may provide an understanding of more general organising principles pertinent to cerebral function in the brain.

APPENDICES

APPENDIX I

Manufacture and Calibration of Standards for Quantitative Autoradiography.

A volume of 50 ml of Spurr's resin was mixed from its constituent parts in accordance with the manufacturer's instructions. To 25 ml was added 100 uCi of [^{14}C] benzoic acid. The liquid resin was heated slightly and mixed with a magnetic stirrer for 60 minutes. Volumes of the radioactive resin mixture were added to varying quantities of the remaining non-radioactive stock and again mixed thoroughly. The resulting [^{14}C]-containing resins were poured individually into a well greased metal mould and cured overnight in an oven at 70^{o}C. The following morning the mould was opened, the sheet of solid resin removed, and applied to X-ray film for seven days. Only if no heterogeneity of OD was observed in the image of the sheets on X-ray film, were the large squares cut into small, 0.5 cm squares.

Five standards of different [^{14}C] concentrations were calibrated according to the method of Reivich et al. (1969). These standards, together with the other nine which were made, were calibrated against those precalibrated and used in Sokoloff's laboratory (these had also been calibrated according to the Reivich

method). The five standards calibrated by both methods produced values which were essentially similar ($\pm$ 4%), although the range of values generated by reference to Sokoloff's standards were those which were adopted.

APPENDIX II

Rates of Glucose Utilisation Following Systemic Injection of Muscimol and THIP.

Glucose utilisation was measured in conscious rats following intravenous administration of saline (n=9), muscimol at doses of 0.15 (n=4), 0.5 (n=4), 1.5 (n=4) and 5.0 mg/kg (n=3), or THIP at doses of 1.0 (n=4), 3.0 (n=4) and 10.0 mg/kg (n=4).

The data are presented as mean glucose use ($\mu mol.100g^{-1}min^{-1}$) $\pm$ SEM in the subsequent five tables. $^{*}p < 0.05$.

APPENDIX II(a)

PRIMARY AUDITORY AND VISUAL AREAS

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF MUSCIMOL AND THIP.

Structure	Saline Control	Muscimol (mg/kg) 0.15	0.5	1.5	5.0	THIP (mg/kg) 1.0	3.0	10.0
Primary Visual								
Visual Cortex, Layer II	72 ± 3	70 ± 3	61 ± 6	48 ± 2*	32 ± 7*	70 ± 3	66 ± 4	42 ± 4*
IV	97 ± 4	86 ± 3	74 ± 7	55 ± 1*	36 ± 6*	96 ± 2	84 ± 4	52 ± 3*
VI	81 ± 4	70 ± 3	66 ± 6	50 ± 3*	35 ± 8*	77 ± 1	72 ± 4	48 ± 2*
Lateral Geniculate Body	78 ± 3	73 ± 6	60 ± 4	49 ± 3*	30 ± 3*	68 ± 3	68 ± 10	58 ± 2*
Superior Colliculus,								
Superficial Layer	84 ± 3	75 ± 2	64 ± 6*	49 ± 2*	35 ± 3*	84 ± 9	89 ± 6	80 ± 4
Deep Layer	82 ± 2	78 ± 1	72 ± 6	67 ± 3*	49 ± 4*	77 ± 5	76 ± 4	64 ± 3*
Primary Auditory								
Auditory Cortex, Layer II	107 ± 6	98 ± 5	93 ± 5	71 ± 3*	42 ± 10*	100 ± 3	109 ± 8	62 ± 1*
IV	142 ± 6	120 ± 9	110 ± 13	82 ± 7*	44 ± 10*	130 ± 5	128 ± 8	68 ± 2*
VI	105 ± 5	94 ± 9	88 ± 8	69 ± 4*	41 ± 10*	99 ± 2	99 ± 6	62 ± 2*
Medial Geniculate Body	108 ± 3	99 ± 2	94 ± 8	94 ± 4*	49 ± 4*	104 ± 2	101 ± 7	66 ± 3*
Inferior Colliculus	177 ± 5	168 ± 3	156 ± 5	163 ± 14	123 ± 9*	176 ± 3	175 ± 9	145 ± 6*
Lateral Lemniscus	107 ± 5	97 ± 3	95 ± 5	95 ± 6	80 ± 7*	96 ± 5	110 ± 6	88 ± 10
Superior Olivary Nucleus	142 ± 6	120 ± 5	130 ± 7	108 ± 11	104 ± 4*	127 ± 8	133 ± 8	101 ± 6*
Cochlear Nucleus	125 ± 6	110 ± 5	99 ± 7	97 ± 5	74 ± 10*	109 ± 2	137 ± 3	110 ± 8

APPENDIX II (b)

CEREBRAL CORTEX

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF MUSCIMOL AND THIP.

Structure	Saline Control	Muscimol (mg/kg) 0.15	0.5	1.5	5.0	THIP (mg/kg) 1.0	3.0	10.0
Sensory-Motor Cortex:								
Layer II	93 ± 4	81 ± 7	72 ± 4*	50 ± 3*	29 ± 4*	85 ± 5	71 ± 4*	47 ± 3*
IV	112 ± 5	96 ± 7	80 ± 4*	55 ± 3*	34 ± 5*	101 ± 6	82 ± 6*	55 ± 4*
VI	87 ± 9	77 ± 7	66 ± 4*	47 ± 3*	30 ± 3*	83 ± 5	67 ± 4*	43 ± 3*
Posterior Parietal Cortex:								
Layer II	87 ± 4	73 ± 6	67 ± 5	47 ± 3*	30 ± 2*	75 ± 4	67 ± 4	47 ± 5*
IV	103 ± 4	87 ± 9	77 ± 7*	53 ± 2*	34 ± 3*	88 ± 4	76 ± 4*	52 ± 5*
VI	72 ± 10	65 ± 4	60 ± 4	44 ± 1*	30 ± 2*	73 ± 2	64 ± 4	42 ± 4*
Frontal Cortex:								
Layer II	86 ± 4	76 ± 4	69 ± 7*	50 ± 3*	30 ± 4*	79 ± 6	67 ± 4*	45 ± 4*
IV	103 ± 4	88 ± 4	77 ± 4*	56 ± 3*	32 ± 5*	97 ± 5	81 ± 6*	51 ± 4*
VI	84 ± 5	78 ± 3	68 ± 4	50 ± 1*	30 ± 3*	84 ± 3	69 ± 3	45 ± 3*
Posterior Cingulate Cortex	111 ± 3	98 ± 10	95 ± 6	81 ± 3*	39 ± 5*	101 ± 4	90 ± 7	74 ± 5*
Anterior Cingulate Cortex	108 ± 4	97 ± 4	96 ± 8	72 ± 6*	39 ± 6*	104 ± 4	105 ± 7	58 ± 3*
Prefrontal Cortex	119 ± 6	98 ± 3*	94 ± 5*	72 ± 3*	39 ± 7*	114 ± 4	104 ± 7	76 ± 1*
Olfactory Cortex	81 ± 3	76 ± 3	74 ± 5	59 ± 3*	32 ± 5*	78 ± 6	71 ± 8	52 ± 4*

APPENDIX II (c)

EXTRAPYRAMIDAL AND SENSORY-MOTOR AREAS

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF MUSCIMOL AND THIP.

Structure	Saline Control	Muscimol (mg/kg) 0.15	0.5	1.5	5.0	THIP (mg/kg) 1.0	3.0	10.0
Caudate Nucleus:								
Medial	105 ± 3	96 ± 8	93 ± 4	69 ± 2*	38 ± 6*	96 ± 5	89 ± 5	62 ± 4*
Lateral	105 ± 3	90 ± 6	83 ± 5*	52 ± 1*	37 ± 4*	93 ± 3	86 ± 6	52 ± 2*
Globus Pallidus	52 ± 2	48 ± 1	49 ± 3	38 ± 4*	28 ± 1*	50 ± 1	50 ± 5	37 ± 1*
Substantia Nigra (pars compacta)	71 ± 3	63 ± 3	60 ± 3	57 ± 1*	43 ± 4*	64 ± 2	65 ± 4	55 ± 2*
Substantial Nigra (pars reticulata)	55 ± 2	50 ± 3	45 ± 4	44 ± 2*	32 ± 1*	54 ± 2	53 ± 1	42 ± 2*
Red Nucleus	73 ± 3	63 ± 2	66 ± 4	62 ± 4*	61 ± 6	71 ± 4	70 ± 2	60 ± 3*
Thalamus (ventrolateral nucleus)	79 ± 4	71 ± 4	68 ± 3	47 ± 2*	40 ± 1*	71 ± 4	69 ± 6	54 ± 4*
Subthalamic Nucleus	85 ± 3	78 ± 7	74 ± 2	71 ± 3*	46 ± 4*	72 ± 2	65 ± 4*	58 ± 2*
Inferior Olivary Nucleus	73 ± 3	69 ± 3	63 ± 4*	61 ± 3*	55 ± 4*	66 ± 2	63 ± 4*	57 ± 2*
Vestibular Nucleus	115 ± 3	104 ± 6	102 ± 10	100 ± 2*	95 ± 5*	98 ± 3*	101 ± 5	97 ± 3*
Cerebellar Nuclei	94 ± 3	95 ± 8	83 ± 10	81 ± 4	62 ± 8*	81 ± 4	81 ± 7	82 ± 4
Cerebellar Hemisphere	54 ± 2	51 ± 2	50 ± 4	47 ± 1	34 ± 4*	50 ± 2	48 ± 2	43 ± 2
Cerebellum Vermis (maximum)	83 ± 2	83 ± 2	79 ± 6	75 ± 2	76 ± 9	83 ± 5	81 ± 9	81 ± 4

APPENDIX II (d)

LIMBIC AND FUNCTIONALLY NON-SPECIFIC AREAS.

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF MUSCIMOL AND THIP.

Structure	Saline Control	Muscimol (mg/kg) 0.15	0.5	1.5	5.0	THIP (mg/kg) 1.0	3.0	10.0
Hippocampus	80 ± 3	73 ± 2	69 ± 5	65 ± 2*	41 ± 9*	75 ± 2	78 ± 4	64 ± 2*
Dentate Gyrus	67 ± 2	63 ± 2	61 ± 5	58 ± 1*	39 ± 6*	59 ± 1	62 ± 4	54 ± 2*
Septal Nucleus: Dorsalateral	51 ± 2	48 ± 5	45 ± 2	46 ± 3	31 ± 4*	51 ± 2	47 ± 3	46 ± 6
Ventromedial	78 ± 3	73 ± 7	71 ± 4	63 ± 1*	37 ± 4*	71 ± 4	66 ± 4	68 ± 6
Nucleus Accumbens	84 ± 2	80 ± 5	74 ± 5	65 ± 2*	35 ± 5*	77 ± 3	81 ± 4	62 ± 2*
Lateral Habenular Nucleus	112 ± 5	100 ± 7	98 ± 6	98 ± 3	80 ± 4*	106 ± 4	100 ± 6	96 ± 7
Medial Habenular Nucleus	79 ± 4	71 ± 7	67 ± 6	63 ± 3	50 ± 5*	73 ± 4	70 ± 5	66 ± 4
Thalamus (mediodorsal nucleus)	105 ± 3	88 ± 6	85 ± 4*	68 ± 3*	43 ± 3*	88 ± 5	86 ± 4*	63 ± 2*
Thalamus (anterior nucleus)	105 ± 3	103 ± 3	97 ± 5	76 ± 3*	43 ± 4*	98 ± 3	81 ± 4	77 ± 4*
Amygdala	43 ± 1	44 ± 4	43 ± 3	37 ± 1	26 ± 4*	42 ± 2	41 ± 3	38 ± 4
Interpeduncular Nucleus	102 ± 3	84 ± 4	87 ± 6	78 ± 6*	67 ± 8*	87 ± 3	90 ± 6	85 ± 6
Ventral Tegmental Area	52 ± 2	44 ± 3	46 ± 4	47 ± 2	36 ± 1*	49 ± 2	49 ± 3	43 ± 2
Dorsal Tegmental Nucleus	103 ± 3	93 ± 6	89 ± 7	77 ± 1*	63 ± 5*	94 ± 4	98 ± 7	90 ± 6
Hypothalamus	53 ± 2	48 ± 2	47 ± 2	46 ± 2	30 ± 3*	46 ± 2	47 ± 4	47 ± 5
Median Raphe Nucleus	93 ± 2	83 ± 2*	78 ± 6*	75 ± 3*	54 ± 5*	88 ± 4	85 ± 5	76 ± 7*
Pontine Reticular Formation	58 ± 2	56 ± 2	51 ± 4	51 ± 4	39 ± 2*	56 ± 2	55 ± 4	47 ± 2*

APPENDIX II (e)

MYELINATED FIBRE TRACTS

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF MUSCIMOL AND THIP.

	Saline Control	Muscimol (mg/kg) 0.15	0.5	1.5	5.0	THIP (mg/kg) 1.0	3.0	10.0
Corpus Callosum	36 ± 2	34 ± 2	30 ± 3	31 ± 2	18 ± 1*	36 ± 3	40 ± 3	30 ± 3
Genu of Corpus Callosum	36 ± 3	34 ± 1	30 ± 2	30 ± 2	20 ± 2*	38 ± 3	38 ± 3	26 ± 2*
Internal Capsule	33 ± 1	32 ± 1	29 ± 3	28 ± 2	18 ± 2*	33 ± 1	31 ± 3	24 ± 2*
Cerebellar White Matter	36 ± 1	34 ± 2	34 ± 2	34 ± 3	25 ± 2*	36 ± 2	35 ± 3	31 ± 2*

APPENDIX III

Local Cerebral Blood Flow and Local Cerebral Glucose Utilisation Following Systemic Injection of Muscimol.

Blood flow and glucose use were measured in different groups of rats following 0.5 and 1.5 mg/kg muscimol (for N values see following tables).

The data are presented as mean blood flow ($ml.100g^{-1}min^{-1}$) $\pm$ SEM and mean glucose use ($\mu mol.100g^{-1}min^{-1}$) $\pm$ SEM in the subsequent two tables. $*p < 0.05$.

APPENDIX III (a)

LOCAL CEREBRAL BLOOD FLOW AND LOCAL CEREBRAL GLUCOSE UTILIZATION FOLLOWING SYSTEMIC ADMINISTRATION OF THE PUTATIVE GABAERGIC AGONIST MUSCIMOL.

Region	Blood Flow $ml.100g^{-1}min^{-1}$			Glucose Use $\mu mol.100g^{-1}min^{-1}$		
	Muscimol mg/kg			Muscimol mg/kg		
	Saline	0.5	1.5	Saline	0.5	1.5
Neocortex						
Visual cortex	143± 9	138± 6	85±14*	83± 3	67± 4*	51± 1*
Auditory cortex	264±14	225±21	177±20	118± 5	97± 6*	74± 3*
Parietal cortex	136± 5	127± 6	87±10*	90± 3	69± 4*	48± 2*
Sensory-motor cortex	138± 4	128± 2	92± 6*	98± 3	73± 3*	51± 2*
Frontal cortex	122± 5	110± 5	80± 5*	91± 3	71± 3*	52± 2*
Prefrontal cortex	150± 7	144±13	121± 7*	119± 6	94± 5*	72± 3*
Olfactory cortex	97± 4	96± 5	93±10	81± 3	74± 5	59± 3*
Diencephalon						
Thalamus: Mediodorsal nucleus	131±10	121± 2	103± 8	105± 3	85± 4*	68± 3*
Thalamus: Ventrolateral nucleus	113± 7	103± 3	100± 3	79± 4	68± 3	47± 2*
Medial geniculate body	209±15	166± 3	139±15*	108± 3	94± 8	94± 4*
Lateral geniculate body	114± 4	105± 7	80± 7*	78± 3	60± 4	49± 3*
Lateral habenula	155± 5	153± 5	145±13	112± 5	98± 6	98± 3
Subthalamic nucleus	127± 5	133± 1	121± 8	85± 3	74± 2	71± 3*
Hypothalamus	86± 4	87± 5	72± 4	53± 2	47± 2	46± 2
Telencephalon						
Nucleus accumbens	116± 8	118± 5	119± 7	84± 2	74± 5	65± 2*
Caudate nucleus	121± 3	111± 3	93± 7*	105± 3	88± 3*	61± 2*
Septal nucleus	79± 4	81± 4	73± 5	51± 2	45± 2	46± 3
Globus pallidus	63± 2	63± 1	48± 5*	52± 2	49± 3	38± 4*
Amygdala	71± 4	73± 2	69± 6	43± 1	43± 3	37± 1
Hippocampus	104± 7	104± 4	101± 7	80± 3	69± 5	65± 2*
Dentate gyrus	98± 5	97± 2	95± 6	67± 2	61± 5	58± 1*

Contd./.....

APPENDIX III (b)

Region	Blood Flow $ml.100g^{-1}min^{-1}$			Glucose Use $\mu mol.100g^{-1}min^{-1}$		
	Muscimol mg/kg			Muscimol mg/kg		
	Saline	0.5	1.5	Saline	0.5	1.5
Mesencephalon						
Superior colliculus	133± 6	117± 5	91± 9*	83± 3	72± 6	67± 3*
Inferior colliculus	244±13	235±19	249±17	177± 5	156± 5	163±14
Red nucleus	122± 8	111± 5	116±10	73± 3	66± 4	62± 4*
Substantia nigra:						
pars compacta	97± 5	97± 3	85± 5	71± 3	60± 3	57± 1
pars reticulata	79± 3	76± 2	65± 5	55± 2	45± 4	44± 2*
Lateral lemniscus	163± 8	147± 4	167±10	107± 5	95± 5	95± 6
Cerebellum:						
Hemisphere	86± 3	84± 4	77± 6	54± 2	50± 4	47± 1
Nuclei	154± 9	130± 7	151±12	94± 3	83±10	81± 4
White matter	46± 1	41± 2	37± 3	36± 1	34± 2	34± 3
Vestibular nucleus	171±10	170±10	178±11	115± 3	102±10	100± 2*
Cochlear nucleus	167±10	162± 8	169±15	125± 6	99± 7	97± 5
Superior olivary nucleus	194±12	171± 5	191±15	142± 6	130± 7	108±11
Inferior olivary nucleus	105± 8	101± 3	110± 8	73± 3	63± 4*	61± 3*
Pons	94± 3	90± 4	91± 7	58± 2	51± 4	51± 4
Myelinated Fibre Tracts						
Corpus callosum	37± 1	38± 3	34± 2	36± 2	30± 3	31± 2
Genu	41± 2	42± 2	37± 3	36± 3	30± 2	30± 2
Internal capsule	46± 2	47± 1	40± 2	33± 1	29± 3	28± 2
n	7	4	5	9	4	4

APPENDIX IV

Rates of Glucose Utilisation Following Systemic Injection of Diazepam.

Glucose utilisation was measured in conscious rats following intravenous administration of saline or diazepam at doses of 0.1, 0.3 or 1.0 mg/kg (n=5 for each group).

The data are presented as mean glucose use ($\mu mol.100g^{-1}min^{-1}$) $\pm$ SEM in the subsequent five tables. $^{*}p < 0.05$.

APPENDIX IV (a)

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF DIAZEPAM

PRIMARY AUDITORY AND VISUAL AREAS

Structure	Saline Control	Diazepam (mg/kg)		
		0.1	0.3	1.0
Primary Visual				
Visual Cortex, Layer II	80 ± 4	78 ± 6	65 ± 6*	58 ± 5*
Layer IV	97 ± 4	98 ± 2	76 ± 7*	63 ± 4*
Layer VI	81 ± 4	82 ± 4	65 ± 4*	56 ± 4*
Lateral Geniculate Body	72 ± 2	71 ± 2	49 ± 2*	45 ± 2*
Superior Colliculus:				
Superficial Layer	84 ± 3	81 ± 5	67 ± 3*	67 ± 3*
Deep Layer	82 ± 2	82 ± 3	56 ± 4*	48 ± 3*
Primary Auditory				
Auditory Cortex, Layer II	105 ± 5	98 ± 3	70 ± 4*	65 ± 4*
Layer IV	142 ± 6	127 ± 8	93 ± 7*	82 ± 7*
Layer VI	105 ± 5	90 ± 9	70 ± 5*	65 ± 5*
Medial Geniculate Body	100 ± 3	99 ± 3	73 ± 5*	67 ± 2*
Inferior Colliculus	178 ± 5	177 ± 8	131 ± 6*	138 ± 4*
Lateral Lemniscus	109 ± 4	103 ± 5	78 ± 5*	80 ± 2*
Superior Olivary Nucleus	143 ± 6	127 ± 7	95 ± 5*	115 ± 9*
Cochlear Nucleus	125 ± 6	117 ± 6	93 ± 2*	93 ± 4*

APPENDIX IV (b)

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF DIAZEPAM

CEREBRAL CORTEX

Structure	Saline Control	Diazepam (mg/kg)		
		0.1	0.3	1.0
Sensory-motor Cortex:				
Layer II	92 ± 4	82 ± 3	67 ± 2*	60 ± 4*
Layer IV	112 ± 4	97 ± 6	79 ± 3*	71 ± 5*
Layer VI	93 ± 5	80 ± 5	65 ± 2*	60 ± 6*
Posterior Parietal Cortex:				
Layer II	83 ± 4	77 ± 7	58 ± 2*	53 ± 4*
Layer IV	103 ± 4	91 ± 7	66 ± 5*	61 ± 5*
Layer VI	82 ± 5	74 ± 5	57 ± 2*	51 ± 5*
Frontal Cortex:				
Layer II	83 ± 4	82 ± 5	65 ± 4*	59 ± 2*
Layer IV	103 ± 4	101 ± 7	78 ± 5*	68 ± 4*
Layer VI	84 ± 5	80 ± 6	65 ± 5*	59 ± 3*
Posterior Cingulate Cortex	111 ± 3	102 ± 7	67 ± 5*	56 ± 2*
Anterior Cingulate Cortex	107 ± 4	104 ± 6	82 ± 7*	71 ± 6*
Prefrontal Cortex	119 ± 5	109 ± 7	99 ± 5*	84 ± 7*

APPENDIX IV (c)

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF DIAZEPAM

EXTRAPYRAMIDAL AND SENSORY-MOTOR AREAS

Structure	Saline Control	Diazepam (mg/kg)		
		0.1	0.3	1.0
Caudate Nucleus: Medial	105 ± 3	96 ± 8	74 ± 4*	72 ± 3*
Lateral	104 ± 3	91 ± 6	76 ± 5*	74 ± 6*
Globus Pallidus	52 ± 2	49 ± 4	39 ± 2*	39 ± 2*
Substantia Nigra (pars compacta)	71 ± 3	68 ± 2	43 ± 3*	42 ± 1*
Substantia Nigra (pars reticulata)	55 ± 2	51 ± 2	38 ± 3*	37 ± 2*
Red Nucleus	76 ± 3	69 ± 2	49 ± 4*	46 ± 2*
Thalamus (Ventrolateral Nucleus)	85 ± 3	78 ± 5	56 ± 2*	52 ± 2*
Subthalamic Nucleus	85 ± 3	81 ± 3	56 ± 2*	52 ± 2*
Inferior Olivary Nucleus	73 ± 2	69 ± 3	54 ± 3*	50 ± 2*
Vestibular Nucleus	115 ± 3	105 ± 3	76 ± 3*	73 ± 1*
Cerebellar Nuclei	94 ± 3	91 ± 4	65 ± 4*	60 ± 1*
Cerebellar Hemisphere	54 ± 2	55 ± 3	42 ± 3*	38 ± 1*
Cerebellum Vermis (maximum)	83 ± 2	76 ± 4	67 ± 5*	53 ± 4*

APPENDIX IV (d)

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF DIAZEPAM

LIMBIC AND FUNCTIONALLY NON-SPECIFIC AREAS

Structure	Saline Control	Diazepam (mg/kg)		
		0.1	0.3	1.0
Hippocampus	80 ± 3	76 ± 4	55 ± 5*	55 ± 3*
Dentate Gyrus	67 ± 2	61 ± 3	47 ± 4*	46 ± 2*
Septal Nucleus: Dorsolateral	51 ± 2	49 ± 4	38 ± 2*	37 ± 1*
Ventromedial	76 ± 4	72 ± 2	47 ± 3*	45 ± 3*
Nucleus Accumbens	84 ± 2	80 ± 4	61 ± 4*	59 ± 3*
Lateral Habenular Nucleus	112 ± 5	110 ± 7	72 ± 5*	71 ± 1*
Medial Habenular Nucleus	79 ± 3	77 ± 4	51 ± 4*	53 ± 3*
Thalamus (Mediodorsal Nucleus)	105 ± 3	102 ± 5	68 ± 3*	60 ± 3*
Thalamus (Anterior Nucleus)	107 ± 3	98 ± 5	77 ± 5*	67 ± 2*
Lateral Amygdala	66 ± 2	64 ± 1	61 ± 1*	56 ± 3*
Medial Amygdala	43 ± 1	40 ± 2	35 ± 1*	35 ± 2*
Mamillary Body	121 ± 5	115 ± 6	55 ± 3*	50 ± 3*
Interpeduncular Nucleus	102 ± 3	98 ± 4	69 ± 6*	66 ± 2*
Ventral Tegmental Area	52 ± 3	49 ± 4	32 ± 3*	32 ± 3*
Dorsal Tegmental Nucleus	103 ± 3	95 ± 4	62 ± 7*	54 ± 3*
Hypothalamus	53 ± 2	52 ± 2	38 ± 2*	36 ± 2*
Median Raphe Nucleus	93 ± 2	84 ± 5	60 ± 5*	52 ± 2*
Pontine Reticular Formation	58 ± 2	57 ± 3	42 ± 4*	39 ± 2*

APPENDIX IV (e)

GLUCOSE UTILISATION FOLLOWING THE ADMINISTRATION OF DIAZEPAM

MYLINATED FIBRE TRACTS

Structure	Saline Control	Diazepam (mg/kg)		
		0.1	0.3	1.0
Corpus Callosum	35 ± 2	36 ± 1	28 ± 2*	28 ± 1*
Genu of Corpus Callosum	38 ± 1	37 ± 1	33 ± 1*	30 ± 2*
Internal Capsule	33 ± 1	32 ± 1	28 ± 1*	26 ± 1*
Cerebellar White Matter	36 ± 1	36 ± 2	32 ± 1*	28 ± 1*

APPENDIX V

Rates of Glucose Utilisation Following Striatal Lesions with Kainic Acid and Subsequent Pharmacological Challenges.

Glucose utilisation was measured in conscious rats 10 days after unilateral striatal lesions with kainic acid or sham lesions, and following either intravenous injection of saline, 1.5 mg/kg muscimol, or 1.0 mg/kg apomorphine (n=5 for each group).

Data are presented as mean glucose use (μmol.100g^{-1} min^{-1}) $\pm$ SEM in the subsequent six tables. *$p < 0.05$ for comparison between sham and lesion treatments; $\phi p < 0.05$ for comparison between saline injected and drug injected treatments.

APPENDIX V (a)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF KAINIC ACID AND SUBSEQUENT PHARMACOLOGICAL CHALLENGES

		Ipsilateral			Contralateral		
		Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg	Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg
Caudate Nucleus	Sham:	82 ± 5	62 ± 6$^{\Phi}$	109 ± 5$^{\Phi}$	86 ± 5	60 ± 7$^{\Phi}$	109 ± 6$^{\Phi}$
	Lesion:	67 ± 3*	57 ± 4	78 ± 5*	92 ± 5	59 ± 4$^{\Phi}$	102 ± 8$^{\Phi}$
Globus Pallidus	Sham:	44 ± 1	38 ± 2	71 ± 5$^{\Phi}$	45 ± 1	41 ± 1	74 ± 7$^{\Phi}$
	Lesion:	80 ± 4*	56 ± 1$^{*\Phi}$	76 ± 6	52 ± 2*	41 ± 1$^{\Phi}$	68 ± 5$^{\Phi}$
Entopeduncular Nucleus	Sham:	42 ± 1	36 ± 2$^{\Phi}$	99 ± 4$^{\Phi}$	42 ± 1	37 ± 2	100 ± 4$^{\Phi}$
	Lesion:	57 ± 2*	45 ± 3$^{*\Phi}$	76 ± 3$^{*\Phi}$	46 ± 3	40 ± 3	82 ± 2$^{\Phi}$
Substantia Nigra, pars reticulata	Sham:	45 ± 1	40 ± 2	80 ± 8$^{\Phi}$	45 ± 1	41 ± 3	83 ± 9$^{\Phi}$
	Lesion:	80 ± 5*	58 ± 1$^{*\Phi}$	98 ± 5$^{*\Phi}$	54 ± 2*	41 ± 3$^{\Phi}$	90 ± 6$^{\Phi}$
Substantia Nigra, pars compacta	Sham:	56 ± 1	50 ± 4	77 ± 8$^{\Phi}$	57 ± 1	50 ± 4	80 ± 8$^{\Phi}$
	Lesion:	62 ± 3	57 ± 1	75 ± 5$^{\Phi}$	65 ± 3*	60 ± 2*	85 ± 4$^{\Phi}$

APPENDIX V (b)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF KAINIC ACID AND SUBSEQUENT PHARMACOLOGICAL CHALLENGES

		Ipsilateral			Contralateral		
		Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg	Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg
Sensory-motor Cortex	Sham:	96 ± 5	46 ± 2$^{\Phi}$	114 ± 5$^{\Phi}$	93 ± 2	45 ± 3$^{\Phi}$	113 ± 9$^{\Phi}$
	Lesion:	101 ± 4	56 ± 5$^{\Phi}$	99 ± 5	107 ± 3	53 ± 3$^{\Phi}$	104 ± 6
Frontal Cortex	Sham:	95 ± 4	48 ± 2$^{\Phi}$	111 ± 7	95 ± 4	48 ± 2$^{\Phi}$	120 ± 5
	Lesion:	81 ± 7	46 ± 2$^{\Phi}$	89 ± 4	85 ± 7	46 ± 1$^{\Phi}$	97 ± 4
Posterior Parietal Cortex	Sham:	98 ± 2	44 ± 1$^{\Phi}$	107 ± 5	98 ± 2	45 ± 2$^{\Phi}$	107 ± 5
	Lesion:	79 ± 5	46 ± 1$^{\Phi}$	100 ± 8	85 ± 4	42 ± 3$^{\Phi}$	105 ± 5
Visual Cortex	Sham:	80 ± 3	46 ± 2$^{\Phi}$	82 ± 4	80 ± 3	46 ± 2$^{\Phi}$	82 ± 4
	Lesion:	75 ± 3	46 ± 2$^{\Phi}$	79 ± 6	78 ± 2	48 ± 2$^{\Phi}$	80 ± 6
Anterior Cingulate Cortex	Sham:	98 ± 3	65 ± 3$^{\Phi}$	65 ± 5$^{\Phi}$	98 ± 3	62 ± 4$^{\Phi}$	71 ± 5$^{\Phi}$
	Lesion:	93 ± 4	60 ± 3$^{\Phi}$	68 ± 5$^{\Phi}$	95 ± 4	64 ± 3$^{\Phi}$	72 ± 5$^{\Phi}$
Prefrontal Cortex	Sham:	110 ± 6	73 ± 5$^{\Phi}$	119 ± 12	110 ± 6	74 ± 3$^{\Phi}$	118 ± 10
	Lesion:	100 ± 7	75 ± 5$^{\Phi}$	115 ± 10	109 ± 5	74 ± 6$^{\Phi}$	120 ± 5

APPENDIX V (c)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF KAINIC ACID AND SUBSEQUENT PHARMACOLOGICAL CHALLENGES

		Ipsilateral			Contralateral		
		Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg	Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg
Thalamus:							
Mediodorsal Nucleus	Sham:	89 ± 3	58 ± 2$^{\Phi}$	97 ± 9	89 ± 3	58 ± 4$^{\Phi}$	105 ± 7
	Lesion:	88 ± 3	63 ± 2$^{\Phi}$	93 ± 5	93 ± 5	65 ± 2$^{\Phi}$	97 ± 6
Ventrolateral Nucleus	Sham:	68 ± 2	47 ± 2$^{\Phi}$	94 ± 4$^{\Phi}$	68 ± 2	48 ± 4$^{\Phi}$	100 ± 5$^{\Phi}$
	Lesion:	77 ± 2*	53 ± 2$^{\Phi}$	108 ± 8$^{\Phi}$	76 ± 3	52 ± 4$^{\Phi}$	112 ± 8$^{\Phi}$
Ventromedial Nucleus	Sham:	89 ± 3	69 ± 2$^{\Phi}$	124 ± 4$^{\Phi}$	88 ± 3	70 ± 3	128 ± 5$^{\Phi}$
	Lesion:	87 ± 3	52 ± 1*$^{\Phi}$	112 ± 5*$^{\Phi}$	106 ± 3*	51 ± 3*$^{\Phi}$	114 ± 5$^{\Phi}$
Parafascicular Nucleus	Sham:	70 ± 2	69 ± 1	109 ± 4$^{\Phi}$	70 ± 2	69 ± 1	109 ± 4$^{\Phi}$
	Lesion:	76 ± 1	65 ± 1$^{\Phi}$	94 ± 4*$^{\Phi}$	86 ± 2*	65 ± 1$^{\Phi}$	94 ± 2*$^{\Phi}$
Anterior Nucleus	Sham:	104 ± 5	77 ± 2$^{\Phi}$	78 ± 6$^{\Phi}$	104 ± 5	72 ± 5$^{\Phi}$	79 ± 6$^{\Phi}$
	Lesion:	97 ± 5	75 ± 2$^{\Phi}$	74 ± 6$^{\Phi}$	102 ± 5	74 ± 3$^{\Phi}$	72 ± 6$^{\Phi}$

APPENDIX V (d)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF KAINIC ACID AND SUBSEQUENT PHARMACOLOGICAL CHALLENGES

		Ipsilateral			Contralateral		
		Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg	Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg
Subthalamic Nucleus	Sham:	73 ± 2	71 ± 2	139 ± 3$^{\Phi}$	76 ± 3	67 ± 5	132 ± 7$^{\Phi}$
	Lesion:	79 ± 3	68 ± 4	111 $\overset{*}{\pm}$ 6$^{\Phi}$	76 ± 3	68 ± 5	121 ± 7$^{\Phi}$
Red Nucleus	Sham:	61 ± 1	63 ± 2	85 ± 4$^{\Phi}$	62 ± 1	57 ± 4	89 ± 5$^{\Phi}$
	Lesion:	71 $\overset{*}{\pm}$ 3	61 ± 4$^{\Phi}$	86 ± 4$^{\Phi}$	74 $\overset{*}{\pm}$ 3	66 ± 5	89 ± 5$^{\Phi}$
Inferior Olivary Body	Sham:	75 ± 4	61 ± 4$^{\Phi}$	84 ± 5	75 ± 4	61 ± 4$^{\Phi}$	82 ± 5
	Lesion:	73 ± 5	65 ± 5	72 $\overset{*}{\pm}$ 2	73 ± 5	62 ± 5	74 ± 2
Cerebellar Hemisphere	Sham:	40 ± 2	44 ± 4	92 ± 3$^{\Phi}$	41 ± 2	46 ± 4	93 ± 8$^{\Phi}$
	Lesion:	42 ± 2	47 ± 3	85 ± 3$^{\Phi}$	42 ± 2	47 ± 3	101 ± 4$^{\Phi}$
Cerebellar Nuclei	Sham:	90 ± 5	78 ± 5	105 ± 3	90 ± 5	84 ± 8	103 ± 6
	Lesion:	87 ± 5	76 ± 3	98 ± 5	85 ± 5	84 ± 5	98 ± 4

APPENDIX V (e)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF KAINIC ACID AND SUBSEQUENT PHARMACOLOGICAL CHALLENGES

		Ipsilateral			Contralateral		
		Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg	Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg
Nucleus Accumbens	Sham:	68 ± 2	66 ± 2	76 ± 4	67 ± 2	65 ± 4	72 ± 5
	Lesion:	78 ± 4*	57 ± 1*◆	62 ± 5	76 ± 3*	58 ± 1	65 ± 6
Ventral Tegmental Area	Sham:	42 ± 1	40 ± 2	48 ± 5	42 ± 1	39 ± 2	48 ± 5
	Lesion:	52 ± 2*	42 ± 5◆	52 ± 3	52 ± 2	44 ± 5	52 ± 3
Lateral Habenula	Sham:	95 ± 5	89 ± 2	66 ± 4◆	95 ± 5	81 ± 6	66 ± 4◆
	Lesion:	117 ± 5*	93 ± 2◆	85 ± 5*◆	102 ± 7	87 ± 5	72 ± 6◆
Amygdala, Medial	Sham:	42 ± 2	38 ± 2	46 ± 3	42 ± 2	41 ± 3	48 ± 3
	Lesion:	40 ± 2	38 ± 2	43 ± 1	42 ± 2	38 ± 3	45 ± 3
Septal Nucleus	Sham:	48 ± 2	48 ± 1	51 ± 2	48 ± 2	44 ± 3	51 ± 2
	Lesion:	51 ± 2	45 ± 2	53 ± 3	48 ± 2	44 ± 1	58 ± 5

APPENDIX V (f)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF KAINIC ACID AND SUBSEQUENT PHARMACOLOGICAL CHALLENGES

		Ipsilateral			Contralateral		
		Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg	Saline	Muscimol 1.5 mg/kg	Apomorphine 1.0 mg/kg
Superior Colliculus:							
Superficial	Sham:	74 ± 3	46 ± 5$^{\Phi}$	79 ± 4	74 ± 3	48 ± 5$^{\Phi}$	79 ± 4
	Lesion:	76 ± 2	59 ± 2$^{\Phi}$	81 ± 9	76 ± 2	49 ± 5$^{\Phi}$	81 ± 7
Deep	Sham:	62 ± 2	59 ± 3	90 ± 4$^{\Phi}$	62 ± 2	57 ± 5	90 ± 4$^{\Phi}$
	Lesion:	73 ± 2	67 ± 2	86 ± 4$^{\Phi}$	81 ± 3*	65 ± 3	90 ± 6
Hippocampus	Sham:	70 ± 1	69 ± 8	70 ± 4	71 ± 2	69 ± 8	70 ± 4
	Lesion:	73 ± 4	65 ± 2	70 ± 4	72 ± 2	65 ± 3	73 ± 5
Lateral Geniculate	Sham:	75 ± 3	49 ± 2$^{\Phi}$	67 ± 3	75 ± 3	51 ± 2$^{\Phi}$	67 ± 3
	Lesion:	70 ± 5	49 ± 2$^{\Phi}$	73 ± 2	73 ± 5	49 ± 2$^{\Phi}$	70 ± 2
Hypothalamus	Sham:	52 ± 3	45 ± 1	49 ± 3	52 ± 3	45 ± 2	52 ± 3
	Lesion:	52 ± 3	45 ± 1	53 ± 4	51 ± 3	45 ± 1	52 ± 4

APPENDIX VI

Rates of Glucose Utilisation Following Intrastriatal Injections of Muscimol.

Glucose utilisation was measured in conscious rats following a single intrastriatal injection of artificial CSF, 100 ng muscimol or 500 ng muscimol (n=5 in each group).

The data are presented as mean glucose use (μmol. $100g^{-1}min^{-1}$) $\pm$ SEM in the subsequent three tables. *$p<0.05$. (+) Densitometric readings for this nucleus were made on the mid-line; therefore, ipsi- and contralateral values are equal.

APPENDIX VI (a)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF MUSCIMOL : MOTOR AREAS.

Structure	Ipsilateral			Contralateral		
	CSF	Muscimol 100 ng	Muscimol 500 ng	CSF	Muscimol 100 ng	Muscimol 500 ng
Caudate: Rostral	83 ± 3	80 ± 3	54 ± 3*	95 ± 3	96 ± 3	79 ± 6
Head	81 ± 2	70 ± 2*	54 ± 3*	96 ± 5	85 ± 4	82 ± 5
Caudal	81 ± 2	82 ± 2	56 ± 3*	81 ± 3	84 ± 3	77 ± 4
Globus Pallidus	45 ± 2	40 ± 2	58 ± 1*	47 ± 1	45 ± 2	47 ± 2
Entopeduncular Nucleus	40 ± 1	48 ± 1*	53 ± 1*	43 ± 2	41 ± 2	43 ± 1
Substantia Nigra, pars reticulata						
Rostral	48 ± 2	42 ± 3	66 ± 2*	47 ± 2	47 ± 2	41 ± 3
Caudal	48 ± 1	44 ± 3	70 ± 1*	48 ± 1	45 ± 2	45 ± 1
Substantia Nigra, pars compacta	54 ± 2	50 ± 2	69 ± 2*	55 ± 1	54 ± 2	56 ± 1
Subthalamic Nucleus	65 ± 3	60 ± 3	53 ± 2*	68 ± 2	65 ± 2	62 ± 3
Red Nucleus	57 ± 2	57 ± 4	64 ± 3	58 ± 2	57 ± 2	60 ± 2
Inferior Olivary Body	57 ± 2	60 ± 3	65 ± 5	56 ± 2	61 ± 2	66 ± 4
Cerebellar Hemisphere	45 ± 2	51 ± 3	50 ± 2	45 ± 2	51 ± 3	50 ± 3
Cerebellar Nuclei	87 ± 4	85 ± 3	90 ± 4	84 ± 4	87 ± 3	92 ± 3
Superior Colliculus:						
Superficial	75 ± 4	75 ± 2	70 ± 3	76 ± 4	73 ± 3	68 ± 4
Deep	67 ± 3	62 ± 3	55 ± 2*	67 ± 3	67 ± 3	66 ± 1

APPENDIX VI (b)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF MUSCIMOL : NEOCORTICAL AND THALAMIC AREAS.

Structure	Ipsilateral			Contralateral		
	CSF	Muscimol 100 ng	Muscimol 500 ng	CSF	Muscimol 100 ng	Muscimol 500 ng
Sensory-motor Cortex	90 ± 3	82 ± 5	48 ± 3*	102 ± 5	91 ± 4	91 ± 3
Frontal Cortex	90 ± 4	85 ± 2	78 ± 6	100 ± 4	102 ± 3	110 ± 4
Posterior Parietal Cortex	75 ± 4	71 ± 3	70 ± 2	86 ± 5	87 ± 4	81 ± 2
Visual Cortex	85 ± 4	79 ± 4	85 ± 4	95 ± 4	85 ± 4	87 ± 4
Anterior Cingulate Cortex	85 ± 4	81 ± 2	72 ± 6	96 ± 4	90 ± 2	85 ± 5
Prefrontal Cortex	105 ± 5	101 ± 6	95 ± 5	115 ± 5	112 ± 5	106 ± 5
Thalamus:						
Mediodorsal Nucleus	80 ± 4	82 ± 3	72 ± 4	85 ± 4	82 ± 2	80 ± 2
Ventrolateral Nucleus	70 ± 2	65 ± 4	42 ± 2*	78 ± 4	72 ± 3	65 ± 2*
Ventromedial Nucleus	94 ± 3	90 ± 3	85 ± 4	98 ± 4	98 ± 4	88 ± 4
Parafascicular Nucleus	70 ± 4	67 ± 3	60 ± 2	73 ± 4	75 ± 4	65 ± 3
Anterior Nucleus	88 ± 4	85 ± 3	80 ± 3	92 ± 5	90 ± 4	83 ± 3
Lateral Geniculate Body	67 ± 4	62 ± 2	63 ± 4	73 ± 3	67 ± 3	67 ± 3
Hypothalamus	46 ± 2	44 ± 2	44 ± 2	47 ± 2	43 ± 2	44 ± 2

APPENDIX VI (c)

LOCAL CEREBRAL GLUCOSE UTILISATION FOLLOWING UNILATERAL INTRASTRIATAL INJECTIONS OF MUSCIMOL : LIMBIC AND WHITE MATTER AREAS.

Structure	Ipsilateral			Contralateral		
	CSF	Muscimol 100 ng	Muscimol 500 ng	CSF	Muscimol 100 ng	Muscimol 500 ng
Nucleus Accumbens	72 ± 2	69 ± 3	73 ± 1	76 ± 2	74 ± 2	73 ± 1
Ventral Tegmental Area	44 ± 3	40 ± 2	36 ± 1*	44 ± 4	41 ± 3	36 ± 1
Lateral Habenula	76 ± 2	83 ± 2	88 ± 2*	78 ± 2	77 ± 3	74 ± 2
Raphé Nucleus (+)	75 ± 3	70 ± 2	79 ± 2	75 ± 3	70 ± 2	79 ± 2
Amygdala, Medial	40 ± 2	40 ± 2	46 ± 3	42 ± 2	42 ± 2	48 ± 3
Hippocampus (Molecular Layer)	75 ± 3	70 ± 4	64 ± 5	75 ± 3	71 ± 4	67 ± 3
Dentate Gyrus	58 ± 3	59 ± 2	50 ± 4	58 ± 3	59 ± 2	52 ± 4
Septal Nucleus	53 ± 2	52 ± 3	47 ± 2	53 ± 2	54 ± 1	53 ± 2
Cerebellar White Matter	28 ± 2	29 ± 1	32 ± 2	31 ± 2	29 ± 1	32 ± 2
Internal Capsule	30 ± 2	30 ± 2	25 ± 2	30 ± 2	32 ± 2	25 ± 2
Corpus Callosum	32 ± 2	31 ± 1	31 ± 2	32 ± 2	31 ± 2	31 ± 2

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PUBLICATIONS ARISING FROM THESIS

1. KELLY, P.A.T. and McCULLOCH, J. Heterogeneous depression of glucose utilisation in the caudate nucleus by GABA agonists. Brain Res., 209, 458-463 (1981).

2. KELLY, P.A.T. and McCULLOCH, J. Differences in the response of the rat superior colliculus to muscimol and THIP. Brit. J. Pharmac., 74, 815P-816P (1981).

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7. KELLY, P.A.T. and McCULLOCH, J. A potential error in modifications of the [^{14}C] 2-deoxyglucose technique. Brain Res. (in press).