CONSEQUENCES OF QUINOLINIC ACID-INDUCED LESIONS OF THE NEOSTRIATUM AND FRONTAL NEOCORTEX IN RATS:

Behaviour, neuroanatomy and neurochemistry

By

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The neostriatum appears to be a well-informed structure.

RGE Öberg and I Divac, 1979, p 291

ABSTRACT

The experiments that have been conducted for this thesis have been directed towards understanding the consequences of quinolinic acid-induced lesions of the medial striatum and (to a lesser extent) the medial prefrontal cortex in the rat; particular attention has been given to the evaluation of these lesions as animal models of Huntington's Disease (HD). The methods that have been used have been drawn from behaviour, neuroanatomy and neurochemistry. Four primary questions have been addressed: 1) is spontaneous nocturnal activity an adequate index of striatal dysfunction?; 2) what is the nature of the behavioural impairment in rats with quinolinic acid-induced lesions of the medial striatum, and can this profile be considered analogous to the neuropsychiatric disturbance in HD?; 3) what is the behavioural profile of rats with quinolinic acid-induced lesions of the medial prefrontal cortex, and does this have implications for the identification of the role of the frontal cortex in the neuropsychiatric disturbance in HD?; and 4) what are the neuroanatomical and neurochemical consequences of chronic excitotoxin-induced lesions of the striatum?

In addressing the first of these questions, spontaneous nocturnal activity was studied after kainate-, ibotenate- and quinolinate-induced lesions of the medial striatum in the rat. Animals with kainate- and ibotenate-induced lesions demonstrated significant nocturnal hyperactivity; however, no such effect was found in the quinolinate lesioned animals. Further, the hyperactivity in the kainate- and ibotenate-lesioned animals attenuated over prolonged testing. This study indicates that spontaneous nocturnal activity may not always be an adequate measure of striatal dysfunction.

Secondly, the behavioural profile of rats with quinolinic acid-induced lesions of the medial striatum was characterized. The lesioned rats were found to be impaired in two

tests of cognitive behaviour, the Morris Water Maze and the spontaneous alternation task, but were apparently unimpaired on a host of motor tests, thus strengthening the interpretation that the impairment demonstrated on the two cognitive tasks was indeed indicative of impaired visuospatial processing. The behavioural data also suggested that the lesioned animals showed impaired cognitive flexibility, and an inability to change learning strategies. Histological analysis revealed significant striatal degeneration seemingly in the absence of cortical degeneration, thus suggesting that lesions largely restricted to the medial striatum are sufficient to produce a behavioural impairment. Comparisons were made with the recognized neuropsychiatric disturbance of Huntington's Disease.

The third question was concerned with investigating the behavioural consequences of rats with quinolinic acid-induced lesions of the medial prefrontal cortex. These animals were found to be impaired in the Morris Water Maze, but were unimpaired on a number of motor tests. Histological analysis demonstrated the presence of marked cortical degeneration; interestingly, striking ventricular dilation was also present in most of these animals. The results were discussed in relation to the pathology of HD.

Lastly, a morphometric study was performed on rats with chronic excitotoxin-induced striatal lesions. Degenerative changes were noted in the striatum, substantia nigra and thalamic nuclei, but no changes were found in the thalamus as a whole or the cortex. The negative cortical finding was supported by a neurochemical study of various neuropeptides in rats with chronic quinolinic acid-induced lesions of the medial striatum: significant degeneration was noted in the striatum, but no changes were found in the cortex in these rats. Again, the results were interpreted in relation to the pathology of HD.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

CHAPTER 1:

INTRODUCTION

INTRODUCTION

Recent research has revealed the highly ordered and detailed neuroanatomy and neurochemistry of the mammalian striatum as well as the recognition of the striatum as an integral part of a sophisticated network, which includes the cortex, thalamus and substantia nigra. New attention has also been paid to the role of the striatum in behavioural function.

The growth in understanding of the normal striatum and basal ganglia has facilitated the investigation of those diseases with known striatal pathology. A case in point in this regard is the recently realized increased comprehension of Huntington's Disease (HD), a progressive degenerative disease of the central nervous system characterized by a movement disorder and neuropsychiatric disturbance and marked striatal, as well as a lesser, extrastriatal, pathology. It is likely that the movement disorder in HD is the outflow of abnormal information from the striatum, as well as the consequence of this defective information on two extrastriatal structures, namely the globus pallidus (internal division) and the subthalamic nucleus. However, little attention has been paid to the neural substrate for the neuropsychiatric disturbance in HD or whether the recognized extrastriatal pathology is primary or secondary to the marked striatal pathology.

The present thesis is primarily concerned with the investigation of these questions through the use of the rodent quinolinic acid model of HD. The specific questions chosen for investigation have been directed by the literature on HD as well as that concerning the frontal lobes and striatum.

CHAPTER 2:

LITERATURE REVIEW:

Huntington's Disease: History, Clinical Aspects and Pathology

I. Historical Aspects
II. Clinical Aspects
III. The Mammalian Basal Ganglia: Neuroanatomy and Neurochemistry
IV. Pathology of Huntington's Disease
    a. Gross Pathology
    b. Neurochemical and Neuroanatomical Pathology
V. Positron Emission Tomography and Huntington's Disease

VI. Excitotoxicity and the Pathogenesis of Huntington's Disease

VII. Animal Models of Huntington's Disease

I. History, Clinical Aspects and Pathology

I. Historical Aspects

Huntington's Disease (HD), a degenerative disease of the human central nervous system (CNS), was first described in 1872 by the American physician George Huntington. Huntington had first noticed the disease as a child while joining his father on his medical rounds in Long Island, New York. Huntington's father and grandfather, who had also been physicians, had noted the disease within their own practices. The observations of three generations were articulated in the 1872 paper in which George Huntington accurately described the disease as an inherited form of chorea; he also noted the presence of mental disturbance and noted the pattern of familial inheritance:

"And now I wish to draw your attention more particularly to a form of the disease which exists, so far as I know, almost exclusively on the east end of Long Island. It is peculiar in itself and seems to obey certain fixed laws. In the first place, let me remark, that chorea, as it is commonly known to the profession, and a description of which I have already given, is of exceedingly rare occurrence there. I do not remember a single instance occurring in my father's practice, and I have often heard him say that it was a rare disease and seldom met with by him." (Huntington, 1872, pg. 320)

"There are three marked peculiarities in this disease: 1. Its hereditary nature. 2. A tendency to insanity and suicide. 3. Its manifesting itself as a grave disease only in adult life." (Huntington, 1872, pg. 320)

Huntington's description of the disease which has come to bear his name was, and still is, regarded as precise, a tribute to his remarkable powers of observation (Hayden, 1981). Indeed, so impressed by Huntington's description was the Canadian physician Sir William Osler that he was moved to comment:

"In the whole range of descriptive nosology there is not, to my knowledge, an instance in which a disease has been so accurately and fully delineated in so few words..... but to Huntingdon's account of the symptomatology, no essential fact has been added." (Osler, 1893, pgs. 97-98)

The clinical signs recognized by Huntington in 1872 are still used for diagnostic purposes today.

Clusters of cases of HD have been found in the USA (as observed by Huntington), Canada, South Africa and Australia, thus pointing towards England, France, Germany or Holland as the source for the original defect. Most cases of HD in North America and Western Europe can almost certainly be traced back to a spontaneous gene mutation arising somewhere in northwestern Europe prior to the Middle Ages (Hayden, 1981).

The spread of the disease from northwestern Europe throughout the world has been linked to historical events. In France, the disease may have been present among the Huguenots who lived in the Loire valley; the revocation of the Edict of Nantes in 1685, the subsequent dispersion of the Huguenots throughout Europe and the further migration of their descendants throughout the world may have contributed to the spread of the disease. In England, the religious intolerance practiced by James I and Charles I may have prompted migration of groups of people carrying the HD gene from England to other parts of the world. Further, at least some of those people executed in the Salem witch trials in 1692 are believed to have suffered from HD (Hattie, 1909; Hayden, 1981).

It is unclear whether all known cases of HD in the world arose from the same genetic mutation. However, there are areas in the world where HD is known to exist, but would seem unlikely to be linked to those cases originating in northwestern Europe. For example, cases are known to exist in Japan, the

Caribbean, India and the Negroid populations of South Africa; detailed epidemiological investigations have not been undertaken in these countries. It is possible that all these cases may be linked to the spontaneous mutation thought to have occurred in Northwestern Europe, but consideration must be given to the suggestion (perhaps a more probable one) that more than one spontaneous mutation, in different areas of the world, has given rise to the modern, worldwide population of HD (Hayden, 1981).

II. Clinical Aspects

HD has an incidence of between 30 to 70 cases per million population (Hayden, 1981). The disease is transmitted through an autosomal dominant mode of inheritance with complete penetrance. The age of onset, characterized by the onset of the choreiform motor disorder, is typically during the fourth or fifth decades of life, although in 3-5% of HD sufferers, the age of onset is prior to the fifteenth year of life (juvenile or Westphal variant) while in another 28% the onset is after the 50th year of life (late onset variant) (Adams and Victor, 1989). Although the occurrence of the disease in men and women is thought to be equal, several authors have noted that there seems to be a higher frequency of the disease in Caucasoid populations residing in North America or Europe than in Caucasoid, Negroid or Mongoloid populations anywhere else in the world (Hayden, 1981; Sanberg and Coyle, 1984).

The triad of symptoms first noted by Huntington in 1872 are still used today in making a positive diagnosis of HD. These are 1) a positive family history for HD (spontaneous mutations are unknown) in which the disease is transmitted in an

autosomal dominant mode of inheritance, 2) a motor disorder which includes chorea, and 3) a certain profile of neuropsychiatric disturbance. (Although diagnosis would seem straightforward, HD is often misdiagnosed as schizophrenia or Alzheimer's Disease due to the absence of family records or the attending physician's inexperience with the variable nature of the motor disorder (Folstein et al., 1986).)

Genetics

"There are three marked peculiarities in this disease: 1. Its hereditary nature..." (Huntington, 1872, pg. 320).

"The hereditary chorea, as I shall call it, is confined to certain and fortunately a few families, and has been transmitted to them, an heirloom from generations away back in the dim past." (Huntington, 1872, pg. 320).

As mentioned, HD is known to be transmitted in an autosomal dominant mode of inheritance with complete penetrance; children who have a parent suffering from HD have a 50% chance of inheriting the disease. Recent research has shown that the defective gene is probably located on the short arm of the fourth chromosome (Gusella et al., 1983). This discovery has allowed predictive testing in persons at risk (Hayden et al., 1988) as well as prenatal testing (Hayden et al., 1987). (The preservation of the gene within the genetic pool can be attributed to the fact that in the majority of cases, the onset of the disease is typically after the childbearing years.) To date, the search for the markers to clone the gene continues (e.g., Davies, 1992).

It has been suggested that the age of onset (defined by the appearance of the motor disorder) of HD is related to the sex of the affected parent. The juvenile form of the disease has an age of onset typically around 13-14 years of age; it has

been found that a large proportion of these patients inherited the disease from their affected father (Myers et al., 1983; Newcombe et al., 1981; Bird et al., 1974). Newcombe et al. (1981) have even found a third-generation effect in which the age of onset is even lower if both the father and grandfather have suffered from HD. If the defective gene is inherited from the carrier mother, the cases were more likely to be of late onset (age 50 or later) (Myers et al., 1983). Further, affected offspring of affected males frequently die before the carrier parent; this is typically not the case for affected offspring of affected females (Bird et al., 1974).

Motor Disorder

1. Chorea and Athetosis

Chorea can be described as "involuntary, arrhythmic movements of a forcible, jerky type" (Adams and Victor, 1989); chorea is also the symptom that first drew George Huntington's attention to the disease:

> "Over fifty years ago, in riding with my father on his professional rounds, I saw my first case of 'that disorder', which was the way in which the natives always referred to the dreaded disease. I recall it as vividly as though it had occurred but yesterday. It made a most enduring impression upon my boyish mind, an impression every detail of which I recall to-day, an impression which was the very first impulse to my choosing chorea as my virgin contribution to medical lore. Driving with my father through a wooded road leading from East Hampton to Amagansett, we suddenly came upon two women, mother and daughter, both tall, thin, almost cadaverous, both bowing, twisting, grimacing. I stared in wonderment, almost fear. What could it mean? My father paused to speak with them and we passed on. Then my Gamaliel-like instruction began; my medical education had its inception. From this point on my interest in the disease has never wholly ceased" (Huntington, 1910, pgs. 255-256).

In HD, the onset of chorea (which is usually taken as the age of onset) is frequently preceded by restlessness. The chorea itself begins only as infrequent tics and jerks of the fingers and toes, then proceeds gradually to involve the limbs

and body axis. Choreic movements may resemble voluntary movements, but are never purposefully organized. The movements may appear exaggerated when superimposed upon voluntary movements. The movements produced by a choreiform disorder are, by definition, discrete, but in severe cases of HD, athetosis contributes a sinuous, writhing quality to the motor disorder, thus leading to the description of the motor disorder as choreoathetotic (Adams and Victor, 1989).

The chorea worsens under periods of psychological stress--indeed, stress is often used by the physician to induce chorea in the clinic. Throughout the course of the disease, chorea may first increase in severity, then lessen or abate altogether (Folstein, 1989; Folstein et al., 1986; Shoulson, 1982).

2. Gait Abnormalities

Koller and Trimble (1985) have observed a gait abnormality in the majority of their HD patients. Biomechanical analysis of gait patterns revealed decreased walking speed, reduced stride and fewer steps per minute. Although haloperidol lessened the chorea in these patients, it did not alleviate the gait abnormality (Koller and Trimble, 1985).

3. Saccades

Saccades have been defined as a "conjugate, ballistic movement of the eyes...that brings the fovea onto a target" (Gouras, 1985, pg. 574). A saccadic abnormality has been noted in HD patients whereby a deficit exists in the initiation of voluntary saccades; saccades to external stimuli are normal (Adams and Victor, 1989; Lasker et al., 1987; Tian et al., 1991). An inability to suppress saccades to

external stimuli, seemingly related to patient distractibility, has also been noted (Lasker et al., 1987).

4. Bradykinesia and Dystonia

Bradykinesia and dystonia have recently been reported to be associated with the later stages of the HD (Hefter et al., 1987; Thompson et al., 1988; Young et al., 1986). Otherwise, the features more commonly associated with Parkinson's Disease (e.g., bradykinesia, rigidity) are found in juvenile HD patients than in adult onset HD (Hayden, 1981; Adams and Victor, 1989; Young et al., 1986; Folstein, 1989).

II. Neuropsychiatric Disturbance

In his 1872 description of the disease, George Huntington recognized that mental disturbance represented an important facet of the illness. Yet, the neuropsychiatric problems associated with HD have only recently begun to be the focus of study. (This might be attributed, at least in part, to the overshadowing of the mental disturbances by the striking choreoathetotic motor disorder.) Examination of the recent literature, reveals a characteristic pattern of mental impairment which can be divided into 2 areas: 1) affective changes, and 2) cognitive decline.

1. Changes in Affect

"There are three marked peculiarities in this disease: .... 2. A tendency to insanity and suicide..." (Huntington, 1872, pg. 320)

"The tendency to insanity, and sometimes that form of insanity which leads to suicide, is marked... As the disease progresses the mind becomes more or less impaired, in many amounting to insanity, while in others mind and body both gradually fail until death relieves them of their sufferings." (Huntington, 1872, pg. 320)

Personality changes in HD patients may precede the onset of the motor disorder by ten to fifteen years (Folstein et al., 1983a, 1983b). After the disease has been diagnosed by the onset of the motor disorder, family members frequently report that for some years prior to the diagnosis, the individual no longer seemed like himself, was easily irritable, suspicious and impulsive (Adams and Victor, 1985; Folstein et al., 1983a, 1983b). Interestingly, similar personality changes have also been reported in patients sustaining frontal lobe damage.

There is a very high suicide rate among HD patients (Schoenfeld et al., 1984). This may be reflective of their insight into their problems (Caine et al., 1978) and

their subsequent gloomy outlook on life (Adams and Victor, 1985); few other chronic, debilitating illnesses are associated with such a high rate of suicide.

As will be detailed later, there are significant changes in the chemoarchitecture of the basal ganglia in patients suffering from HD. This is particularly interesting in light of a recent study examining rhesus monkeys that have undergone social deprivation in early life and displayed abnormal social, motor and cognitive behaviours in adulthood. Post-mortem analysis found changes in the chemoarchitecture of the basal ganglia in these socially deprived animals (Martin et al., 1991). This suggests that the neural basis for changes in affect in HD may be related to striatal pathology.

2. Cognitive Decline

2a. Linguistic Abilities. General speech abilities are preserved in patients with HD; however, recent research has revealed subtle linguistic abnormalities that may not be immediately obvious. For example, a comparison of control subjects, HD and Parkinson's Disease (PD) patients on a variety of timed verbal tasks indicated that although HD patients were not impaired in speech planning or initiation, they demonstrated reduced syllable duration and pauses between phrases as well as reduced syllable repetition rates; these deficits were not shared by the two other groups (Ludlow et al., 1987). Gordon and Illes (1987) analyzed speech samples from HD patients and subjects at risk for HD. The HD patients were found to be significantly impaired, in comparison to the at-risk subjects, showing decreases in the number of words produced, reduced syntactic complexity, melodic line reductions, phrase length

reductions, decreased articulatory agility, increased number of paraphasic errors and word-finding difficulty. Unfortunately, no neurologically normal control group was included, so the at-risk subjects could not be compared to normal controls and it could not be definitively determined whether or not the at-risk group was perhaps impaired in some way. Another study analyzed samples of spontaneous language from PD, Alzheimer's (AD) and HD patients as well as normal controls. Within each disease group, 5 subjects were in the early stages of the disease and 5 subjects were in the middle stages of the disease. While general comunicative ability was preserved the linguistic analysis showed that each group was characteristically impaired. The HD patients demonstrated hastened speech, numerous self-correction, many interjections and temporal speech interruptions, and a reduction in syntactic complexity. With the exception of temporal speech interruptions (which were also found in AD patients), the other two groups did not share these impairments (van der Loos, 1987; Illes, 1989).

Another recent study has revealed that HD patients are impaired in the comprehension and discrimination of linguistic prosody. Speedie et al. (1990) compared HD patients with right or left hemisphere stroke patients and normal control subjects on the ability to comprehend affective prosody (i.e., determination of the tone of voice of a speaker as happy, angry, etc.) and propositional prosody (the ability to determine whether a sentence is a question or command, etc.). Early HD patients (chosen because they were still able to function within society) were impaired on both tasks, in comparison to controls. A second experiment attempted to determine which aspects of prosody might be impaired in early HD patients. The patients were

compared to subjects at-risk for HD on the comprehension and discrimination of prosody as well as on a task of tonal memory (Seashore Test of Musical Abilities) and a rhythm discrimination test. HD patients were impaired, in comparison to subjects at-risk on the comprehension and discrimination of prosody, as well as on the tonal memory task, but not on the rhythm discrimination task. The authors suggest that the tonal aspect of prosody may present particular problems for HD patients, and that the problems that HD patients have in general with prosodic aspects of language may contribute to the social problems experienced by HD patients early on in the disease (Speedie et al., 1990).

2b. Recall and Recognition Memory. Examination of memory in HD patients reveals a unique deficit: while recall memory is usually severely impaired (Caine et al., 1977; Weingartner et al., 1979; Massman et al., 1990; Hodges et al., 1990), recognition memory for the same material (especially for verbal material) can appear almost normal (Butters et al., 1985, 1986, 1987; Granholm and Butters, 1988; Heindel et al., 1988, 1990; Weingartner et al., 1979).

Heindel and his colleagues have tested recognition memory for verbal (1988) and pictorial information (1990): HD, Alzheimer's Disease (AD) and Parkinson's Disease (PD; demented and nondemented) patients as well as control subjects were presented with word lists (e.g., MOTEL, ABSTAIN) and were later asked to complete lists of three-letter stems (e.g., MOT, ABS). If the stems had been seen previously in the form of a complete word, HD and non-demented PD patients were able to perform as well

as controls in completing the words while AD and demented PD patients were significantly impaired (Heindel et al., 1988). However, if the stems had not been seen previously, performance of the HD group was significantly worse than that of controls. This is again reminiscent of the relative preservation of general language abilities in HD patients. Similarly, HD, AD and control subjects were presented with groups of picture fragments, were later presented with larger groups of fragments (which included those previously seen) and were asked to state what they thought the fragment represented. If the fragment had been seen before, the performance of controls and HD patients was not significantly different, while AD patients demonstrated a significant impairment (Heindel et al., 1990). Again, if the fragment had not been seen previously, the performance of the HD group was significantly worse than controls.

In one particularly striking study, control subjects, HD and Korsakoff's patients were asked to learn to read mirror images of words and were subsequently given a test of recognition memory for the words used. While the HD patients had difficulty in acquiring the skill of reading mirror images, they demonstrated normal recognition memory for the stimuli. (The Korsakoff's patients demonstrated normal acquisition rates of the mirror reading task, but poor recall and recognition memory) (Martone et al., 1984).

Also characteristic of the memory impairment in HD is the seemingly uniform deficit in remote memory. That is, memory for past events (e.g., personal history, general knowledge, or visuospatial information), whether in the distant or recent past,

is uniformly poor. This is quite unlike the graded memory deficit demonstrated by Korsakoff's or AD patients (Albert, Butters and Brandt, 1981; Beatty, 1989; Beatty et al., 1988; Caine and Fisher, 1985).

Attempts have been made to characterize the nature of the memory deficit in HD. It has been proposed that the memory deficit in HD is suggestive of a problem of retrieval of previously stored information as opposed to a problem in encoding of information (e.g., Brandt and Butters, 1986).

2c. Deficits in Processing of Visuospatial Information

It is commonly reported that HD patients suffer from deficits related to the processing of visuospatial information. Several authors have found that HD subjects demonstrated lower performance IQ (PIQ) than verbal IQ (VIQ) subscale scores on the WAIS-R (Josiassen et al., 1983; Caine et al., 1978; Fedio et al., 1979; Sax et al., 1983). Specifically, impairment on the performance subscale seems to be related to deficits on the Block Design, Object Assembly, Picture Arrangement and Picture Completion tests. Fedio et al. (1979) have reported HD patients to be impaired on the Mosaic Comparisons Test, the Rey-Osterrieth Complex Figure Test and the stylus maze test, all considered tests of visuospatial skills. Moses et al. (1979) found that patients were impaired on the Visual scale of the Luria-Nebraska Neuropsychological Test Battery, a test that includes the mental rotation of objects.

Fedio et al. (1979) also administered the Money Road Map Test to HD patients (in this test, subjects are required to draw a specified path on a map twice; on the second

trial the position of the map is shifted 180 degrees relative to the subject) and found an impairment in these subjects when compared to controls. A dissociation for the test was found by Brouwers et al. (1984). Controls, HD and AD patients were given the Money Road Map Test (as well as a variety of other tests): HD patients were found to be impaired on this test while AD patients and controls were not.

Beatty (1989) asked HD patients and normal controls to indicate places on two maps: the first, in the region of the US were they were born and raised and second, on a map of California, where the subjects currently resided. HD patients demonstrated impaired performance, in relation to controls, on both test maps. This was interpreted as indicating impaired judgement for visuospatial information (as well as similarly impaired remote and recent memory, which has been mentioned previously).

Some of the studies described previously have also included evidence of deficient processing of visuospatial information in HD subjects. For example, Martone et al. (1984) reported that HD patients demonstrated difficulties in reading mirror images of words. This difficulty was not shown by Korsakoff's patients given the same task. Similarly, Heindel et al. (1989) have shown that HD (but not AD nor non-demented PD) patients demonstrated difficulty in the acquisition of a motor task which required the maintenance of contact between a hand-held stylus and a rotating metal disk. Butters et al. (1985) found that advanced HD patients (as well as Korsakoff's patients) could not acquire the Tower of Hanoi puzzle; this same deficit was not exhibited by HD patients in the early stages of the disease or neurologically-normal controls.

In a rare study that did not involve the commonly administered pencil and paper

tests, Potegal (1971) required normal controls, HD and PD patients to observe the location of a target and after the target was hidden by an overlying sheet of paper, to indicate the location of the hidden target. Patients with HD demonstrated impaired judgement (both in relation to controls as well as PD patients) in this task when they were required to move a step sideways after the object had been observed and hidden, and then indicate the location of the target. Thus, it seemed that a change in the HD subject's position resulted in an inability to compensate for the change in the location of the object with respect to self. Potegal (1971) attributed this deficit to caudate degeneration; however, recent research has described the degeneration of other neural structures in HD (Vonsattel et al., 1985, 1987).

2d. Similarity in cognitive impairments in patients with HD and those with frontal lobe damage

There is a striking similarity in the symptoms demonstrated by HD patients and those observed in subjects with frontal lobe damage. As is the case with patients who have sustained frontal lobe damage, HD patients demonstrate problems in the planning, organizing and sequencing of information as well as in the use of cognitive flexibility to form alternate search strategies; this is reported by the patients themselves when commenting upon the problems they have with coping with the disease, and it has also been noted in deficits in subtests of the Performance IQ (PIQ) scale of the WAIS-R which require such skills (e.g., block design, picture completion, picture arrangement) (Caine et al., 1978; Josiassen et al., 1983; Knopman and Nissen,

1991).

HD patients show impairments on other commonly used tests requiring the aforementioned skills such as the word fluency test, the Wisconsin Card Sorting Test, the Stroop Test and the Porteus Mazes test (Josiassen et al., 1983). Butters et al. (1985) asked HD patients in the early (EHD) and advanced (AHD) stages of the disease, along with controls and Korsakoff's patients, to solve the Tower of Hanoi puzzle, which requires the use of planning, problem-solving and skill-learning abilities. Where AHD and Korsakoff's patients were impaired on the task, EHD and controls were able, with practice, to learn the task. On a somewhat similar test, HD patients were asked to demonstrate certain movement sequences (i.e., use a key, sip through a straw) and were found to be impaired (Shelton and Knopman, 1991). A similar deficit has been shown in patients with left or right frontal cortical excisions (Kolb and Milner, 1981a).

Further behavioural investigations on patients with HD may reveal more similarities. For example, it has long been noted that patients with HD demonstrate reduced emotional spontanaeity, although this has not been studied systematically. A reduction in spontaneous facial expression has been noted in patients with frontal cortical excisions (Kolb and Milner, 1981b); it is tempting to think that the deficit casually described in HD patients may be similar to that studied in patients with frontal cortical excisions.

HD: A Disease of Gradual Onset and a Triad of Symptoms

"It is attended generally by all the symptoms of common chorea, only in an aggravated degree, hardly ever manifesting itself until adult or middle life, and then coming on gradually but surely, increasing by degrees, and ofteen occupying years in its developemnt, until the hapless sufferer is but a quivering wreck of his former self" (Huntington, 1872, pg. 320).

Thus, HD is not a disease of sudden onset, even though onset is usually taken to be the time at which choreiform movements appear. It has been observed that impairment (motor disorder and neuropsychiatric disturbance) in HD patients develop over time, becoming gradually more marked. Further, certain abilities seem to be impaired before other abilities; this effect may even be detectable in subjects at risk for HD. Chorea is preceded by fine motor abnormalities as well as the beginning of mental disturbance. After the onset of chorea, other motor abnormalities, such as bradykinesia and dystonia appear (Thompson et al., 1988; Penney et al., 1990). As Penney and his colleagues have described (1990) it may be possible to predict the time to diagnosis in persons at risk, given the severity of early motor abnormalities. Mental capacities in HD are also believed to grow progressively worse (Lyle and Gottesman, 1977; Fedio et al., 1979; Josiassen et al., 1983).

It is important to recognize that the presence of only one of the triad of symptoms (i.e., motor disorder, autosomal pattern of inheritance, neuropsychiatric disturbance) is not sufficient for a clinical diagnosis of HD. Any one of the triad is symptomatic of other diseases. For example, chorea is hardly unique to HD; it is present in a host of other disorders, including benign hereditary choreoacanthocytosis, olivopontocerebellar atrophy and dentatopallidorubrosubluysian atrophy, to name only a few (Adams and

Victor, 1985). Further, many of the neuropsychiatric disturbances associated with HD are characteristic of other syndromes, particularly of patients who have sustained frontal lobe damage. Lastly, there are many genetic diseases which are passed on in an autosomal dominant mode of inheritance. As recognized by George Huntington in 1872, HD is the presence of all three symptoms together.

III. The Mammalian Basal Ganglia: Neuroanatomy and Neurochemistry

HD is primarily a disease of the striatum. In order to understand its pathology, it is first necessary to understand the anatomy of the basal ganglia. As mentioned previously, recent research has done much to elucidate the neurochemical anatomy of the striatum and its connections.

Neuronal Types of the Mammalian Striatum

The availability of specialized equipment and techniques (e.g., electron microscopy, Golgi and gold-toned neuronal stains) has permitted the identification of various cell types within the mammalian neostriatum. At the simplest level, a distinction may be made between spiny and aspiny striatal neurons, so named for the relative density of dendritic spines (as observed through electron microscopy) associated with each neuronal type. Examination of Golgi-stained neurons of the monkey neostriatum has revealed two types of spiny neurons (spiny I and II) and at least two types of aspiny neurons (aspiny I and II) (DiFiglia, Pasik and Pasik, 1976). The spiny type I ("medium spiny") neuron possesses a high density of dendritic spines; by contrast, the spiny type II neuron demonstrates a somewhat lower dendritic spine density. Both have long axons, thus enabling spiny neurons with the capability of carrying messages from the striatum to other neural structures. Both type I ("medium aspiny") and type II aspiny neurons are similar in that they possess short axons (neurons with short axons are sometimes referred to as Golgi type II cells) and are contained entirely within the striatum; when compared to either type of spiny neuron, aspiny neurons possess relatively few dendritic spines (DiFiglia, Pasik and Pasik, 1976).

Closer examination of these spiny and aspiny neurons has revealed that they may be distinguished through characteristics other than spine density. The medium spiny neurons is the most numerous striatal neuron: 90% of the total striatal neuronal population in the rat are thought to be of the medium spiny type. Under the electron microsocope, the medium spiny neurons have been shown to possess large, unindented nuclei and chromatin aggregates with little cytoplasm within the organelles. By contrast, the medium aspiny neurons are far fewer in number: Graveland et al. (1985) have estimated that 4-5% of the rat striatal neuronal population is of the medium aspiny type. Under the electron microscope, the medium aspiny neurons demonstrate indented nuclei with large amounts of cytoplasm, particularly in the Golgi apparatus and rough endoplasmic reticulum (DiFiglia, Pasik and Pasik, 1980).

Spiny and aspiny neurons may also be distinguished on the basis of associated neurotransmitters. The medium spiny neurons are known to be associated with the peptides substance P, methionine (met)-enkephalin and dynorphin, and the amino acid gamma-amino butyric acid (GABA). Similarly, the medium aspiny neurons are known to be associated with the peptides somatostatin, neuropeptide Y (NPY), the enzyme nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) as well as the gas nitric oxide; the large aspiny neurons are associated with acetylcholine (ACh) (Graybiel and Ragsdale, 1983; Nieuwenhuys et al., 1985; Cooper, Bloom and Roth, 1991; Gerfen, 1992a; Vincent and Kimura, 1992).

Nuclei of the Basal Ganglia

The basal ganglia is composed of a number of nuclei. These include the striatum (caudate and putamen), globus pallidus (external and internal divisions),

nucleus accumbens, subthalamic nucleus and substantia nigra (pars compacta and pars reticulata).

Afferents and Efferents of the Basal Ganglia

The nuclei of the basal ganglia communicate with each other through four circuits:

1a. Cortex-striatum-globus pallidus-thalamus-cortex. Each neocortical area projects to the CPu via the corticostriatal tract. Specifically, neurons located in cortical layers V and VI provide somatotopically organized output to the striatum from the cortex (Nieuwenhuys et al., 1988). The transmitters of the corticostriatal tract are the amino acids glutamate (predominantly) and aspartate (Graybiel and Ragsdale, 1983; Nieuwenhuys, 1985). The terminals of the corticostriatal tract synapse upon the medium spiny cells of the striatum, which in turn, send a projection to the internal division of the globus pallidus (GPi) (Nieuwenhuys et al., 1988). The transmitters in this striatopallidal projection are gamma-amino butyric acid (GABA, an amino acid), substance P, enkephalin and dynorphin (neuropeptides) (Nieuwenhuys, 1985; Haber and Elde, 1981; Graybiel and Ragsdale, 1983; Anderson and Reiner, 1990) (Graybiel and Ragsdale, 1983) as neurotransmitters. The primary projection from the GPi is directed to the ventroanterior (VA) and ventrolateral (VL) nuclei of the thalamus. The loop is completed by the projection of the thalamic nuclei to the motor and premotor cortex (i.e., Brodmann's area 4 and 6) (Côté and Crutcher, 1985; Nieuwenhuys et al., 1988; Gerfen, 1992a). (Refer to figure 2.1).

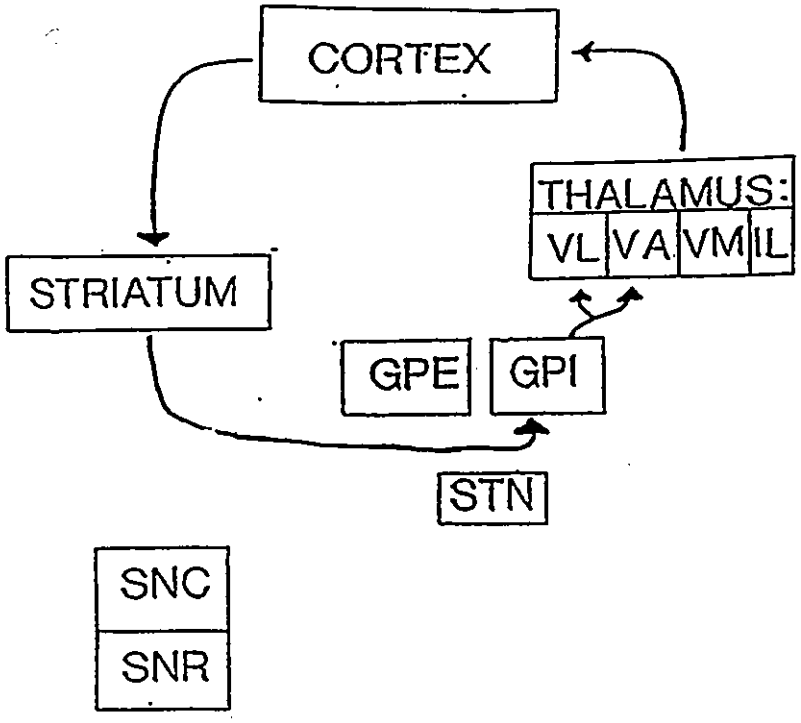
1b. Cortex-striatum-substantia nigra-thalamus-striatum. As mentioned above, all cortical areas project to the striatum. From this point, the medium spiny cells form

a projection to the SNr. The transmitters associated with this pathway are GABA and substance P. Subsequently, the cells of the SNr send a projection to the VA and ventromedial (VM) thalamic nuclei. In turn, these areas of the thalamus send a projection to the intralaminar nucleus of the thalamus, which projects back to the striatum (Côté and Crutcher, 1985; Nieuwenhuys et al., 1988; Gerfen, 1992a). Refer to figure 2.2.

2.Globus pallidus-subthalamic nucleus-globus pallidus. A reciprocal connection exists betweent the GPe and STN; the GPe sends a projection to the STN, which in turn sends a projection back to all areas of the globus pallidus (Côté and Crutcher, 1985; Nieuwenhuys et al., 1988; Gerfen, 1992a). The transmitter involved in this pallido-subthalamic pathway is GABA (Graybiel and Ragsdale, 1983; Niewenhuys, 1985). Refer to figure 2.3.

3. Striatum-substantia nigra-striatum. The striatum sends a projection to the SNr; these SNr neurons synapse upon the dendrites of the neurons of the SNc (Nieuwenhuys et al., 1988). The transmitters involved in the striatonigral pathway are GABA, substance P and dynorphin (an opiate peptide) (Graybiel and Ragsdale, 1983; Nieuwenhuys, 1985; Anderson and Reiner, 1990). In turn, the compacta neurons project to the striatum; the nigral neurons terminate on those striatal neurons which send efferents towards the GP and also having a presynaptic influence on the corticostriatal glutamatergic afferents (Nieuwenhuys et al., 1988). The predominant transmitter involved in the nigrostriatal pathway is dopamine (a type of catecholamine), although other transmitters are thought to be involved in a minor way (Graybiel and Ragsdale, 1983; Gerfen, 1992a). Refer to figure 2.4.

Figures 2.1 and 2.2 Schematic depiction of the cortico-striato-pallido-thalamo-cortical circuit (2.1) and cortico-striato-nigro-thalamo-striatal circuit (2.2).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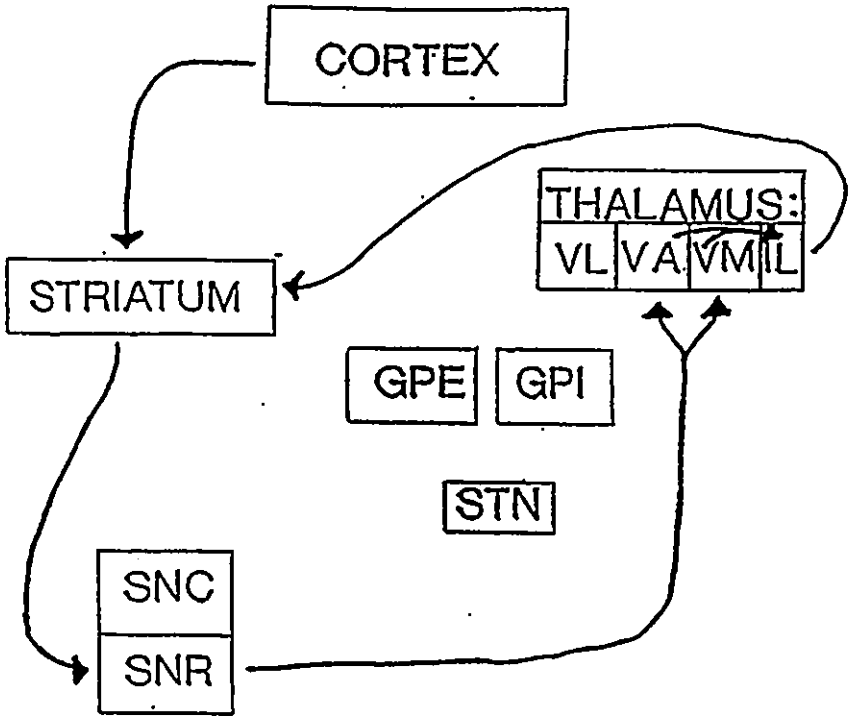
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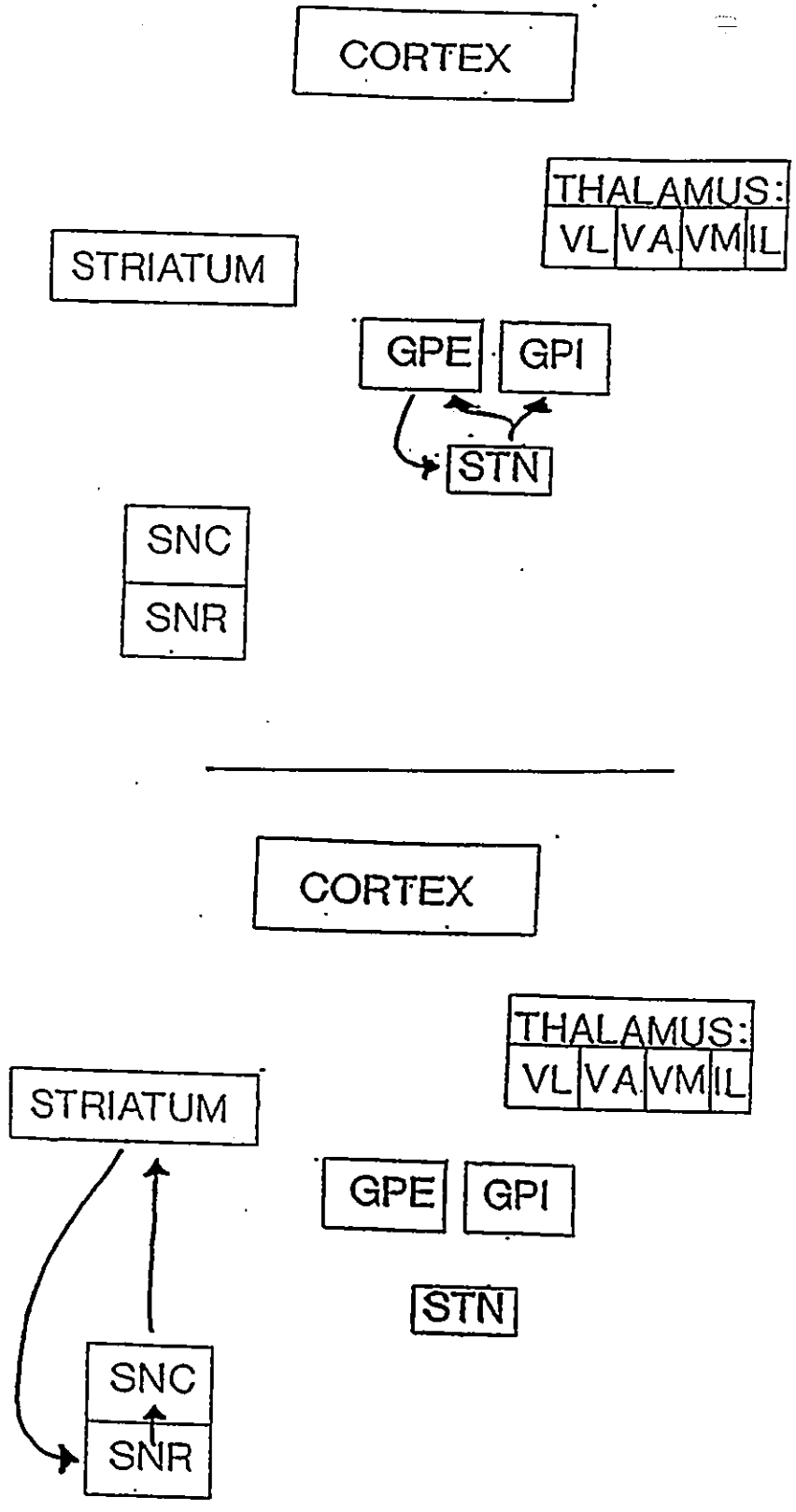
Figures 2.3 and 2.4 Schematic depiction of the pallido-subthalamo-pallidal circuit (2.3) and the striato-nigro-striatal circuit (2.4).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Basal ganglia-thalamocortical circuits

Older ideas of diverse cortical areas converging upon the basal ganglia and being "funnelled" back to the precentral motor cortex have largely been supplanted by the idea of the existence of a number of independent, parallel, basal ganglia-thalamocortical circuits. Each circuit represents a closed loop, beginning with 1) neocortex projecting to a specific area of striatum, 2) this area of striatum projecting to certain areas of globus pallidus and substantia nigra, 3) these areas of pallidum and nigra projecting to certain areas of thalamus, and 4) the thalamic areas projecting to a specific area of cortex (Alexander et al., 1986, 1990). The four circuits of the primate brain are described below (refer to figures 2.5-2.6).

1. Motor circuit

The primary motor cortex, the arcuate premotor area and the supplementary motor all project to the putamen in a somatotopically organized fashion. The putamen projects to the caudal and ventral two thirds of both divisions of the globus pallidus, as well as the caudolateral SNr. The GPi projects to the VLo nuclei (pars oralis of the ventrolateral thalamic nucleus) as well as the lateral VApc (pars parvocellularis of the ventral anterior thalamic nucleus) and the CM (centromedial thalamic nuclei). The caudolateral SNr which received projections from the putamen sends afferents to the VAmc (pars magnocellularis of the ventroanterior thalamic nucleus). The VLo and VAmc project back to the supplementary motor area, the VApc and VLo send projections to premotor cortex, and the CM sends projections to the primary motor cortex. As well as the execution of motor movements, this circuit is thought to be involved in preparatory aspects of motor control (Alexander et al., 1990).

2. Oculomotor circuit

The frontal eye fields, the supplementary eye fields (dorsal to the arcuate sulcus), the dorsolateral prefrontal cortex and the posterior parietal cortex all project to the body of the caudate; this area in turn sends efferents to the ventrolateral SNr and the caudal and dorsomedial GPi. This area of the SNr projects to VAmc and MDmf (pars multiformis of the dorsomedial nucleus of the thalamus), while the dorsomedial GPi sends its efferents to the lateral VApc. The thalamic nuclei closes the circuit through projections to the frontal and supplementary eye fields. This circuit is believed to be involved in the control of eye movements (Alexander et al., 1990).

3a. Prefrontal circuit: dorsolateral prefrontal

Dorsolateral prefrontal cortex projects to the entire rostrocaudal extent of the dorsolateral head of the caudate. Rostral areas of this portion of the caudate project to the dorsomedial third of the GP and rostral SNr. This area of the GP sends projections to the medial portion of the VApc; the aforementioned rostral SNr pprojects to the pars parvocellularis of the medial dorsal nucleus of the thalamus. The circuit is closed by each of the thalamic nuclei sending efferents back to dorsolateral prefrontal cortex. This circuit is thought to be involved in visuospatial cognition and memory; most of the evidence has come from cortical lesions in primates (Alexander et al., 1990).

3b. Prefrontal circuit: lateral orbitofrontal

Lateral orbitofrontal cortex (Brodmann's area A10, Walker's area A12) projects to the ventromedial caudate, in its entire rostrocaudal extent; in turn, this area of the caudate projects to rostromedial SNr, and dorsomedial GPi. The rostromedial

SNr projects to the pars magnocellularis of the medial dorsal nucleus of the thalamus; the dorsomedial GPi projects to the VApc; both thalamic nuclei return projections to the lateral orbitofrontal cortex. An inability to change behavioural sets and tendencies towards perseveration results from lesions to the lateral orbitofrontal cortex; again, functional evidence for this circuit comes primarily from the study of cortical lesions in primates (Alexander et al., 1990).

4. Limbic circuit

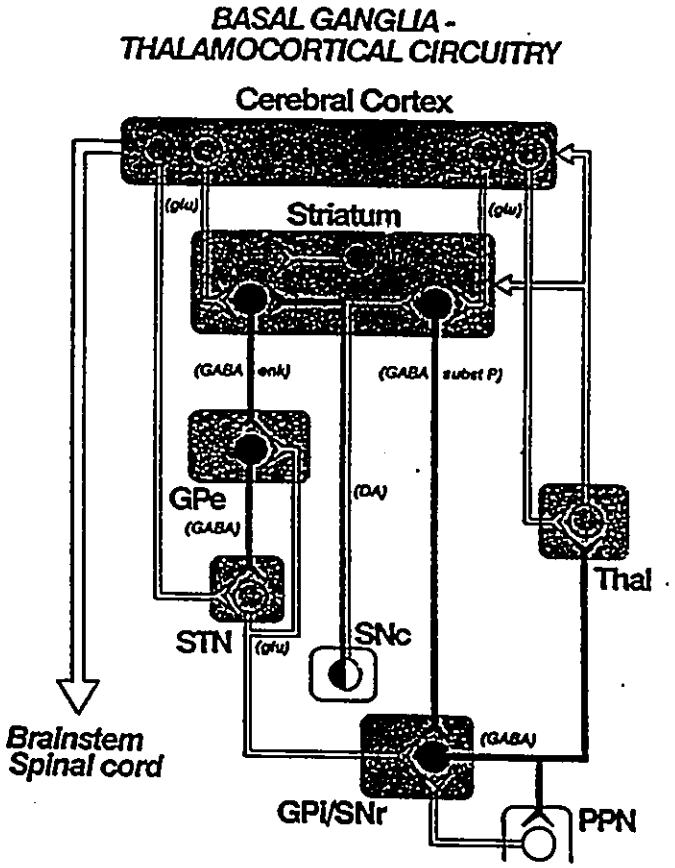
The anterior cingulate area, medial orbitofrontal cortex and temporal lobe (as well as the hippocampus, entorhinal cortex and perirhinal cortex) project to the ventral striatum; this striatal area projects to the ventral pallidum which in turn, projects to the MDmc. This circuit is closed by the projection from the thalamic nucleus to the anterior cingulate area and medial orbital prefrontal cortex. The function of the limbic circuit is a good deal less well known than the other three, but the best-known role for this circuit is in emotional and motivational processes (Alexander et al., 1990).

Largely speaking, the precise function of these circuits are still theoretical; most of the evidence has come from anterograde and retrograde labelling studies. The validation of functional aspects of the circuit (as well as further elucidation of the anatomy) awaits detailed functional and anatomical experimentation, but a certain body of experimental literature does support the theory of parallel processing in the basal ganglia (Alexander et al., 1990).

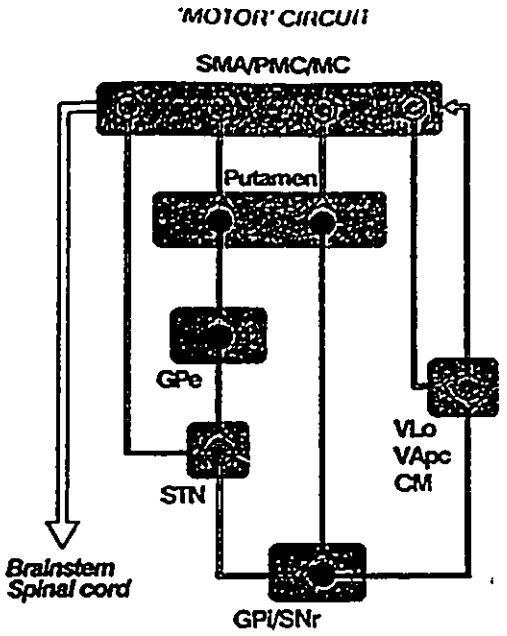
Other Afferents of the Basal Ganglia

In addition to the internal connections of the basal ganglia, a number of other

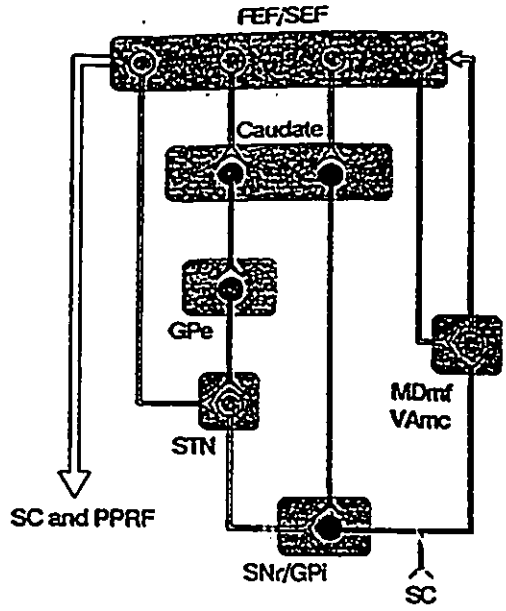
Figures 2.5 and 2.6 Schematic diagram of hypothesized basal ganglia-thalamocortical circuitry. From Alexander GE, Crutcher MD, and DeLong MR. 1990. Basal ganglia-thalamocortical circuits: parallel substrates for the motor, oculomotor, "prefrontal" and "limbic" functions. In HBM Uylings, CG Van Eden, JPC de Bruin, MA Corner and MGP Feenstra (eds). The Prefrontal Cortex. Its Structure, Function and Pathology, Progress in Brain Research, 45, pgs. 121, 126, 129, 132, 134.



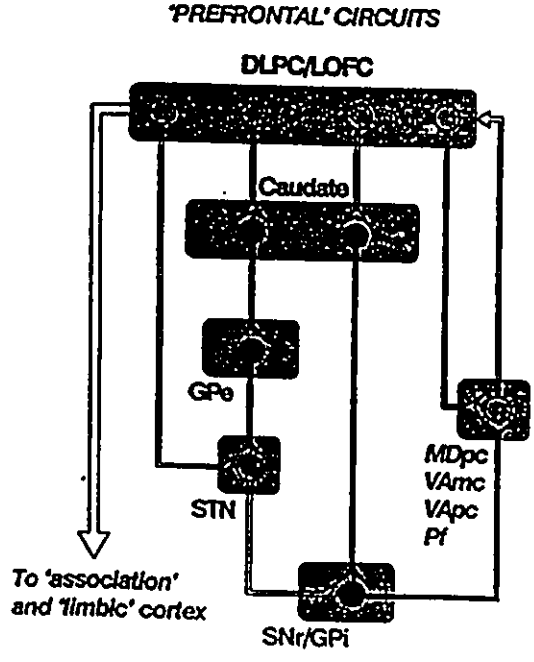
. Schematic diagram of the circuitry and neurotransmitters of the basal ganglia-thalamocortical circuitry. See text for discussion. (A few of the known connections have been omitted for the sake of clarity; e.g., striatal projections from the dorsal raphe, the STN and PPN.) Conventions are the same as in Fig. 2. ACh, acetylcholine; DA, dopamine; enk, enkephalin; GABA, gamma-aminobutyric acid; glu, glutamate; PPN, pedunculopontine nucleus; SNc, substantia nigra pars compacta; Thal, thalamus.



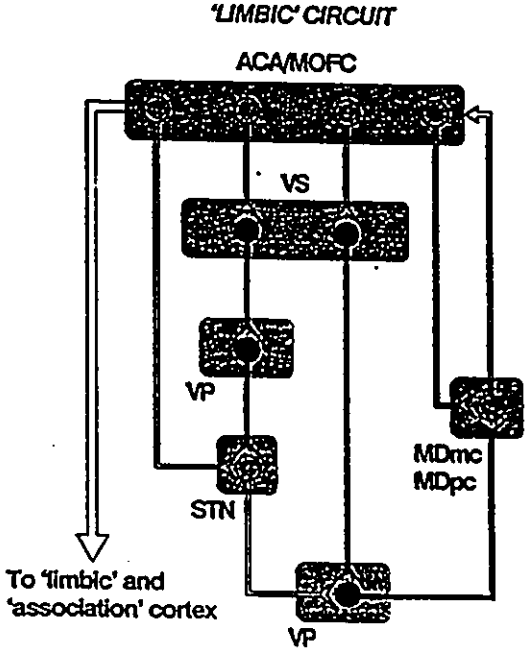
. Simplified diagram of the "motor" circuit. The cortical areas shown projecting to the putamen include only the "closed loop" portion of the "motor" circuit. Additional "open loop" corticostriatal inputs to the "motor" circuit arise from the arcuate premotor area, primary somatosensory cortex and somatosensory association cortex. Inhibitory neurons are filled; excitatory are unfilled. CM, n. centrum medianum; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VApc, n.ventralis anterior, pars parvocellularis; VLo, n.ventralis lateralis, pars oralis.



Simplified diagram of the closed loop portion of the "oculomotor" circuit. Conventions are the same as in Fig. 2. MDmf, n. medialis dorsalis pars multiformis; PPRF, paramedian pontine reticular formation; SC, superior colliculus; VAmc, n. ventralis anterior pars magnocellularis.



Simplified diagram of the closed loop portions of the "prefrontal" circuits. Conventions are the same as in Fig. 2. MDpc, n. medialis dorsalis pars parvicellularis; Pf, n. parafascicularis.



Simplified diagram of the closed loop portions of tl "limbic" circuit. Conventions are the same as in Fig. 2. MDm N. medialis dorsalis pars magnocellularis; VP, ventr pallidum; VS, ventral striatum.

structures send projections to the basal ganglia. Apart from the dopaminergic projection from cell group A9 to the striatum, cell groups A8 (retrorubral nucleus) and A10 (ventral tegmental area of Tsai) also send dopaminergic projections to the striatum; CCK is co-localized in this pathway (Nieuwenhuys, 1985).

Cell groups B6, B7 and B8 (dorsal raphe, nucleus centralis superior; located within the raphe nucleus) send serotonergic projections to the CPu, as well as to the globus pallidus, nucleus accumbens and substantia nigra. Not all the projection from the raphe is serotonergic; as much as one quarter of the projection is thought to involve other transmitters (Nieuwenhuys, 1985; Lavoie and Parent, 1990).

Cell groups A6 (located within the locus coeruleus) and A5 (also located caudal to the locus coeruleus) send projections to the caudate, while cell groups A1 and A2 (located caudal to the locus coeruleus) send projections to the nucleus accumbens. All these projections are noradrenergic (Nieuwenhuys, 1985).

Corticostriatal Organization

With the advent of sophisticated neuroanatomical techniques, concepts involving striatal organization have changed in the past few years; the ideas surrounding corticostriatal organization have been no exception. The possibility of the existence of a corticostriatal tract first arose in 1891 from the pioneering work of Ramón y Cajal, but until the 1940s, its existence remained unconfirmed. Although experimental evidence for a corticostriatal tract was first provided in the 1940s, the study of Kemp and Powell (1970) was largely responsible for establishing its existence. Using the phenomenon of retrograde fibre degeneration

following cortical lesion, Kemp and Powell (1970) suggested that the entire cortex projects to the striatum in a topographically organized fashion, such that more anterior areas project to the head of the caudate, while more posterior areas project to the tail of the same structure.

Kemp and Powell's very influential study was regarded as dogma until Goldman and Nauta (1977) , using the new autoradiographic tracer techniques, obtained evidence to the contrary. In contrast to Kemp and Powell's notion of anterior-posterior organization of corticostriatal projections, Goldman and Nauta found that the principal sulcus of the primate cortex sends projections to the entire rostro-caudal extent of the caudate nucleus. Furthermore, they were also able to demonstrate that corticostriatal terminations formed ring-like clusters, the core of which was bare of autoradiographic label. It was suggested that the difference between these two studies might be explained through the increased sensitivity known to be associated with autoradiographic tracer techniques (Goldman and Nauta, 1977). This concept of a single cortical area projecting to many and various areas in the caudate and putamen has been replicated and extended by many groups of researchers both for primates (Kunzle, 1975, 1977) as well as rodents (McGeorge and Faull, 1989).

Compartmentalization of the Striatum

Although the old concept of the striatum has been one of a homogeneous subcortical structure, recent research using immunocytochemical techniques has led to a radical change in this view. As described previously, the neuronal population of the mammalian striatum consists largely of medium spiny neurons and a smaller

population of large and medium aspiny neurons. These cells can be characterized on the basis of the associated transmiter as well as on the basis of their efferents; axons of the spiny neurons form projections from the striatum, while the aspiny neurons are the striatal interneurons.

The detailed a sophisticated circuitry of the striatum is well illustrated by the recently discovered patch/matrix or striosome/matrix organization of the striatum. Although this compartmentalization is most striking in the caudate and putamen, it is also thought to be present in most other nuclei of the basal ganglia. First noted as a general organizational principle by Goldman and Nauta (1977), it is now widely recognized that certain neurotransmitters are organized into three-dimensional transmitter-rich and transmitter-poor networks within the nuclei of the basal ganglia (Gerfen, 1992). The first neurotransmitter found to conform to such an organization was acetylcholine, as identified by immunohistochemical staining for acetylcholinesterase (AChe) or cholineacetyltransferase (ChAT). Enzyme staining for AChe and has revealed low immunoreactivity in the striatal compartment referred to as the "patch" or striosomes (striatal bodies) accompanied by high immunoreactivity in the striatal compartment referred to as the "matrix" (Graybiel and Ragsdale, 1978). This pattern of immunoreactivity has also been found for the neuropeptide somatostatin (Chesselet and Graybiel, 1986), as well as the terminals for the catecholamine dopamine (as inferred by staining for tyrosine hydroxylase (Graybiel, Hirsch and Agid, 1984)) and the indoleamine serotonin (Lavoie and Parent, 1990). Staining for the neuropeptide substance P, on the other hand, has revealed the opposite pattern with higher immunoreactivity in the striosomes and low immunoreactivity in the matrix (Bolam et al., 1988). Not all

transmitters conform to this organization; GABA, for example, does not seem to fit into any sort of patch/matrix organization.

Although the functional significance of the patch/matrix organization of the striatum is unknown, a recent study has demonstrated a conservation of the patch/matrix ratio across three different species. Johnston et al. (1990) have discovered that a 15%:85% patch:matrix ratio is demonstrated in the rat, rhesus monkey and man. Additionally, the number of patches is also relatively similar across these three species. The authors suggest that the maintenance of a similar patch:matrix ratio across species may be necessary to preserve the proportion of limbic and sensory-motor input to different areas of the striatum that is necessary for various kinds of learning (Johnston et al., 1990).

IVa. Pathology of Huntington's Disease: Gross Neuropathology

"I know nothing of its pathology." (Huntington, 1872, pg. 321)

The gross neuropathology of HD was first studied in the late nineteenth century, most notably by Meynert and Golgi. Even at this early date, the vulnerability of the striatum to the disease process was already recognized (Vonsattel et al., 1985). Subsequent neuropathological studies were provided by Jelgersma in 1909 (Bruyn et al., 1979), Alzheimer in 1912 and Dunlap in 1927 (Vonsattel et al., 1985). By this time, it was well recognized that the striatum, particularly the caudate, sustains the greatest degeneration in HD, although other structures, such as the cortex, globus pallidus, centrum semiovale and substantia nigra, also seems to be affected (Vonsattel et al., 1985). Other corroborative studies have since been provided (e.g., McCaughey, 1961; Lange et al., 1976), but the most authoritative and detailed description of the gross neuropathology of HD is from a series of studies by Vonsattel and his colleagues, beginning in 1985.

Through systematic study of a series of 163 post-mortem brain specimens of diagnosed HD, Vonsattel et al. (1985, 1987) were able to confirm and extend the observations of previous researchers in finding that 1) the caudate nucleus undergoes greater degeneration than any other neural structure and also that 2) the anteromedial aspects of the caudate are more affected than the lateral, posterior aspects. A similar degenerative pattern was found for the putamen, although, as

mentioned previously, this structure did not sustain as much degeneration as the caudate (refer to figures 2.7 and 2.8).

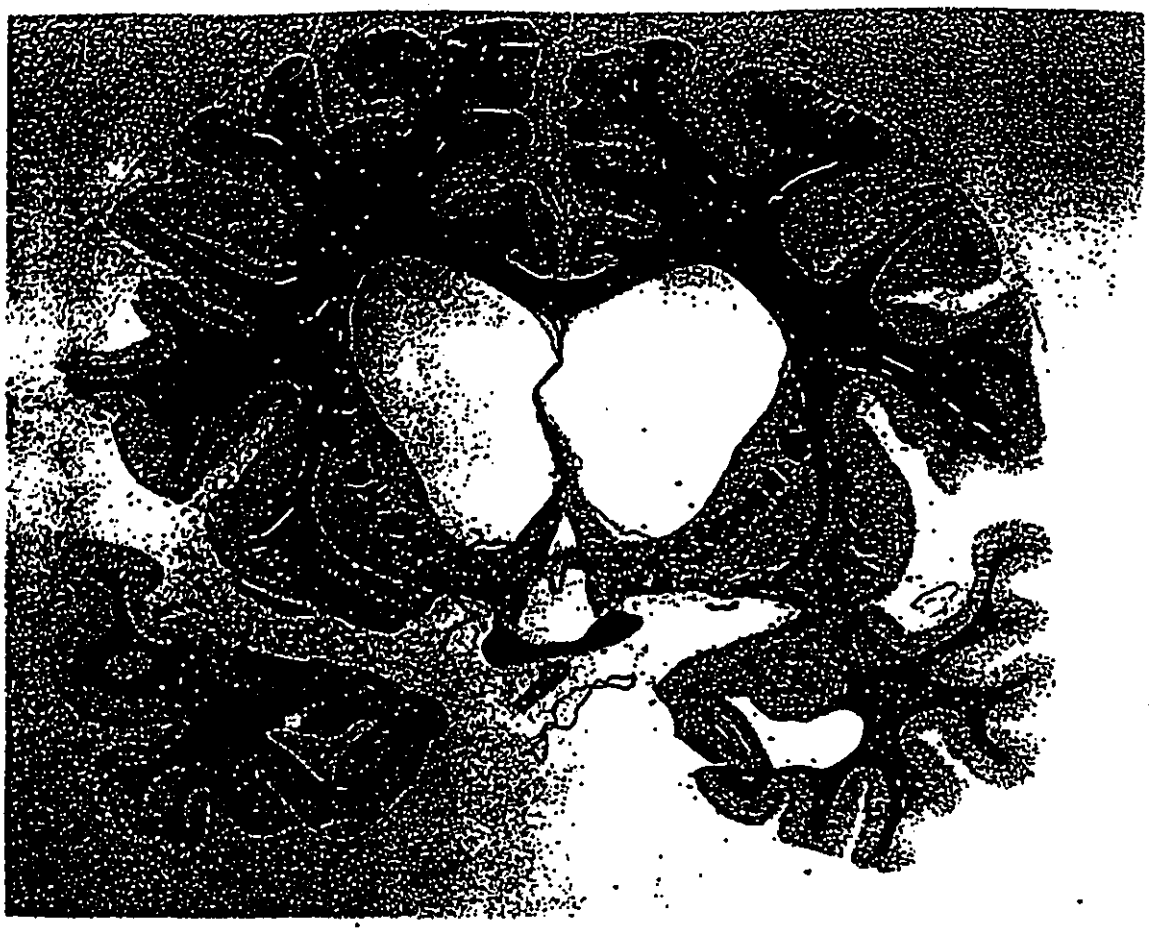
From this series, a rating scale of pathological severity (grades 0 through 4) was established, based on macroscopic and microscopic examination of the striatum. Cases assigned a grade of 0 demonstrated no macroscopic or microscopic abnormalities, although a clinical diagnosis of HD had been made. Cases with the first signs of degenerative changes in the caudate and putamen were assigned a grade of 1; subsequent, progressive degeneration in these two structures was evident in those cases assigned to more severe grades of pathological involvement (e.g., grades 2,3, and 4) (Vonsattel et al., 1985, 1987).

Although the lateral ventricles were slightly enlarged in grade 2 cases, the medial aspect of the caudate maintained its normal convex outline in grades 1 and 2. In those cases assigned to the third grade, however, the medial aspect of the caudate was no longer convex, but flat; in those cases assigned to the fourth grade, the medial aspect of the caudate was concave. Cell counts in the caudate revealed a loss of 50% of caudate neurons in grade 1 and a 95% loss in grade 4. Astrocyte counts in the caudate were increased by 16% in grades 1 and 2 and by 28% in grades 3 and 4 (Vonsattel et al., 1985, 1987).

Degeneration of the globus pallidus was not evident in grades one or two, only in grades three and four. The nucleus accumbens appeared normal until grade 4, at which point it demonstrated a slight reduction in size (Vonsattel et al., 1985, 1987).

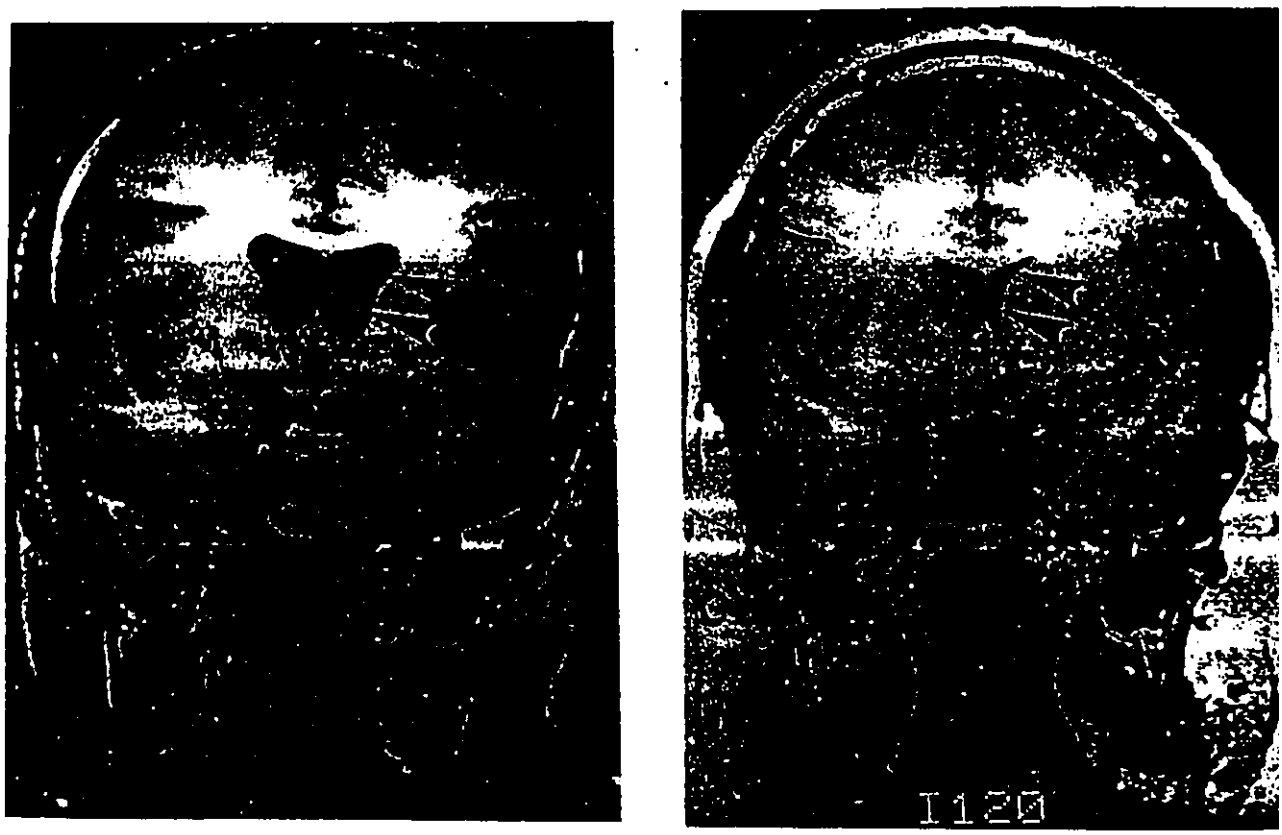
A subsequent study of a smaller series of postmortem HD brains by these same investigators further quantified the degeneration associated with various CNS structures, and confirmed earlier reports that mean brain weight in these HD

Figure 2.7 Coronal section of brain from a patient who had died with advanced Huntington's Disease. Note gross wasting of the striatum and marked ventricular dilation. From Earle KM. 1973. Advances in Neurology, 1, pg. 344.



Transverse section of the brain of a patient with Huntington's chorea. Courtesy K. M. Earle, M.D., Washington, D.C.

Figure 2.8 MRI scans age-matched normal control (right) and Huntington's Disease patient (left).Note marked ventricular dilation, caudate and cortical atrophy in HD patient. From Folstein S. 1989. Huntington's Disease. A disorder of families. Baltimore: Johns Hopkins University Press, pg. 78.



Magnetic resonance imaging (MRI) scan of a 31-year-old HD patient (*left*) and an age-matched control subject (*right*). Caudate atrophy can be appreciated by comparing the patient's scan with the control's. The medial border of the right caudate is marked in each figure by arrows.

postmortem cases was significantly lower than that of controls (de la Monte et al., 1987, 1988). Analyses of coronal sections showed that the cross-sectional area of the cerebral cortex, white matter, caudate, putamen and thalamus was significantly smaller in the HD cases than in the controls. The reduction in cross-sectional area was greater in caudate, putamen and globus pallidus (approximately 60%) than in the cortex, white matter, hippocampus, amygdala and thalamus (approximately 20-30%). Further, the atrophy in the former structures was progressive across grades while the atrophy in the latter structures was uniform across grades (de la Monte et al., 1988). With respect to the cortical area in particular, tissue atrophy ranged from 21-29% across various areas of the cortex.

Statistical analysis of the entire collection of 163 cases revealed several significant correlations. Significant negative correlations were found between assigned grade and hemispheric weight of the fixed tissue, neuronal count, as well as the premorbid rating of disability (Vonsattel et al., 1985, 1987).

Two studies have examined the status of the substantia nigra in HD post-mortem tissue. Both Ferrante et al. (1989) and Oyanagi et al. (1989) have found that the area of the substantia nigra in HD is significantly smaller than that in control patients. Zweig et al. (1992) have examined the locus coeruleus in HD and have found significant reduction in rostro-caudal length of this nuclei (but no changes in the dorsal raphe) when compared to controls.

IVb. Pathology of Huntington's Disease: Neurochemical Pathology

1. The medium spiny neurons: Met-enkephalin, dynorphin, substance P and GABA

As mentioned previously, the medium spiny neurons form the vast majority of the total striatal neuronal population. The transmitters thought to be associated with these cells are methionine-enkephalin (met-enkephalin), dynorphin and substance P, each co-localized with GABA.

GABA and glutamate were among the first neurotransmitters to be measured in HD post-mortem tissue. Perry et al. (1973) was the first to measure a wide variety of amino acids in HD basal ganglia and cortex in a series of eight HD and eight control brains. GABA levels were found to be significantly reduced in HD substantia nigra, putamen-globus pallidus as well as occipital and temporal cortex, but not frontal cortex or cerebellum (Perry et al., 1973). GAD activity was significantly reduced in HD caudate, putamen and globus pallidus, but no different from controls in the frontal cortex (Bird et al., 1973). This basic finding has been confirmed by other researchers (Bird et al., 1973; McGeer et al., 1973; Stahl and Swanson, 1974; McGeer and McGeer, 1976; Spokes, 1980; Ellison et al., 1987; Pearson et al., 1990).

An early paper suggested that disease severity may influence levels of GAD depletion (Stahl and Swanson, 1974). This suggested influence of clinical state upon post-mortem analysis was confirmed by Ellison et al. (1987) in an extensive study of graded HD cases. In this detailed study, GABA demonstrated a gradient of depletion across the three striatal nuclei, whereby the caudate demonstrated the greatest loss of GABA, with the putamen and nucleus accumbens demonstrating

lesser losses, respectively. Similar changes were found for the two divisions of the pallidum and the substantia nigra. Cortical areas were also measured for the first time, showing significant depletions of glutamate were found in Brodmann's areas 3-1-2 (postcentral strip), 6, 9, and 17, but no other cortical areas demonstrated any significant change (Ellison et al., 1987).

The known involvement of subcortical basal ganglia nuclei in HD as well as reported decreases of GABA in HD post-mortem tissue led researchers to assay HD postmortem tissue for SP. Decreases in substance P levels were found in the SNc and SNr in HD brain when compared to controls (Kanazawa et al., 1977; Emson et al., 1980); a similar decrease was found in HD globus pallidus, caudate and putamen (Kanazawa et al., 1979; Emson et al., 1980). Nonsignificant decreases were noted in frontal cortex (Brodmann's area 32), lateral globus pallidus, thalamus and hypothalamus. This basic profile of SP in HD CNS post-mortem tissue has been confirmed by other researchers (Arai et al., 1987; Aronin et al., 1983; Kanazawa et al., 1985; Gale et al., 1978).

In order to clarify the relationship between pathological severity and neurochemical data, an examination of SP levels was undertaken in a series of pathologically graded HD brains (Beal et al., 1988a). In general, the previous findings of reductions in SP levels in various subcortical nuclei were confirmed. For the first time, a significant reduction in SP was noted in the subthalamic nucleus, and the bed nucleus of the stria terminalis. The depletions in all these areas were found to increase with pathological severity, and correlated significantly with the percentage of neuronal loss in each grade (according to the rating scale previously described in the Gross Pathology section). In contrast to previous

studies, Beal et al. (1988a) found small, but significant increases in SP levels in HD frontal cortex (Brodmann's areas 6, 8 and 9). It has been suggested that SP in cortical areas is co-localized with NPY- or SS-positive neurons, which are known to be preserved in HD frontal cortex. If true, this would account for the increased cortical levels of SP.

Neurochemical analyses have also been conducted for the other neuropeptides localized to medium spiny neurons, met-enkephalin and dynorphin. These studies have revealed levels in HD tissue to be below that of controls, particularly in caudate, putamen, and globus pallidus (Emson et al., 1980; Seizinger et al., 1986; Dawbarn et al., 1986).

Several neuroanatomical studies have examined HD striatum using immunocytochemical techniques. Analysis for met-enkephalin or substance P has usually revealed reduced levels of immunoreactivity in HD striatum when compared to normal controls (Marshall et al., 1983; Zech and Bogerts, 1985; Kanazawa et al., 1985; Grafe et al., 1985; Ferrante et al., 1986).

In summary, the medium spiny striatal neurons sustain severe degeneration as revealed by lowered levels or a decrease in staining for the associated transmitters.

2. The medium aspiny neurons: neuropeptide Y, somatostatin and NADPH-d

The biochemical marker nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) has been found to be co-localized with neuropeptide Y and somatostatin neurons in the medium aspiny neurons of the cortex and striatum of various mammalian species. In the medium aspiny striatal neurons, this localization is believed to be exclusive (Vincent et al., 1983).

In the first two studies of SS levels in HD brain, SS was found to be significantly increased in the caudate, putamen, nucleus accumbens, internal (GPi) and external globus pallidus (GPe) (Aronin et al., 1983; Nemeroff et al., 1983).

Beal et al. (1988b) have found significant increases in SS levels in caudate, putamen and nucleus accumbens when compared to control values. In agreement with Aronin et al. (1983) SS levels in the GPe were significantly higher than in relation to controls, but in contrast, no difference in SS levels was found in the GPi. For the first time, SS was measured in HD substantia nigra; SS levels were significantly elevated in the SNr in HD grades 3 and 4. SS levels were also significantly increased in the red nucleus and locus coeruleus. A wide number of HD cortical areas were assayed and SS levels were found to be increased in Brodmann's areas 6, 8, 9, 10, 11, 21 and 45 (frontal and temporal cortex). This is in contrast to Aronin et al. (1983) who found no difference in SS levels in Brodmann's areas 8 and 11.

As with somatostatin, NPY levels were found to be significantly increased in the caudate, putamen and nucleus accumbens. NPY levels were also found to be significantly increased in the GPe, SNc, subthalamic nucleus, anterior and dorsomedial thalamus, claustrum, bed nucleus of the stria terminalis, red nucleus and locus coeruleus. Further, Brodmann's areas 6, 8, 9, 10, 11, 45, 47, 20, 21, 22 of cerebral cortex also demonstrated increased levels of NPY (Dawbarn et al., 1985; Beal et al., 1988b). (Additionally, neurochemical assays of NADPH-d activity revealed a pattern of significantly higher levels of immunoreactivity in HD striatal tissue than in controls.)

Use of the diaphorase histochemical stain as well as immunohistochemical

techniques for the localization of NPY and SS in HD post-mortem material has demonstrated a striking preservation of the medium aspiny cells, even in severe grades of HD. Careful analysis has demonstrated that even where the total number of neurons was significantly reduced in HD postmortem striatal tissue, the number of NADPH-d neurons was significantly greater, in relation to controls, in terms of absolute number, percentage of the total number of neurons, as well as density. Cellular abnormalities in the medium aspiny neurons were only noted in the most severe grades of HD (Ferrante et al., 1985a, 1985b). A similar result has been provided by Dawbarn et al., (1985). Using an immunocytochemical stain for NPY, these investigators demonstrated significantly more NPY-positive neurons in the caudate and putamen of HD cases, when compared to controls. The same study also revealed significantly increased striatal levels of NPY and somatostatin, (as measured by radioimmunoassays) in HD tissue; the pathological profile thus provided further support for the notion of selective neuronal sparing.

NPY neurons as well as glutamatergic neurons have recently been examined in HD cortex. The density of NPY immunoreactive neurons in superior frontal cortex was no different between HD patients and age-matched controls, while that of nonphosphorylated neurofilament immunoreactivity (specific for glutamate pyramidal neurons in the cortex) was significantly reduced in HD patients when compared to controls; the HD data was suggestive of a relative sparing of NPY immunoreactive neurons in cortex (Cudkowicz and Kowall, 1990).

In summary, levels of somatostatin, neuropeptide Y and NADPH-d are significantly higher in HD striata than control tissue; neuroanatomical analyses reveal that these neurons are preserved in the face of marked striatal degeneration.

3. The large aspiny neurons: acetylcholine

Acetylcholine (ACh) is localized to the large aspiny neurons of the mammalian caudate and putamen. Due to its rapid hydrolysis, ACh cannot be measured directly in post-mortem brain: the markers used to study cholinergic neurons are either the synthetic enzyme cholineacetyltransferase (ChAT) or the less specific degradative enzyme acetylcholinesterase (AChe) (Nieuwenhuys, 1985; Cooper, Bloom and Roth, 1991).

The first study to measure ChAT levels in HD striatal tissue found that the caudate and putamen of the HD cases demonstrated a marked loss in ChAT activity when compared to the control subjects (McGeer et al., 1973a, 1973b). This finding has subsequently been confirmed by a number of researchers (Stahl and Swanson, 1974; Aquilonius et al., 1975; Bird, 1980; Bird and Iversen, 1974; McGeer and McGeer, 1976; Spokes 1980; Hammond and Brimijoin, 1988).

Using a histochemical stain for AChe, relative preservation of the large aspiny cholinergic neurons has been demonstrated in HD striatal postmortem tissue. (Ferrante et al., 1987a). The general pattern of cholinergic patch/matrix organization is preserved in HD, although some abnormalities have been noted. Specifically, the area of the matrix zone was found to be reduced in size, while that of the patch zones were found to be no different from that of controls (Ferrante et al., 1987a).

In the same study, Ferrante et al. (1987a) noted that while levels of ChAT were significantly reduced in HD striata (via neurochemical analysis), there was a relative preservation of cholinergic cell bodies as revealed by staining for AChe. This difference in results for the two cholinergic markers in HD striatum is not

well understood but may be related to the distribution of AChe and ChAT among neuronal elements since it has been suggested that the AChe in nerve cell bodies is preserved while the ChAT-containing axons and terminals degenerate (Ferrante et al., 1987a).

Muscarinic cholinergic receptor binding has been examined in HD brain and compared with ChAT activity. Decreases in ChAT activity as well as of cholinergic receptor binding has been found in HD caudate, putamen (Hiley and Bird, 1974; Wastek et al., 1976) and globus pallidus (Wastek et al., 1976). No significant differences were found in other areas examined such as hypothalamus, cerebellum, pons, hippocampus, substantia nigra, red nucleus and frontal cortex (Wastek et al., 1976).

In summary, ChAT levels are markedly reduced in HD striata while enzyme staining or neurochemical analysis for AChe yield a pattern of preservation.

4. Subcortical striatal afferents: dopamine and noradrenaline

One of the most important neostriatal afferents is the dopaminergic projection originating primarily from the substantia nigra, pars compacta. Dopamine (DA) activity in HD post-mortem tissue was first measured by Bird and Iversen (1974) who found no difference between HD cases and controls, in the DA levels of the caudate and putamen, a finding that was subsequently confirmed by others (via T-OH levels by McGeer and McGeer, 1976). However, other authors have reported a significant increase in DA levels in one or more nuclei of the HD basal ganglia (Bernheimer, 1973; Bird, 1980; Spokes, 1980; Melamed et al., 1982; Kish et al., 1987). These differences have been attributed, by Kish et al., to differences

in handling of post-mortem tissue, particularly in the time elapsed to freezing tissue after death as well as the division of striatal tissue into different areas (Kish et al., 1987). The preservation or increase in levels of DA in structures that otherwise undergo atrophy suggest a preservation of dopaminergic terminals accompanied by atrophy of surrounding tissue.

Ferrante et al. (1987b) studied striatal dopaminergic terminals (via immunoreactive staining for tyrosine hydroxylase) in HD post-mortem tissue revand reported that in control tissue, tyrosine hydroxylase immunoreactivity and acetylcholinesterase patch/matrix organization demonstrated a high degree of coincidence. This pattern was preserved in HD postmortem striatal tissue, in spite of marked striatal atrophy. As was the case with acetylcholinesterase staining, the area of the tyrosine-hydroxylase-defined matrix was significantly decreased in comparison to controls, while that of the patches was normal. Accompanying neurochemical studies of tyrosine hydroxylase activity have revealed normal or only slightly elevated levels in HD tissue. This finding of preservation is different in magnitude from that associated with SS/NPY levels, which are found to be increased 3-5 times in HD postmortem striatal tissue. Taken in conjunction with the neurochemical and neuroanatomical sutdies of T-OH in HD striatal tissue, it is probably the case that T-OH immunoreactivity, and presumably DA terminals, are lost in proportion to striatal atrophy (Ferrante et al., 1987b).

One of the few studies to measure noradrenaline (NA) in post-mortem tissue has been that of Spokes (1980) who found significant increases in HD caudate, lateral pallidum, nucleus accumbens and SNr. Although tissue atrophy might partially explain these increases, Spokes (1980) proposed that this explanation

might be insufficient as it did not explain why NA levels were normal in putamen and medial pallidum, both of which undergo atrophy in HD.

V. Positron Emission Tomography and Huntington's Disease

Positron emission tomography has proven to be very useful in the study of HD. Because of the tool's relative novelty as well as the rarity of HD patients, not many studies are available, but those few existing are largely in accordance with each other.

Fluorodeoxyglucose is the radioisotope that has been most commonly used in PET studies of HD patients. Typically, PET reveals a hypometabolism of the striatum, regardless of the severity of the disease. In patients in the earliest stages of the disease, hypometabolism of striatal tissue precedes tissue loss (typically assessed by computerized axial tomography), thus affording physicians increased diagnostic sensitivity not previously available. Of particular interest is the finding that hypometabolism is detectable in the caudate prior to the putamen (Young et al., 1986), presumably suggesting a temporal pattern of striatal degeneration. Further correlations have been noted between striatal hypometabolism and the various symptoms of HD. Interestingly, only putamen metabolism has been found to correlate with such motor abnormalities as chorea, fine motor co-ordination and oculomotor abnormalities, while caudate metabolism has been found to correlate with overall functional capacity and bradykinesia/rigidity (Young et al., 1986).

Whether or not the cortex demonstrates hypometabolism has proven to be more controversial. Some researchers have found no changes in glucose metabolism in the cortex (Young et al., 1986; Hayden et al., 1985, 1986) while others have found cortical hypometabolism of varying degrees (Kuhl et al., 1982; Garnett et al., 1984; Kuwert et al., 1989, 1990; Martin et al., 1992). It has been suggested that the studies

demonstrating negative findings in the cortex may have been testing subjects in the early stages of the disease and vice versa; more recent investigations have also had the advantage of PET scanners with increased powers of resolution (Kuwert et al., 1989, 1990).

The sensitivity of PET to the earliest stages of HD has led researchers to investigate the role of PET as a diagnostic tool for individuals at-risk for HD. In contrast to the studies on patients with diagnosed HD, these results have been subject to more variability. One group of researchers has failed to find striatal abnormalities for glucose metabolism in at-risk subjects (Young et al., 1987) while others have noted a suggestion of abnormalities in some of these subjects (Hayden et al., 1985; Kuhl et al., 1982, 1984). The variability in these studies may well be due to the length of time to disease onset in these at-risk individuals. This problem might be partially resolved by separating subjects into groups on the basis of the age of onset of the disease in the affected parent.

VI. Excitotoxicity and the pathogenesis of Huntington's Disease

The pathogenesis of HD is unknown, but a recent accumulation of evidence suggests that pathogenesis of HD may involve NMDA-receptor mediated excitotoxicity. Prior to examining this evidence, it will first be useful to review the molecular mechanisms for NMDA-receptor mediated excitotoxicity.

Subtypes of glutamate receptors

There are three subtypes of ionophore-linked glutamate receptors: the kainate receptor (KA), the quisqualate/AMPA receptor (Q/AMPA) and the N-methyl-D-aspartate receptor (NMDA). Each is stimulated by glutamate, but also selectively by their eponymic analogues. The KA and Q/AMPA receptors mediate sodium (Na^+) and potassium (K^+) conductance while the NMDA receptors also mediate calcium (Ca^{2+}) conductance. Glutamate also activates a metabotropic receptor which is linked to a second messenger system involving hydrolysis of phosphatidyl inositol (Watkins and Olverman, 1987; Choi, 1988; Choi and Rothman, 1990) (refer to figure 2.9).

The NMDA receptor

The NMDA receptor is both chemical- and voltage-dependent: at rest, the ionophore is blocked by magnesium (Mg^{2+}), thus preventing any inflow or outflow, but when a certain level of depolarization has been achieved (by activation of adjacent KA or Q/AMPA receptors), the Mg^{2+} ion leaves the ion channel, thus permitting the influx of Ca^{2+} and Na^+ in response to NMDA receptor stimulation (refer to figure 2.10). The NMDA receptor can be stimulated by a variety of endogenous compounds, including quinolinic acid (QUIN). The NMDA receptor can be selectively antagonized by 2-aminophosphonovalerate (2-APV) or nonselectively by zinc (Zn^{2+}),

phencyclidine, MK-801 or ketamine (Choi, 1988; Choi and Rothman, 1990).

Once the NMDA receptor has been stimulated, Ca^{2+} and Na^{+} ions enter the cell through the NMDA receptor-linked ionophore. Glutamatergic stimulation of the metabotropic receptor results in the activation of diacylglycerol (DAG) and inositol triphosphate (IP_3) within the cell; IP_3 mobilization results in Ca^{2+} liberation from the endoplasmic reticulum. If intracellular Ca^{2+} levels are extremely high, this may result in breakdown of intracellular nucleic acids, and cell membrane components (the production of free radicals also enhances the breakdown of the cell membrane), the end result being cell death (Choi, 1988; Choi and Rothman, 1990).

Quinolinic Acid and Huntington's Disease

The discovery that QUIN occurs endogenously within the mammalian CNS, coupled with the finding that it selectively stimulates the NMDA receptor, has aroused interest in the possibility that it may somehow be involved in certain diseases states, HD among them.

Quinolinic acid (2,3-pyridine dicarboxylic acid; QUIN) is a heterocyclic amino acid, long known to be an intermediary in the metabolism of tryptophan in the kynurenine pathway. The precursor, 3-hydroxyanthranilic acid gives rise to QUIN via the synthesizing enzyme 3-hydroxyanathranilate oxygenase (3-HAO). The breakdown of QUIN via the degradative enzyme quinolinic acid phosphoribosyltransferase (QPRT) yields the metabolite nicotinic acid mononucleotide (Schwarcz et al., 1989; Wolfensberger et al., 1983; Bruyn and Stoof, 1990; Whetsell et al., 1989).

In the early 1980s, it was discovered that this metabolite, when injected intrastriatally, could produce axon-sparing lesions in rats (Schwarcz and Kohler, 1983; Schwarcz et al., 1983). The similarity of this damage to previous rat models of HD (as produced by KA and ibotenic acid)

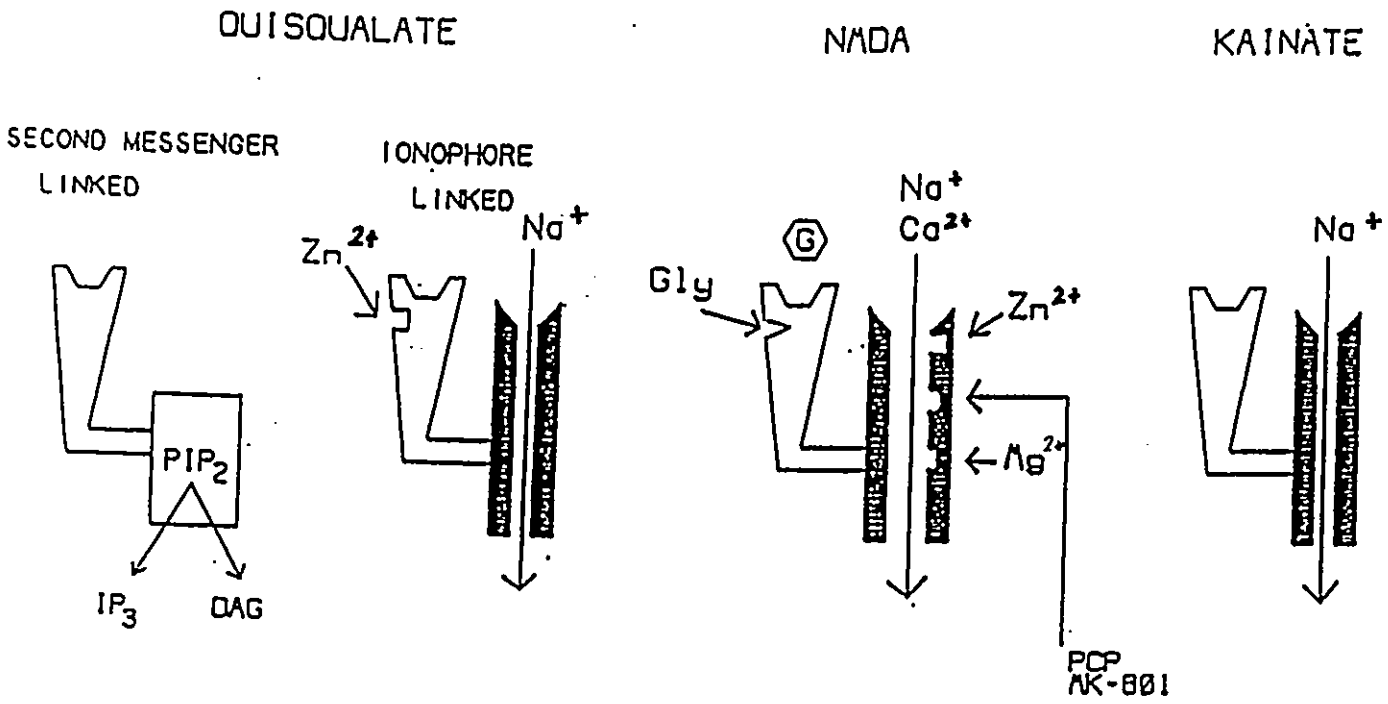
led to a more systematic evaluation of QUIN as a model for the disease. Neurochemical analysis suggested that a QUIN-induced striatal lesion appeared to spare the medium aspiny striatal neurons while destroying the medium spiny neurons (Beal et al., 1986). The similarity between this neurochemical profile and that of HD led researchers to assay HD brain for QUIN and related compounds. If QUIN were indeed instrumental in the pathogenesis of HD then perhaps some abnormalities of these compounds could be detected in HD brains.

Shortly after QUIN was shown to produce axon-sparing lesions in rats, Wolfensberger et al. (1983) reported the presence of QUIN in both normal human and rat brain. In human brain, 3 areas were measured. QUIN levels were found to be highest in frontal cortex, followed by cerebellar cortex and caudate, respectively (Wolfensberger et al., 1983).

QUIN was first measured in HD post-mortem CNS tissue (e.g., frontal cortex and putamen) by Reynolds and colleagues who found no difference between HD patients and neurologically normal controls (Reynolds et al., 1988; Pearson and Reynolds, 1989). Schwarcz et al. (1988) measured the anabolic enzyme for QUIN, 3-HAO, in HD and control brains and reported levels to be higher in HD tissue in a wide variety of brain regions (e.g., substantia nigra, globus pallidus, cerebellum, thalamus, frontal and parietal cortex), particularly the caudate and putamen. Along similar lines, Reynolds and Pearson (1989) have reported increased cortical levels of 3-hydroxykynurenine, the precursor of 3HAO in HD patients.

Further supportive evidence of potential involvement of QUIN in HD has come from three recent reports. The first, by Greenamyre et al. (1985) found reduced glutamate binding in HD caudate and putamen but normal cortical levels when compared to normal controls and Alzheimer's patients, suggesting the likely loss of the cells that bear glutamate receptors

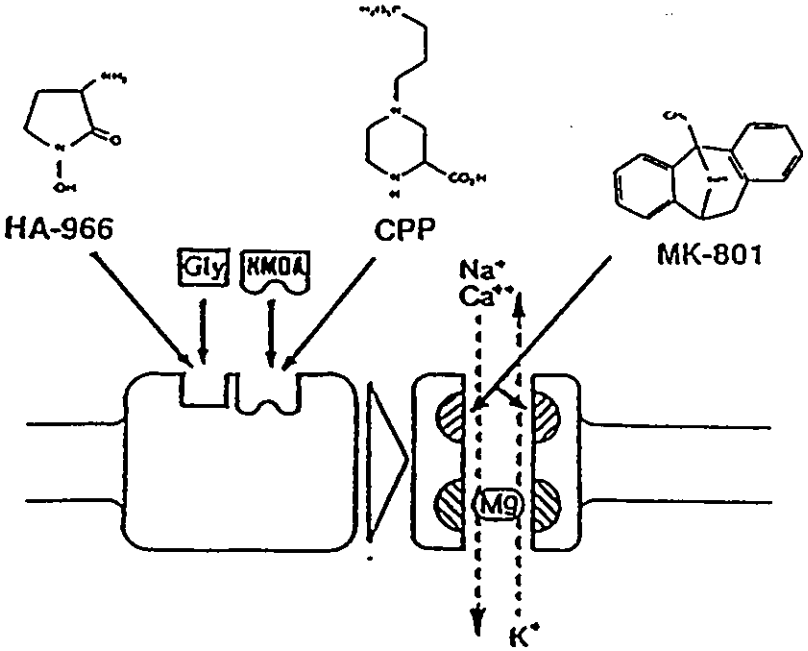
Figure 2.9 Schematic of the known subtypes of glutamate receptors. From Choi DW. 1988. Glutamate neurotoxicity and diseases of the nervous system. Neuron, 1, pg. 624.



Classes of Glutamate Receptors

One type of quisqualate receptor stimulates the formation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol-4,5-biphosphate (PIP_2); the other is linked directly to a Na^+ ionophore. Activation of the quisqualate receptor-ionophore complex can be potentiated by Zn^{2+}. The NMDA receptor opens a channel permeable to Ca^{2+} as well as Na^+; this receptor-channel complex has several modulatory sites discussed in the text. The kainate receptor opens an ionophore permeable to Na^+.

Figure 2.10 Detailed schematic of the NMDA receptor. From McDonald JW and Johnston MV. 1990. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. Brain Research Reviews, 15, pg 43.



This diagram outlines current information about the components of the NMDA receptor/channel complex based on biochemical and electrophysiological evidence. The NMDA recognition site is coupled to a cationic channel that is permeable to both Ca^{2+} and Na^{+}. A glycine modulatory site (distinct from the classical inhibitory glycine site) is closely associated with the NMDA receptor; glycine is required for channel activation and enhances NMDA responses. Mg^{2+} blocks the channel in a voltage-dependent manner; at relatively negative membrane potentials Mg^{2+} blocks the channel and blockade is relieved by depolarization. Thus NMDA receptor channel activation requires both NMDA and glycine receptor activation and concomitant membrane depolarization. Also, Zn^{2+} reduces NMDA responses by binding to a site on the external face of the membrane (not illustrated). NMDA responses can be blocked pharmacologically in at least 3 ways: competitive NMDA antagonists, such as CPP, compete for binding at the NMDA recognition site; competitive glycine receptor antagonists, such as HA-966, block the glycine site and reduced NMDA mediated responses; and non-competitive NMDA receptor antagonists such as MK-801 and dissociative anesthetics bind within the ionophore to the PCP receptor and prevent ion fluxes.

(Greenamyre et al., 1985). The second, by Young et al. (1988), found that NMDA receptor binding was reduced by 93% in HD putamen, when compared to controls (the magnitude of this decrease was greater than that for the other receptor bindings sites). Cortical binding levels were unchanged. Young et al. (1988) are careful to indicate the although this finding may be suggestive of QUIN overproduction and a preferential loss of neurons bearing NMDA receptors, other explanations must be considered. Albin et al. (1990) have also noted a 50% decrease in NMDA receptor density in the putamen of a post-mortem case study of a patient belived to have been at risk for HD. However, while Dure et al. (1991) have found significant decreases of NMDA receptor binding in HD caudate, decreases of similar magnitude were also found for binding levels of other glutamate receptor subtypes.

To date, the involvement of QUIN in the pathogenesis of HD is far from clear. Investigations have been hindered by technical difficulties associated with the assay as well as small sample size, the absence of pathological grading of cases, and the general rarity of HD patients. Although the majority of those few studies have not provided unequivocal support for the involvement of QUIN in HD, it is important, as indicated in every paper on the subject, as well as a recent review by Bruyn and Stoof (1990) to consider possible mechanisms of action of QUIN that might contribute to pathogenesis but which would still be in keeping with the observed findings. Certainly, one potential explanation may be that the neuronal degeneration in HD is the result of transient increases in QUIN. In this regard, it would be very interesting to investigate QUIN levels in patients at-risk for HD. A second possibility is that prolonged exposure to only slightly increased levels of QUIN over long periods gives rise to HD. This idea has been supported by the recent study which demonstrated that cell cultures of the corticostriatal system or caudate

exposed to 100 micromoles of QUIN for periods up to seven weeks produced gradual neuronal deterioration (Whetsell and Schwarcz, 1989). Given the inter-patient variability associated with QUIN levels, nonsignificant increases in QUIN levels would be difficult to detect in HD post-mortem brain.

VII. Animal Models of Huntington's Disease: Evaluation in relation to the pathologic state

The creation of striatal lesion via intrastriatal injection of a given excitotoxin is the most popular and most widely used animal model of Huntington's Disease. Prior to the discovery of kainic acid, electrolytic striatal lesions were used, but the nonselective damage produced was regarded as a primary disadvantage, and thus limited the utility of this model.

Kainic Acid-Kainic acid, a rigid analogue of glutamate, was isolated from the seaweed Digenea simplex in 1953. The unisolated product had been used in the Oriental pharmacopeia for many years previous, and attempts to further purify the seaweed extract led to the isolation of kainic acid (Takemoto, 1978).

The use of kainic acid in the creation of an animal model of Huntington's Disease (in rats) was first reported in 1976, both by Coyle and Schwarcz (1976) and by McGeer and McGeer (1976). The primary advantage of kainic acid was perceived to be the following: unlike electrolytic lesions, it produced selective damage in that intrastriatal injections of KA were thought to destroy striatal neurons while sparing axons which terminate in the striatum or fibre bundles passing through the striatum (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). Neurochemical analysis of KA-lesioned striata revealed reductions in striatal GABA, GAD, and choline acetyltransferase, all markers of striatal neurons, accompanied by preservation or increases in levels of tyrosine hydroxylase or dopamine, contained in the terminals of the nigrostriatal projection (Beal et al., 1985, 1986; Coyle and Schwarcz, 1976; McGeer and McGeer, 1976; Schwarcz and

Coyle, 1977; Coyle et al., 1978). The advent of radioimmunoassays for the neuropeptides has revealed that kainic acid intrastriatal injections also produce significant depletions of substance P, somatostatin and neuropeptide Y (Beal et al., 1985, 1986, 1987). In essence, kainic acid injections into the striatum appear to destroy the medium spiny and medium aspiny populations of striatal neurons and deplete levels of associated transmitters, namely GABA, substance P, somatostatin and neuropeptide Y; whether the large aspiny cholinergic neurons are destroyed is less certain. The depletion of GAD, GABA, substance P and ChAT, with preservation of tyrosine hydroxylase parallels the pattern of pathology seen in HD. However, the parallel between the kainic acid model and HD is not maintained when one considers the data obtained from radioimmunoassays and immunocytochemistry for somatostatin and neuropeptide Y. In HD, the medium aspiny striatal neurons to which somatostatin and neuropeptide Y are colocalized (as well as the biochemical marker NADPH-d) are preserved in the face of marked striatal atrophy. These results underscore the limitation of the the kainic acid model, in which levels of somatostatin and neuropeptide Y are significantly decreased (Beal et al., 1986, 1987).

The behavioural consequences of kainic acid lesions followed a few years after the original reports. Rats treated with kainic acid were found to be impaired in a delayed alternation paradigm (Divac, Markowitsch, and Pritzel, 1978) as well as in the reliable alternation of speed in the late trials of an alternately reinforced runway task (Pisa et al., 1981). Anteromedial caudate lesions produced by kainic acid served to impair the acquisition and reversal of spatial position learning (Dunnett and Iversen, 1981). Kainic acid-lesioned rats were found to demonstrate marked

nocturnal hyperactivity (Fibiger, 1978; Mason and Fibiger, 1979a, 1979b; Sanberg et al., 1986).

The data on learned behaviours in kainic acid lesioned animals have provided some parallels between the model and the human disease. In a rare and extremely useful series of papers, Oscar-Berman and her colleagues have taken tasks used in the animal literature and applied them in studies involving human patients. Although the focus of her work has been Korsakoff's patients, groups of Huntington's patients have been included for purposes of comparison. In particular, she has found that patients with HD are impaired on the primate version of the delayed response task (Oscar-Berman et al., 1982); this is similar to the impairment found by Divac, Markowitsch and Pritzel (1978) in kainic acid-lesioned rats performing the rodent version of the same task. Additionally, HD patients have been reported to be impaired on tests similar to the delayed response test (e.g., Wisconsin Card Sorting Test, Stroop Test) which require changing behavioural responses in consideration of the changing demands of the task (Josiassen et al., 1983). While it is difficult to compare typical neuropsychological tests and those used with rodents, it is probably fair to suggest that there is some similarity in the cognitive strategy required for such tests as the Stroop/Wisconsin Card Sorting Test and the delayed alternation test that has been used in humans, primates and rodents; this provides another parallel between the kainic acid model and the disease state. Further, the impairment in speed alternation on a runway task has been interpreted as being analogous to the deficit in serial memory performance expressed by HD patients (Pisa et al., 1981).

One of the most important drawbacks of the kainic acid model is the

extrastriatal damage known to be caused by intrastriatal injection. The goal has always been to limit KA-induced damage to the striatum, but numerous studies have shown that intrastriatal injection of kainic acid will produce gross damage to many sites distal to the striatum (Köhler and Schwarcz, 1983; Schwarcz et al., 1989; Zaczek and Coyle, 1982; Zaczek, Simonton and Coyle, 1980). This is particuarly important when considering the neural substrate for the neuropsychiatric disturbance in HD. It becomes very difficult to attribute behavioural deficits observed in the KA model to the striatal lesion if gross degeneration is observed in areas such as the hippocampus, amygdala and cortex.

It is important to remember that analysis of the kainic acid model was limited by what was known about Huntington's Disease in the late 1970s. For example, in the 1970s, it was not yet known that medium aspiny neurons degenerate while the aspiny neurons are spared. The "shortcomings" of the kainic acid model are largely associated with these new findings. Regardless, the major drawback of the kainic acid model remains that of the marked extrastriatal damage after intrastriatal injection; this greatly limits the interpretation of the model.

Ibotenic acid-Ibotenic acid, another analogue of glutamate, is derived from mushrooms. The first isolation was extracted from Amanita strobiliformis, but today it is more commonly derived from Amanita muscaria and, to a lesser extent, Amanita pantherina (Takemoto, 1978). The use of ibotenic acid as an excitotoxin began in the 1980s; the primary investigators have been Isacson and his colleagues, who have given attention to the role of neural transplants in the ibotenic acid model.

The search for a new excitotoxin was generated by the recognition that kainic

acid injected into the striatum produces considerable extrastriatal damage; ibotenic acid became popular because it was initially thought (and is still widely believed) to produce only local damage--whether or not this is actually the case will be discussed in a later section. First reported in 1984, intrastriatal injections of ibotenic acid were found to produce severe depletion of the general neuronal population in the striatum as well as significant depletions in levels of glutamic acid decarboxylase (GAD) (Isacson et al., 1984, 1986). Further studies confirmed the findings of the first report as well as noting significant losses of choline acetyltransferase activity (ChAT), GABA, substance P, somatostatin and NPY (Isacson et al., 1985; Beal et al., 1985, 1986), while dopamine and vasopressin levels (i.e., markers for striatal afferents) were spared (Beal et al., 1986).

Rats with IBO-induced lesions of the striatum are impaired on the delayed-alternation task. Intrastriatal transplants will attenuate these deficits (Isacson et al., 1986). The place task version of the Morris Water Maze has revealed that animals with ibotenic acid-induced lesions of the medial striatum are impaired in the performance of this task (Whishaw et al., 1987). Apomorphine or methamphetamine treatment after unilateral ibotenic acid-induced striatal lesion produces marked rotational behaviour; a bias to the paw ipsilateral to the lesion when reaching for food with one paw and a drop in reaching success when both paws are used is also demonstrated after unilateral lesion (Dunnett et al., 1988). Further, hyperactivity of ibotenic-acid lesioned animals has been noted.

As with the kainic acid model, the ibotenic acid model parallels the human disease state in that both result in significant depletions in striatal GAD, GABA, ChAT, and substance P, while dopamine is spared (see above for appropriate HD

references). Similarly, ibotenic acid may result in impairments on the delayed alternation task, just as in HD (Oscar-Berman et al., 1982). However, as with the kainic acid model, administration of ibotenic acid results in the depletion of striatal somatostatin and NPY (Beal et al., 1986), which is not the case in HD. Further, although it is widely believed that ibotenic acid produces only local damage, there is reason to believe that this may not be the case (see Saji and Reis, 1987; Volpe and Baker, 1989).

Quinolinic acid-In 1983, it was discovered the the tryptophan metabolite quinolinic acid was capable of producing axon sparing lesions of the rat striatum. Further, neurochemical analysis revealed that intrastriatal injection of quinolinic acid produced dose-dependent decreases in GAD and ChAT as well as TOH (Schwarcz, Whetsell and Mangano, 1983). These similarities with the kainic and ibotenic acid models led to the further exploration of the consequences of intrastriatal injection of quinolinic acid. Beal et al. (1986) reported that intrastriatal injection of 240 nanomoles of quinolinic acid produced significant depletions of substance P and GABA while levels of somatostatin, neuropeptide Y, dopamine and vasopressin were no different from controls (as measured by radioimmunoassay and HPLC analysis). This finding begins to approximate the neurochemical finding of HD post-mortem tissue in which somatostatin and NPY levels are significantly increased; the major difference between the kainic/ibotenic acid models and the quinolinic acid model appears to be the depletion of neuropeptide Y/somatostatin in the former and the sparing of the same neurotransmitters in the latter.

Neuroanatomical analysis of the quinolinic acid model via immunocyto-

chemistry and histochemistry has proven to be more controversial. In particular, the controversy has focused around the sparing of the medium aspiny neurons (those in which somatostatin, NPY and NADPH-d are co-localized) after intrastriatal injection of quinolinic acid. One group of researchers has reported that these neurons are not spared (Davies and Roberts, 1987; Boegman et al., 1987; Boegman and Parent, 1988), while others have claimed the opposite (Beal et al., 1986, 1987, 1991a; Koh et et al., 1986; Koh and Choi, 1988).

The discrepancy may arise from the interpretation of the available data. Davies and Roberts (1987) and Boegman and Parent (1988) have based their reports upon the absence of neuronal sparing in the lesioned area directly surrounding the needle tract; however Boegman and Parent (1988) have conceded that there is neuronal sparing in the perimeter of the lesion core, although the spared neurons appear abnormal. This is exactly the interpretation of Beal and his colleagues (1986, 1987, 1991b) (Davies and Roberts (1987) do not seem to have examined the area surrounding the core lesion area).

The behavioural consequences of quinolinic acid intrastriatal injection have been limited to the study of spontaneous nocturnal motor activity. Sanberg and his colleagues (Sanberg et al., 1989; Giordano et al., 1990) have suggested that rats with quinolinic intrastriatal injections demonstrate transient nocturnal hyperactivity for a very limited part (i.e., one hour) of the nocturnal testing session. While this may be the case, it is to be noted that nocturnal hyperactivity demonstrated by kainic and ibotenic acid-lesioned animals is considerably greater than that expressed by quinolinic acid lesioned animals.

Purpose of an Animal Model of a Disease

The ultimate aim in creating an animal model of a disease is to find a cure or preventive therapies for the disease. A more immediate goal is to gain better comprehension of the pathophysiology and pathogenesis of the illness. The adequacy of a given animal model may be evaluated in relation to its ability to mimic the clinical disorder. Once a model has been established, different variables may be manipulated to further understanding of the pathogenesis, and ultimately, to find preventive therapies. Other benefits, such as a better understanding of underlying neural mechanisms, may also arise in the creation and study of an animal model. The accuracy of any model is limited by the extent of the existing body of knowledge concerning the disease as well as the existing technology. A perfect representation is an uncommon occurrence, but this should not be a barrier to research; imperfect models are bound to precede closer representations. A given animal model should be evaluated in as many ways as possible, in order that the strengths and weaknesses of the model may be recognized. This recognition may be particularly important when it comes time to evaluate potential therapies for humans suffering from the disease.

Rats with quinolinic acid-induced striatal lesions have been proposed as an animal model of HD. I have attempted to evaluate this model in several ways, using behavioural, neuroanatomical and neurochemical techniques.

CHAPTER 3

BEHAVIOURAL ANALYSIS OF QUINOLINIC ACID-INDUCED LESIONS IN RATS:

I. Spontaneous Nocturnal Activity After Intrastriatal Injection of Kainic Acid, Ibotenic Acid and Quinolinic Acid: A Comparative Study

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Introduction

Striatal lesion produced by intrastriatal injection of kainic acid was the first widely used model of HD. The suggestion that the intrastriatal kainic acid lesions might provide an animal model of HD had been generated by the discovery that intrastriatal injections of kainic acid into the rat striatum produced a biochemical and anatomical profile that was interpreted as analogous to the pathological state found in HD (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). At the time, the great advantage of kainic acid was that it was thought to spare axons of passage, thus offering a considerable advance over electrolytic lesions as far as selectively lesioning striatal neurons was concerned. The study of the behavioural characteristics of kainic acid lesioned animals came a few years later with the work of Fibiger and colleagues. One of the behavioural characteristics to be noted was that animals lesioned with kainic acid were significantly hyperactive in relation to controls. Because of a perceived similarity in behavioural response, it was suggested that the hyperactivity in rats might be analogous to the motor abnormalities in HD patients (Mason and Fibiger, 1979a; Fibiger, 1978; Sanberg et al., 1986; Giordano et al., 1988).

Uncertainties over the limitations of kainic acid lesions (particularly the discovery that intrastriatal injections of kainic acid consistently produced damage in extrastriatal structures) led to the search for new excitotoxins. In the early 1980s, ibotenic acid was reported to have the same local degenerative effects as kainic acid, but with the reported advantage that there was no apparent damage distal to the site of injection (Guldin and Markowitsch, 1981, 1982; Köhler and Schwarcz, 1983). Again, it was found that intrastriatal injections of ibotenic acid produced a pattern of hyperactivity that was most marked at night and under a regimen of food deprivation (Isacson et al., 1984, 1986). Additionally, it was once again suggested that this hyperactivity might be considered analogous to the motor abnormalities of HD (Isacson et al., 1986). This suggestion seems to have gone largely unchallenged, due, at least in part, to the difficulty in comparing human and animal behaviours.

The excitotoxin used most recently in the creation of an animal model of HD is quinolinic acid, a metabolite of the tryptophan pathway, and therefore endogenous to the mammalian central nervous system. Based on the hyperactivity profiles described previously for the KA and IBO models, I initiated a series of experiments to determine whether or not rats with quinolinic acid-induced striatal lesions were spontaneously hyperactive. At the time, my interest was in using a graft to ameliorate any hyperactivity. However, the preliminary experiments did not reveal any differences between control and QUIN-lesioned animals where spontaneous nocturnal activity was concerned, and as a result, the present study has taken a different turn. The present

experiments were initiated to replicate the reports of previous experimenters who had reported hyperactivity after KAIN- and IBO-induced striatal lesions, examine these animals in relation to a cohort of QUIN-lesioned animals, and to follow the spontaneous activity profiles over a prolonged testing period.

Methods and Materials

Surgery

Experiment 1. Male, Sprague-Dawley rats (200-250 g; Charles-River, Quebec) were housed in plastic cages with free access to food and water in a room with a 12h:12h light:dark cycle. At the time of surgery, the rats were anaesthetized and placed into a Kopf small animal stereotaxic apparatus. All rats received unilateral injections of one microlitre of vehicle or excitotoxin into the right hemisphere at the following co-ordinates: A/P +0.7; M/L 2.5; D/V 5.5 (from skull). The vehicle and excitotoxins were delivered via a 10 ul Hamilton syringe (Hamilton Company, Reno, NV) fitted with a 30 gauge blunt-tipped needle.

The first group of eight rats received 0.9% saline (CONT), the second group received 10 nm of kainic acid (KAIN) and the third group received 240 nm of quinolinic acid (QUIN) (both excitotoxins were delivered in one microlitre of solution). Following the protocols of previous researchers, the saline and QUIN were delivered at a rate of one ul per minute (i.e., Beal et al., 1986); the KAIN was delivered at 0.25 ul per minute (Fibiger, 1978).

Experiment 2. The second experiment was similar to the first, but also characterized the long-term effects of ibotenic acid-induced striatal lesions. Surgical procedures were similar to that of Experiment 1. Animals were injected unilaterally at the following co-ordinates: A/P: +0.2; M/L 2.7; D/V 6.0, with one ul of vehicle (0.9% saline) or one of three excitotoxins: 10 nm of KAIN, 240 nm of QUIN or 190 nm of ibotenic acid (IBO) were delivered at a rate of one ul per minute. (All excitotoxins, purchased from Sigma, were mixed with 0.9% saline and brought to pH 7.4 with NaOH.)

Testing Protocol All animals were allowed one week recovery time before behavioural testing. Prior to the commencement of a testing session, all animals were food deprived for 24 hours, following the procedures of previous researchers (Isacson et al., 1986). Testing consisted of measuring various components of spontaneous nocturnal motor activity via the Digiscan Animal Activity Monitors (Model RXYZCM(16), Omnitech Electronics, Columbus, OH). Each monitor consisted of an acrylic cage (41.5 cm^2, 30 cm deep) surrounded by two tiers of photocells which monitored the animal's activity. The data was processed by the Digiscan Analyzer connected to a microcomputer.

All animals were tested through the late afternoon and evening (4pm-8am); each testing period consisted of 16 hours (which was further divided into 32 half hour samples), the last twelve of which were in the dark. A selected group of samples (10 pm-4am) was chosen for statistical analysis. Each animal was tested over a period of up to thirty weeks.

Histology. One year post-surgery, the animals in both experiments were sacrificed by intracardial perfusion of 0.9% saline followed by 4% paraformaldehyde. Brains were post-fixed overnight in 4% paraformaldehyde and sunk in 30% sucrose. Each brain was cut, using a cryostat, in coronal sections (40 um) through the striatum; sections were collected on chrom-alum subbed slides. In order to analyze the size of the remaining striatal tissue, the area of the striatum in all groups was measured. To do this, sections at 3 coronal planes were selected (A/P +1.7, +1.2, -0.3); these sections were expanded and traced using a Bausch and Lomb projector. The striatum was measured according to the boundaries defined in figure 3.1. Striatal area was assessed using a Bioquant System IV software package (R and M Biometrics, Nashville, Tennessee). The individual tracings were placed on a a Hipad Digitizer software tablet (Houston Instruments, Austin, Texas) connected to an IBM Personal Computer. Area was measured using a cursor (Houston Instruments) attached to the software tablet. For each individual structure, two measurements were taken and the mean of the two was calculated.

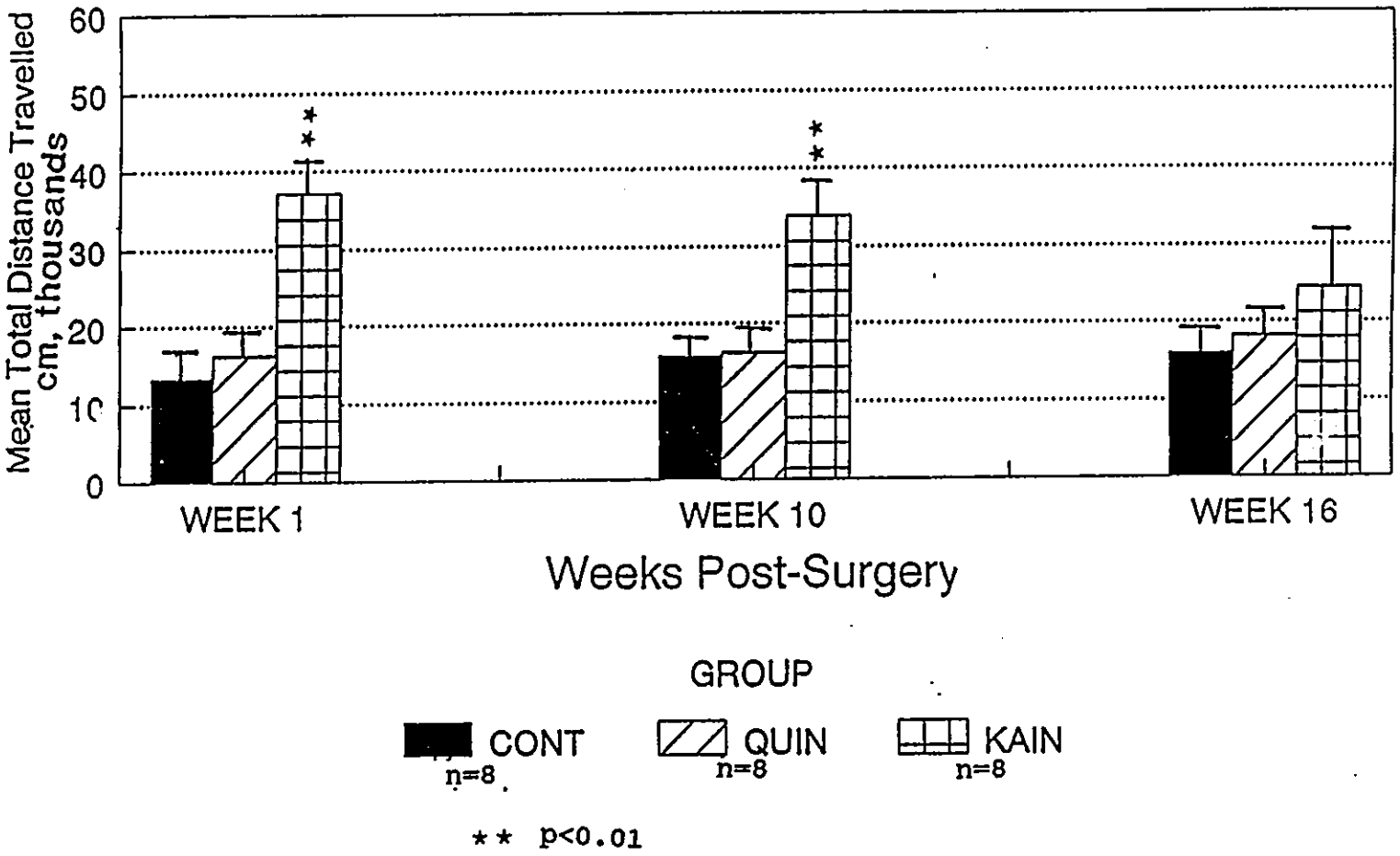
Results

Experiment 1. Statistical analysis revealed that significant nocturnal hyperactivity, as measured by the dependent variable total distance travelled was present in the KAIN group (refer to figure 3.2) at one week post-surgery ($F(2,18)=18.570$, $p<0.001$) and persisted for at least ten weeks ($F(2,18)=12.523$, $p<0.01$) post-surgery. At sixteen weeks post-surgery, and thereafter, there was an attenuation of hyperactivity such that

Figure 3.1 Spontaneous Nocturnal Activity, experiment 1. Mean total distance travelled.

Experiment 1:

Mean Total Distance Travelled and S.E.s



the KAIN animals were no different from the other two groups. A similar pattern was present for the dependent variable number of movements (the number of discrete horizontal movements separated by breaks of one second or more); hyperactivity was present in the KAIN group at the commencement of testing ($F(2,18)=21.370$, $p<0.001$) and up to ten weeks post-surgery ($F(2,18)=3.813$), $p<0.05$), but was not present at sixteen weeks or thereafter (figure 3.3). Where average speed was considered, no group differences were found. At no time did the QUIN group differ from the CONT group.

Experiment 2. As in Experiment 1, KAIN-induced hyperactivity, as measured by total distance travelled (figure 3.4), was present immediately ($F(3,23)=4.254$, $p<0.01$); post-hoc analysis revealed that only the kainate group was significantly different from the control group. In contrast to experiment 1, this hyperactivity disappeared after one week of testing. This pattern was also reflected for number of movements (figure 10) which demonstrated significance at the commencement of testing ($F(3,23)=4.807$, $p<0.01$); only the KAIN group was significantly different from controls. IBO-lesioned animals were not hyperactive immediately; a trend for total distance travelled was present at 7 weeks post-surgery ($p=0.06$) and significance was achieved at 12 weeks post-surgery for total distance travelled ($F(3,23)=10.521$, $p<0.01$). This hyperactivity also disappeared by 30 weeks post-surgery, at which time the four groups were indistinguishable from each other. As in Experiment 1, QUIN animals failed to demonstrate significant hyperactivity at any time. Where the variable number of movements was concerned, only the KAIN group demonstrated hyperactivity

immediately after surgery ($p<0.01$); this disappeared by the second week post-surgery. IBO- and QUIN-lesioned animals demonstrated trends towards hyperactivity, but significance was never achieved. Again, when average speed was considered, no group differences were found (figure 3.5).

Histology. Histology confirmed the presence of striatal lesion in all the experimental groups; the control animals demonstrated no sign of damage (refer to figures 3.6 to 3.9). The analysis for striatal area showed that the striatal lesion was somewhat bigger in the QUIN group than the other two experimental groups (tables 3.1-3.4).

Discussion

These experiments have accomplished the following: 1) replicated the findings of previous researchers as a result of noting nocturnal hyperactivity in rats with KAIN- and IBO-induced striatal lesions; 2) observed that this hyperactivity develops and attenuates over a period of several months; and 3) noted that intrastriatal injection of quinolinic acid in rats does not produce significant nocturnal hyperactivity within the testing periods utilized. The last two points (that of development and attenuation of hyperactivity in the KAIN- and IBO-lesioned animals and absence of hyperactivity in QUIN-lesioned animals) have not, to the best of my knowledge, been previously reported. Histological analysis has confirmed the presence of striatal lesions in the experimental groups.

Figure 3.2 Spontaneous Nocturnal Activity, experiment 1. Mean number of movements.

Experiment 1

Mean Number of Movements and S.E.s

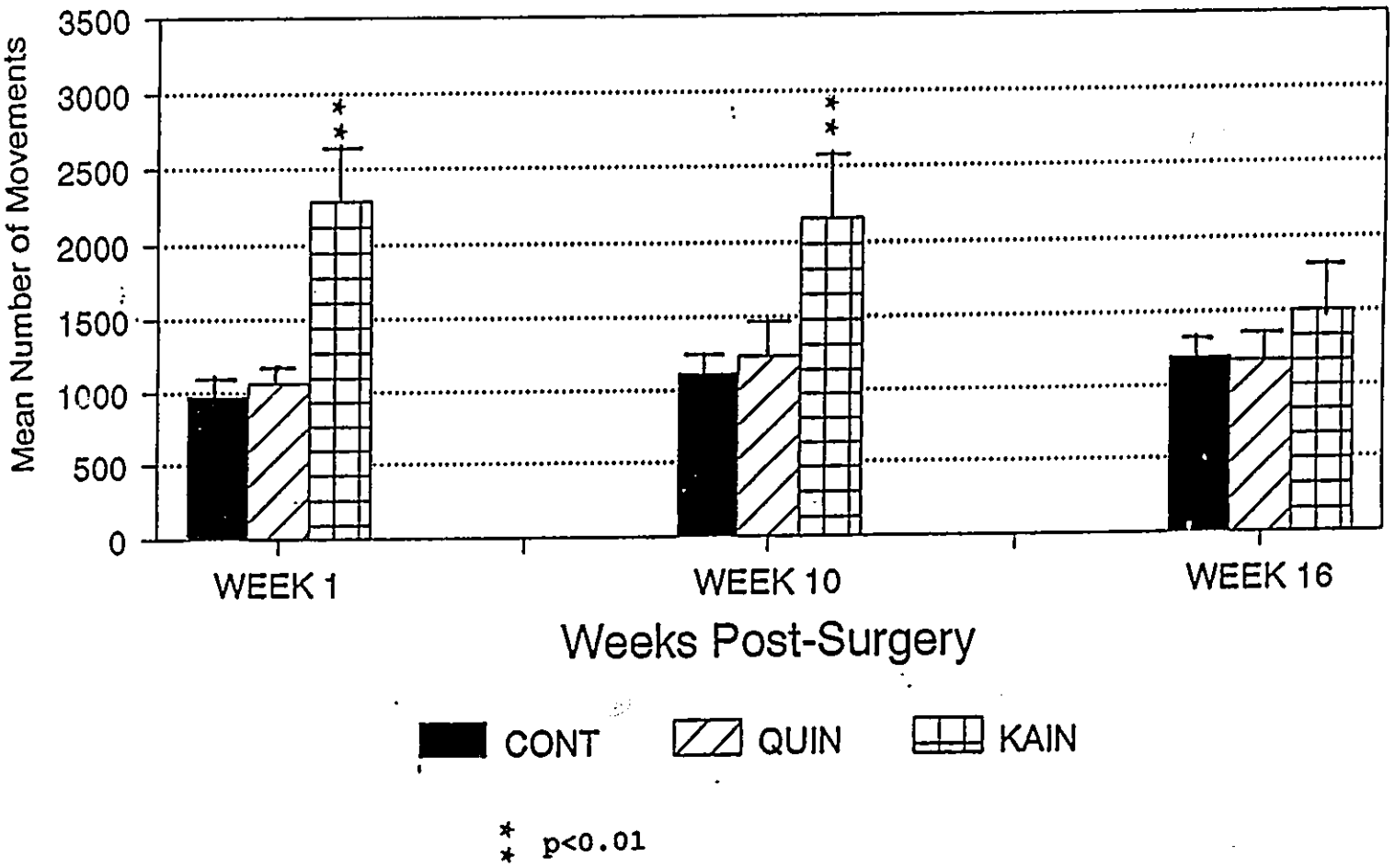


Figure 3.3 Spontaneous Nocturnal Activity, experiment 2. Mean total distance travelled.

Experiment 2

Mean Total Distance Travelled and S.E.s

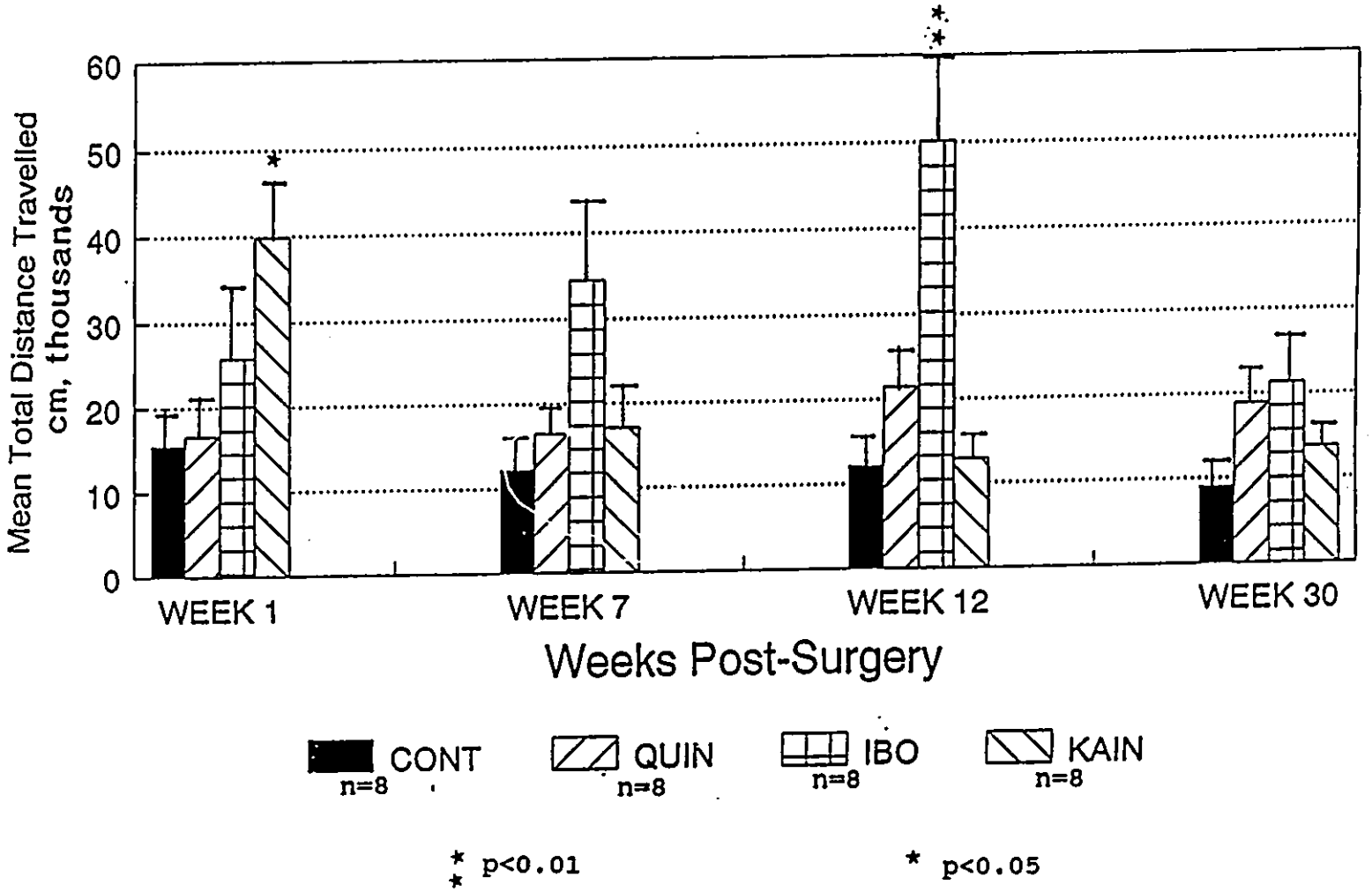


Figure 3.4 Spontaneous Nocturnal Activity, experiment 2. Mean number of movements.

Experiment 2

Mean Number of Movements and S.E.s

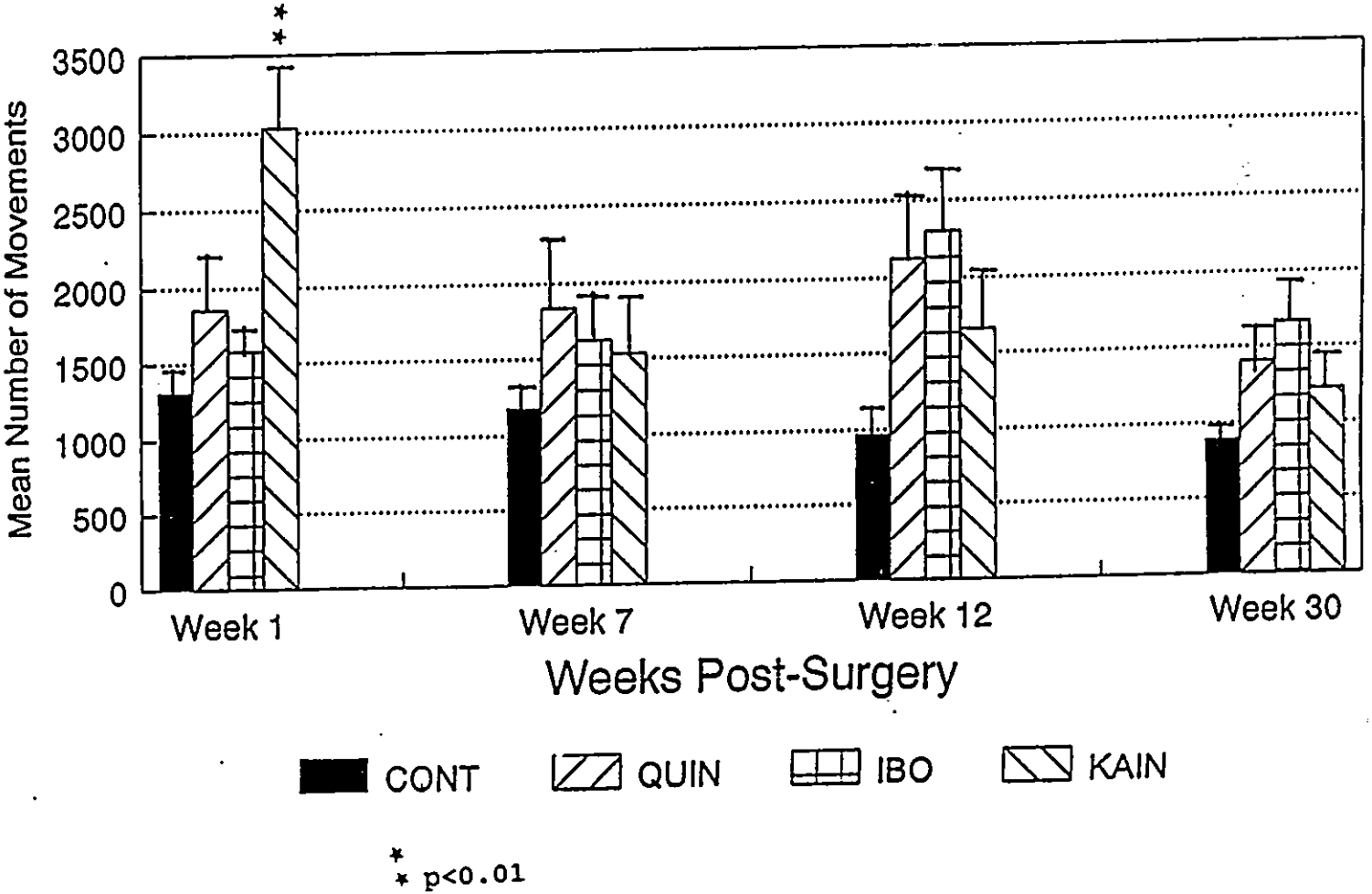


Figure 3.5 Controls, Nissl stain

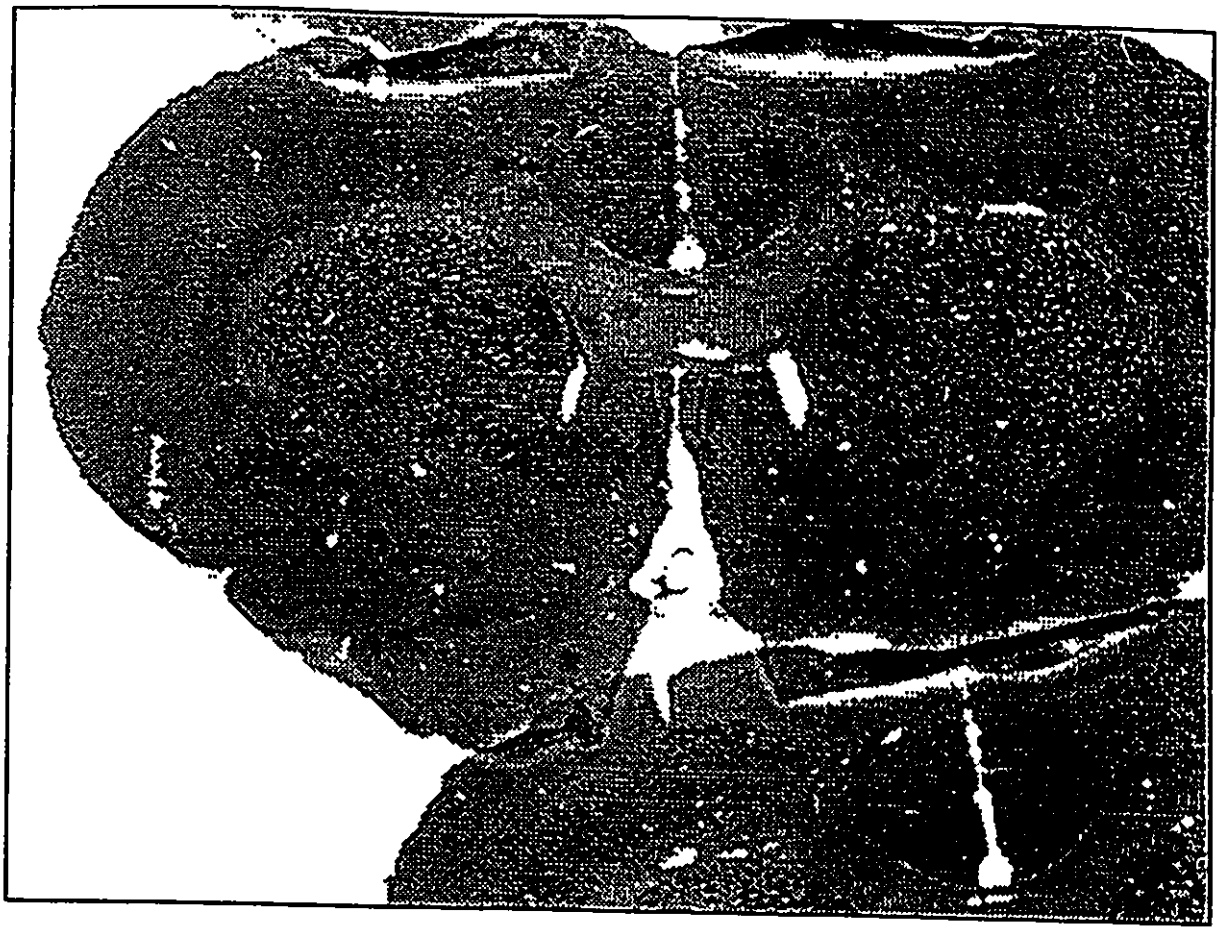


Figure 3.6 Unilateral kainate-induced striatal lesion, sacrificed one year post-surgery. Representative section.

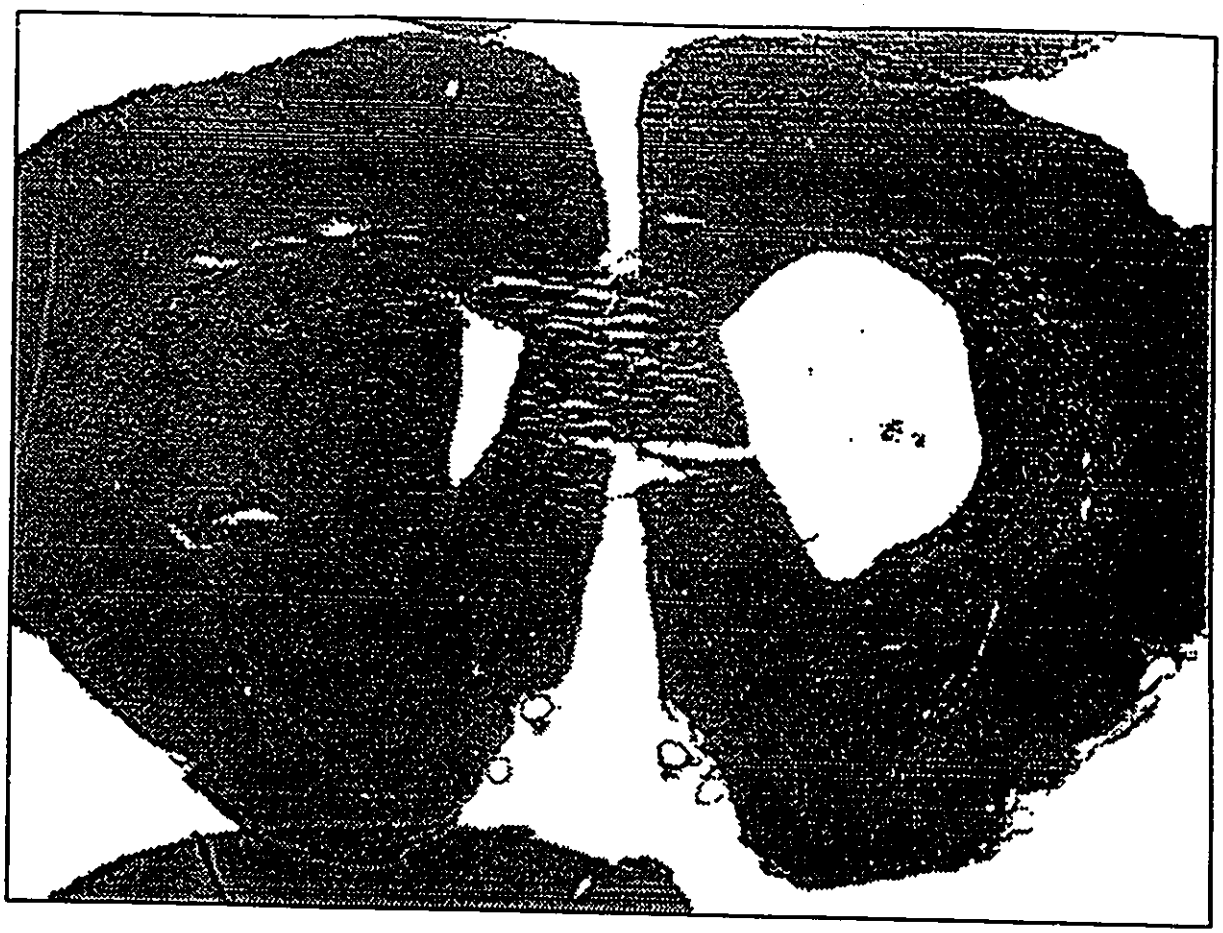
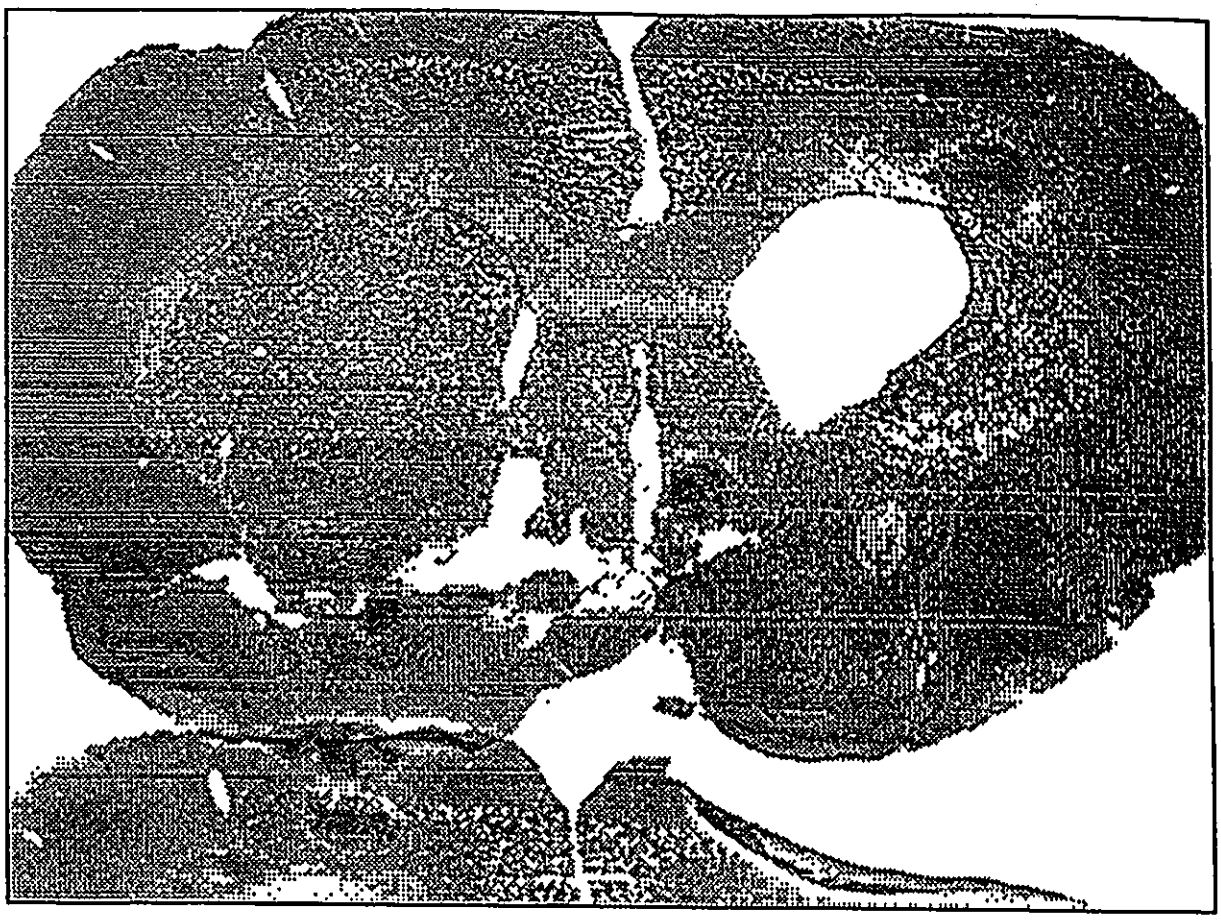
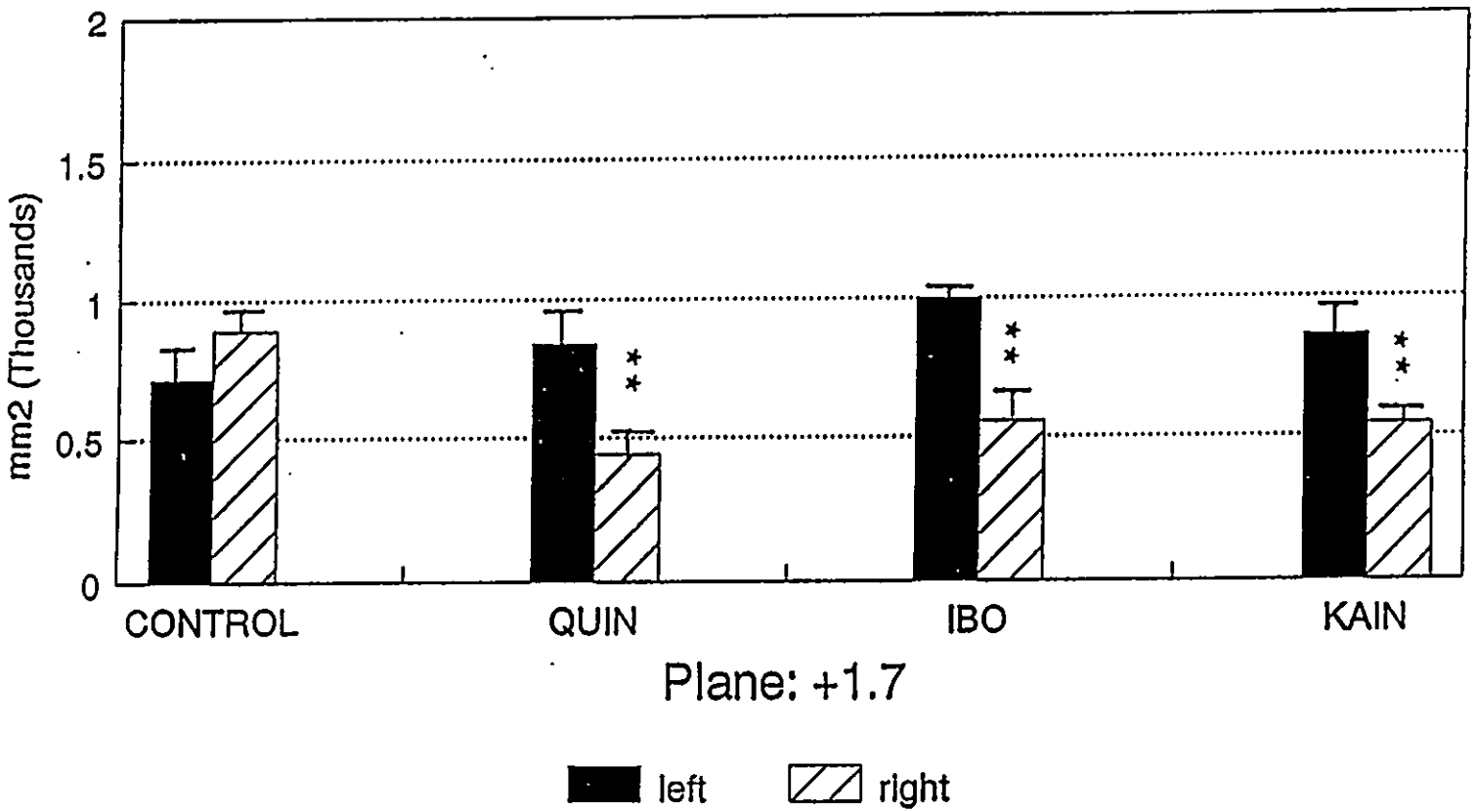


Figure 3.7 Unilateral ibotenate-induced striatal lesion, sacrificed one year post-surgery. Representative section.



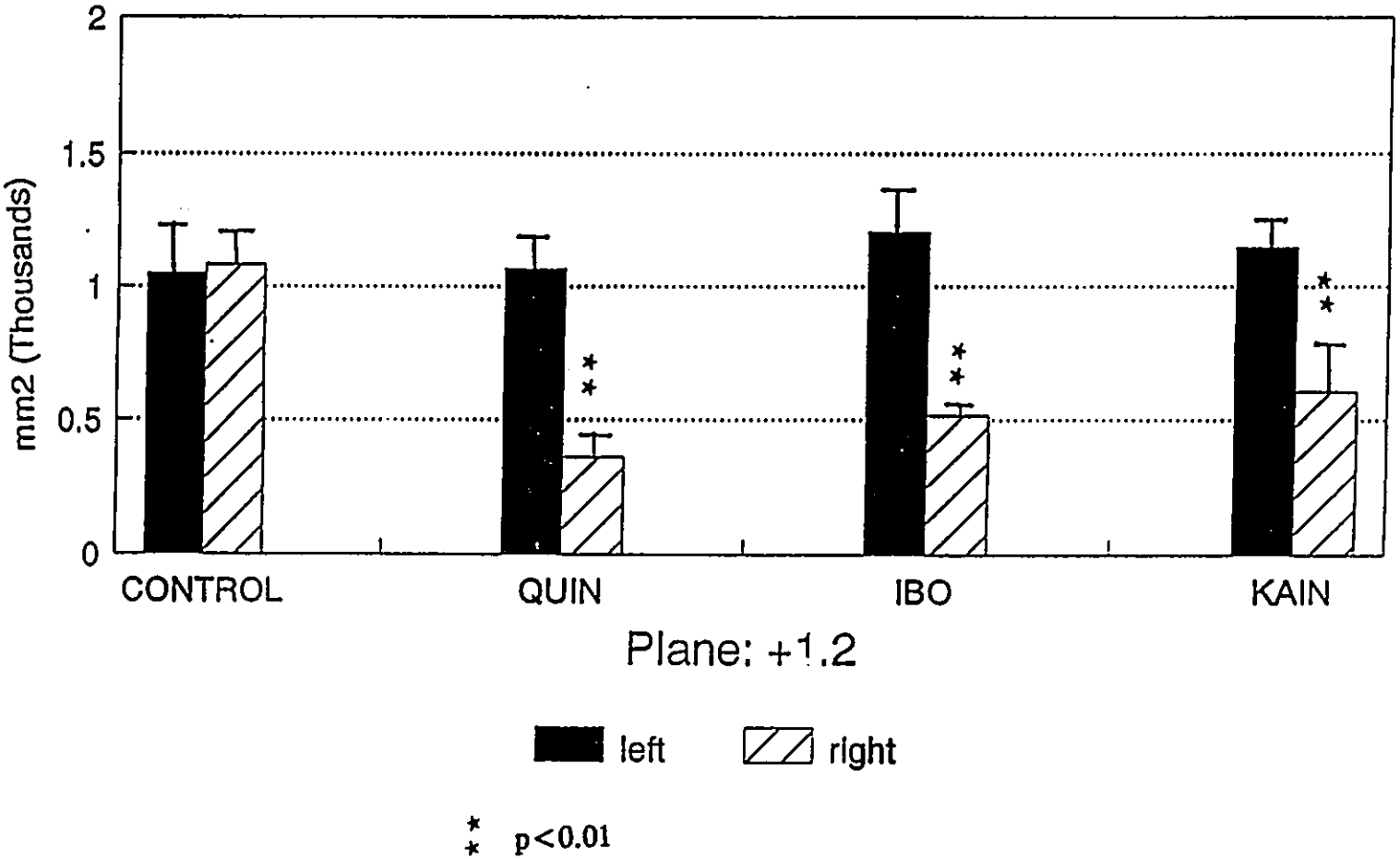
Figures 3.9-3.11 Graphic description of striatal area in CONT and three experimental groups.

Morphometry: Striatum

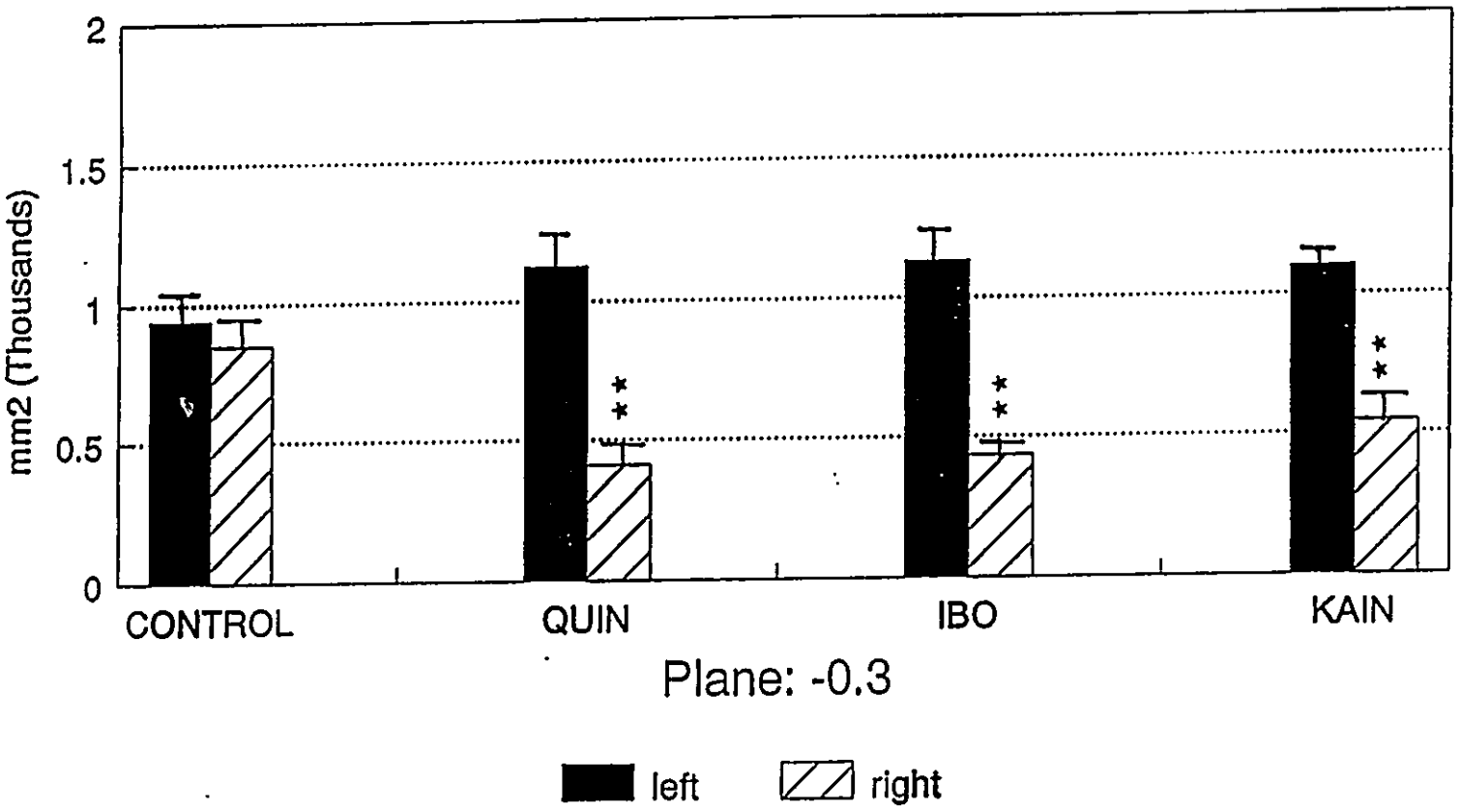


* * p<0.01

Morphometry: Striatum

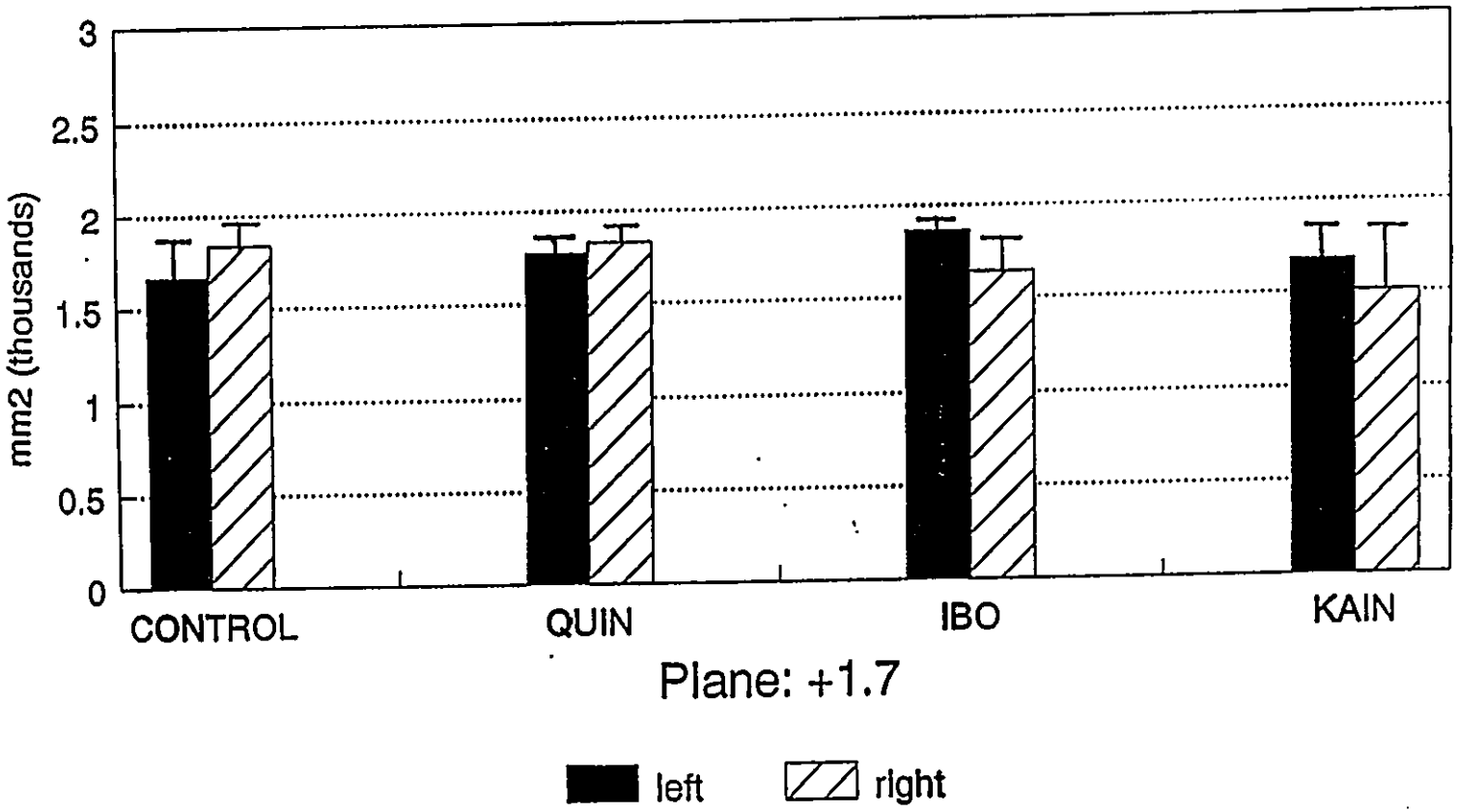


Morphometry: Striatum

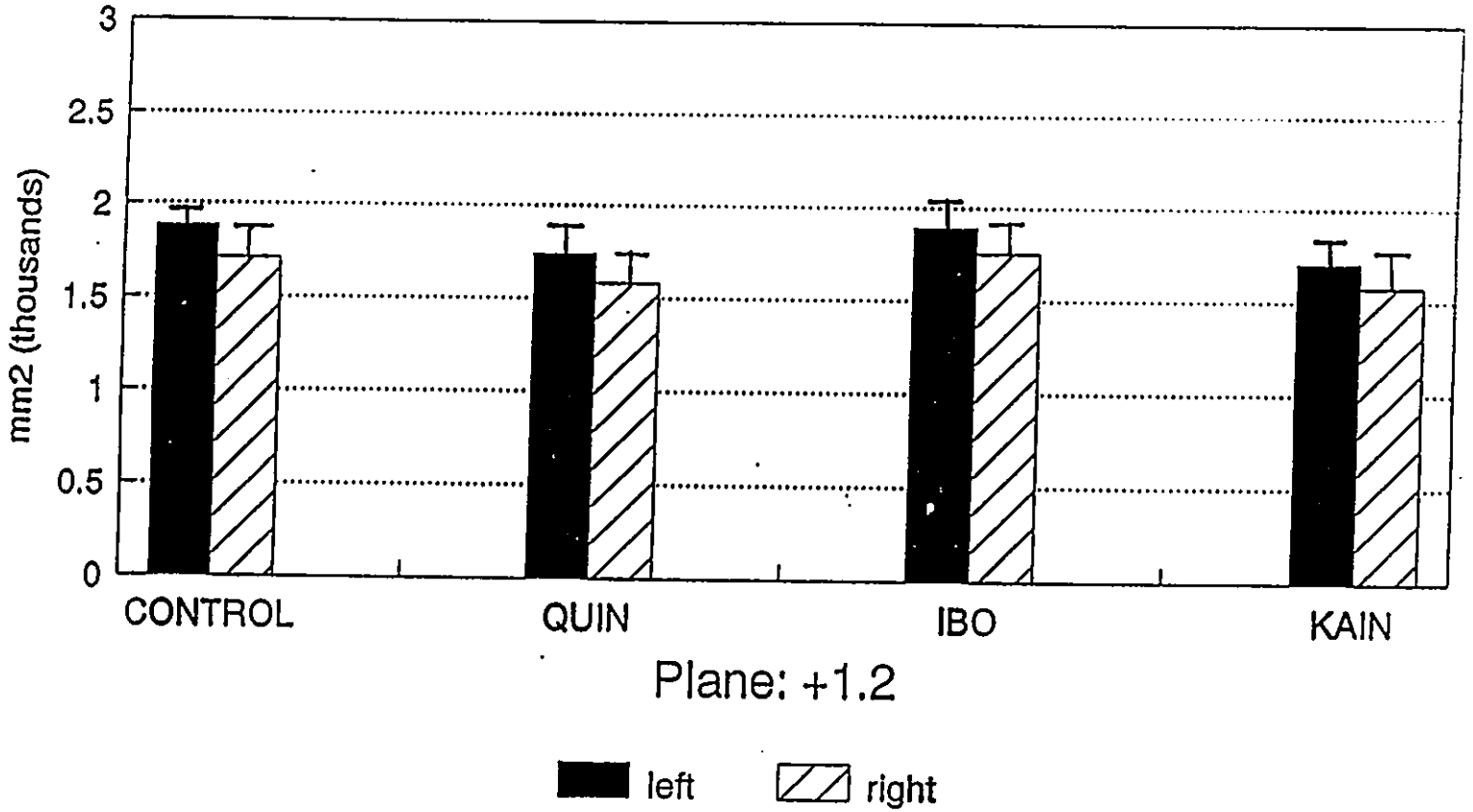


*
* $p<0.01$

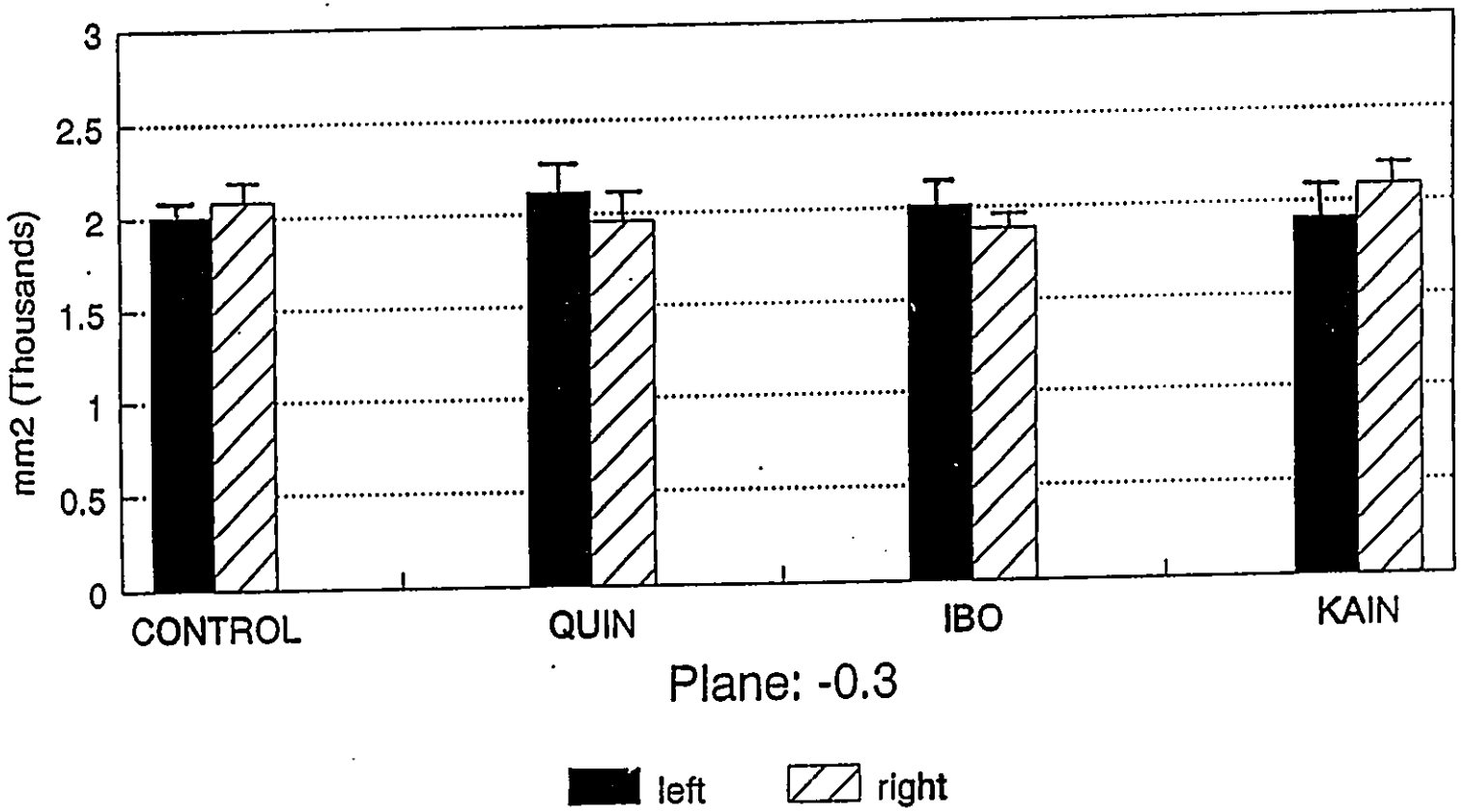
Morphometry:Cortical Area



Morphometry: Cortex



Morphometry: Cortex



Tables 3.1-3.4 Striatal area and percent lesion (right/left) in CONT and three experimental groups.

Group

CONT

Plane	Striatal area Hemisphere left	right	%lesion (r/l)
+1.7	713.84	891.15	125
	25.9	55.62	
+1.2	1051.3	1083.3	103
	175.2	21.9	
-0.3	940.3	849.4	90
	103.2	82.4	

Mean area (mm2) and associated SEs

Group

QUIN

Plane	Striatal area Hemisphere left	right	(r/l)
+1.7	839.9	441.2	53
	81.9	67.01	
+1.2	1067.7	362.22	34
	90.4	117.4	
-0.3	1125.4	410.9	36
	73.9	70.1	

Mean area (mm2) and associated SEs

Group

IBO

Plane	Striatal area Hemisphere left	right	(r/l)
+1.7	1000.5	555.71	63
	44.1	92.2	
+1.2	1207.7	516.1	53
	63.4	45.8	
-0.3	1131.1	434.9	49
	68.9	58.5	

Group

KAIN

Plane	Striatal area Hemisphere left	right	% lesion (r/l)
+1.7	862.01	541.8	56
	52.9	62.1	
+1.2	1149.9	608.7	43
	55.9	119.8	
-0.3	1101.6	543.2	38
	41.6	85.3	

Quinolinic acid has been reported to produce a striatal lesion that is better reflective of the neuropathological profile of HD than kainic acid lesions (e.g., Beal et al., 1986, 1989). (Ibotenic acid lesions have not been characterized in as much detail.) This knowledge, coupled with the finding from the present study that QUIN-lesioned animals were not significantly different from controls in their activity profiles, would seem to cast some doubt on the analogy that has been drawn between the motor abnormalities of HD and the nocturnal hyperactivity observed in animals with striatal lesions produced by kainic acid or ibotenic acid. Specifically, it may be suggested that if hyperactivity were truly analogous to HD motor abnormalities, then hyperactivity should have been present in animals lesioned with quinolinic acid, given that quinolinic acid produces a profile closer to that of the human pathological state.

Neural Mechanisms of Spontaneous Hyperactivity

When considering potential neural mechanisms underlying hyperactivity in KAIN and IBO-lesioned rats, it is important to consider whether the hyperactivity induced by intrastriatal injection of KAIN and IBO is the result of local striatal degeneration or damage to extrastriatal areas. It has long been recognized that intrastriatal administration of KAIN produces marked extrastriatal damage (Köhler and Schwarcz, 1983; Zaczek, Simonton and Coyle, 1980; Schwob et al., 1980) and doubts have been raised over the extent to which axons of passage are spared (Mason and Fibiger, 1979b; Schwob et al., 1980). It is generally acknolwedged that IBO does not cause as much extrastriatal damage as KAIN (Guldin and Markowitsch, 1981, 1982; Köhler and

Schwarcz, 1983; Schwarcz et al., 1979). It was initially believed that IBO-induced neuronal damage was limited to the injection site, but more recent work has shown that intracerebral injections of ibotenic acid do indeed produce damage distal to the injection site: Saji and Reis (1987) and Pasinetti et al. (1991) have both reported that intrastriatal injection of ibotenic acid in rats produces neuronal degeneration in the substantia nigra, and Volpe and Baker (1989) have found that injections of ibotenic acid into the anterior dorsal hippocampus produces a significant loss of choline acetyltransferase positive cells in the septum. The discrepancy in findings between these two studies and those which have reported the absence of extrastriatal damage following intrastriatal ibotenic acid injection may be related to time to sacrifice. With the exception of Schwarcz et al. (1979), all the studies which have failed to find extrastriatal damage (Guldin and Markowitsch, 1981, 1982; Köhler and Schwarcz, 1983) had short postsurgical survival times (not longer than 10 days); in the single study (Schwarcz et al., 1979) that permitted long post-surgical survival time (e.g., 1 year), the sample size was very small (n=2) (all other animals in this study were sacrificed at 10 days post-surgery). By contrast, thosed studies that have reported extrastriatal damage after intrastriatal excitotoxin-induced lesion have permitted longer post-surgical survival times. For example, Saji and Reis (1987) sacrificed their animals up to 21 days post-surgery, Pasinetti et al. at three months post-surgery, and Volpe and Baker (1989) sacrificed their animals up to four months post-surgery. Thus, the absence of extrastriatal effects after intrastriatal excitotoxin injections which have been reported by some researchers are possibly more apparent than real; longer post-surgical

survival times after intrastriatal excitotoxin injection will probably reveal damage to extrastriatal areas.

(At first glance, the presence of extrastriatal damage after intrastriatal injection might seem to be a serious drawback to a putative model of HD, given that the intention has always seemed to be one of limiting damage to local areas. However, one must consider that neuronal damage in Huntington's Disease is not limited to the striatum; as described in the literature review, it has been known, for at least seventy years, that the neural degeneration in Huntington's Disease also involves the cortex, globus pallidus, and substantia nigra as well as the caudate and putamen (Vonsattel et al., 1985; Bruyn et al., 1979). Detailed studies by Vonsattel and his colleagues have reported additional degenerative changes of a lesser magnitude in the white matter, hippocampus, amygdala and thalamus accompanied by progressive ventricular dilation (Vonsattel et al., 1985, 1987; de la Monte et al., 1987, 1988). These neuroanatomical studies have been complemented by the many neurochemical studies of post-mortem tissue which have found changes in neurotransmitter levels in extrastriatal areas when compared to controls. Thus, although this remains to be fully investigated, the extrastriatal damage produced by excitotoxins such as ibotenic acid may prove to be a serendipitous feature of the models.)

As for the question of whether hyperactivity induced by intrastriatally-injected kainic/ibotenic acid is the result of local (i.e., striatal) or extrastriatal damage, my data from the QUIN-lesioned animals would suggest that a striatal lesion alone is insufficient to produce hyperactivity. Nevertheless, nocturnal motor activity has been

used as an index of the ability of embryonic striatal transplant to restore normal function. When run in parallel with a sham-lesioned control group and an excitotoxin lesioned control group, the lesion-plus-transplant group has been shown to be no different from controls, while the rats in the lesion-only group were significantly hyperactive (Isacson et al., 1984, 1986; Giordano et al., 1988; Sanberg et al., 1986). This amelioration of hyperactivity as a result of transplantation has been attributed to establishment of neuronal connections between the transplant and the host striatum. Anatomical study of these grafts has shown that the implanted tissue resembles normal striatal tissue in terms of gross appearance as well as more subtle aspects such as striosome/matrix organization (Isacson et al., 1987a; DiFiglia et al., 1988; Roberts and DiFiglia, 1988) and well-established neuronal connections have been reported between host striatum and transplanted striatum (Pritzel et al. 1986). However, a similar establishment of connections has been noted between the transplant and other striatal efferent and afferent target areas such as the globus pallidus, substantia nigra and cortex (Clarke et al., 1988; Pritzel et al., 1986; Srinathsinghji et al., 1988; Wictorin and Björklund, 1989; Wictorin et al., 1988, 1989, 1990). Thus, whether the observed amelioration of hyperactivity is the result of the establishment of connections between host and transplant in the striatum or in other areas remains unresolved.

The use of diazepam may be useful in determining whether or not hyperactivity arises from extrastriatal or striatal damage after striatal excitotoxin injection. Ben-Ari and his colleagues (1979) have reported that diazepam treatment prior to kainic acid injections into the amygdaloid nucleus will produce local damage similar to that found

in controls; however, hippocampal damage was attenuated in the diazepam-treated animals while it was present in the kainic acid-only animals. Thus, treatment with diazepam seemed to produce a profile of attenuation of distant damage accompanied by maintenance of local damage. If the phenomenon is reliable and valid, diazepam may be useful in attenuating distal damage and thus determining the neural structures responsible for hyperactivity after excitotoxic lesion.

Attenuation of Excitotoxin-Induced Hyperactivity

Another finding of these experiments has been that kainic acid/ibotenic acid induced hyperactivity attenuates over time to the point where these animals are no different from controls in terms of nocturnal activity. To the best of my knowledge, this particular phenomenon has not been previously described. Consideration must be given to potential underlying neural mechanisms. It is possible that the observed attenuation is a property of unilateral striatal lesions and that with time, the unlesioned side somehow serves to restore normal behavioural function. However, even if this is the case, the question concerning the underlying neural mechanism remains unanswered.

One interesting possibility is that the observed attenuation of hyperactivity is the result of spontaneous recovery of the host. Such an occurrence has been reported, both within the clinical literature (i.e., recovery of function after brain damage) and the experimental literature. With respect to the latter, Kolb, Reynolds and Fantie (1988) have tested rats with frontal cortex lesions in the Morris Water Maze at two or twenty-

eight days following surgery and found that the longer post-surgical recovery period led to performance comparable to that of unlesioned controls. As stated by the authors, the mechanism for this effect is uncertain, and the general phenomenon is probably deserving of much more attention.

A recent paper by Roberts and DiFiglia (1990) also points to spontaneous regeneration after excitotoxic lesion. Chronic quinolinic acid-induced striatal lesions were shown to demonstrate increased synaptic density and reorganization of other neuronal elements over long periods of time (e.g., 30 weeks). The exact mechanism remains unknown, but it is possible that growth factors play a role in the apparent regeneration (Roberts and DiFiglia, 1990).

With the introduction of the technique of tissue transplantation, many researchers have shown that a given behavioural abnormality (i.e., rotation, hyperactivity) produced by a lesion in a given area of the brain can be relieved by transplantation of grafts from animals of embryonic age. The assumption has always been that it is interaction of the neurons contained within these grafts with the lesioned host that is responsible for the given amelioration. Recent research has suggested that the observed ameliorative effects may be the result of growth factors within the transplanted tissue interacting with the host brain. For example, Bohn et al. (1987) treated mice with the neurotoxin MPTP and subsequently implanted adrenal medulla grafts into one striatum. Although the grafts did not appear to survive, tyrosine hydroxylase immunoreactivity was demonstrated in the host striatum containing the graft. This effect was not demonstrated in the contralateral striatum which was also lesioned (since the MPTP

was given subcutaneously) but which did not receive a transplant. This suggested that the grafts of adrenal medulla served to provide some sort of neurotrophic factor or mechanism to the host tissue, thus leading to the recovery of fibres previously damaged by MPTP (Bohn et al., 1987). It has recently been found that cografts of adrenal medulla cells and glioma cells reduce abnormal rotational behaviour produced in rats with 6-OHDA lesions to a degree that is greater than that produced by adrenal medulla or glioma grafts alone (Bing et al., 1990), suggesting a synergistic effect of the two types of grafts.

Further examination of the transplantation phenomenon has suggested that the ameliorative effects found after grafting may be due, at least in part, to a host response to the surgical procedures themselves. For example, Fiandaca et al. (1988) administered MPTP systemically to Cebus monkeys and followed with intrastriatal implantation of adrenal medulla cells. One of their surgical methods involved placing the adrenal cells within a metal carrier and implanting both the carrier and the tissue within the striatum. Surprisingly, the control animal which had been treated with MPTP but which only received implantation of the metal tissue carrier (no adrenal cells) showed the same enhanced production of tyrosine hydroxylase immunoreactivity as those animals which had received both the carrier and the adrenal medulla cells. The exact mechanism for this effect is uncertain, but one possibility is that macrophages and microglia known to be present in brain tissue treated in this way, may release trophic substances, which may in turn result in enhanced tyrosine hydroxylase immunoreactivity (Fiandaca et al, 1988). Gash and his colleagues

(Levivier et al., 1989; Pearlman et al., 1990) have also recently discovered that transplantation of fetal striatal tissue and even the transplantation of gelfoam alone prior to injection of quinolinic acid into the striatum will result protect from apomorphine-induced rotational behaviours observed in rats treated with quinolinic acid alone. This protective effect is greater with fetal striatal transplants but the similar protective effect of the gelfoam-only group would suggest a host-response produced by the surgical procedure.

Thus, it seems that growth factors may play a role in the recovery of function, at least after transplantation of embryonic tissue. As far as excitotoxins are concerned, it is a long standing observation that the excitotoxin injection into the striatum induces glial proliferation, commonly referred to as gliosis (e.g., Isacson et al., 1987b). In keeping with this observation, some older literature has indicated that glia taken from kainic acid-lesioned striatum are capable of maintaining continuous culture; this capability is not present in glia derived from the contralateral unlesioned striatum taken from the same animal (Singh and Van Alstyne, 1978). Additionally, it has also been demonstrated that growth factors in glia derived from kainic acid-lesioned rat corpus striatum are capable of supporting NGF- and NGF-insensitive neurons (Lindsay, 1979). Whether growth factors derived from excitotoxin-induced lesions are involved in the observed attenuation of hyperactivity (in a way similar to that seemingly produced by growth factors from grafts or as a host-generated response) remains an intriguing possibility, as yet uninvestigated.

Interestingly, Graveland, Williams and DiFiglia (1985) have reported the

simultaneous degeneration and regeneration of spiny neuronal elements in HD. The precise explanation for the regenerative effect is unclear, but abnormal metabolism or the influence of trophic factors have been suggested. These regenerative changes in HD (Graveland, Williams and DiFiglia, 1985) point to another parallel between the excitotoxin model of HD and the disease state.

Absence of Hyperactivity in Rats with QUIN-induced striatal lesions

A third finding of this investigation is that rats with QUIN-induced lesions of the striatum do not demonstrate any discernable nocturnal hyperactivity, even when tested over long periods of time, and even when hyperactivity has been observed in accompanying cohorts of KA/IBO-lesioned animals. This suggests that nocturnal activity may not always be an adequate index of striatal dysfunction. The reason for the difference betweeen QUIN and KA/IBO lesioned animals where hyperactivity is concerned is not certain. It cannot be suggested that the difference between the QUIN and KA/IBO groups is due to smaller lesions in the QUIN group because the histological analysis for striatal area showed that the QUIN group had somewhat larger lesions than the other two groups.

The negative finding associated with the QUIN group accompanied by hyperactivity in parallel cohorts of KAIN- and IBO-lesioned animals is somewhat analogous to the findings of Dunnett et al. (1987b) and Robbins et al. (1989a, 1989b) who have reported that quisqualate-induced lesions of the nucleus basalis or the

substantia innominata in the rat produce no or very mild behavioural impairments when compared to IBO-induced lesions of the same structure. For example, IBO-induced lesions of the nucleus basalis will produce nocturnal hyperactivity, but quisqualate-induced lesions of the same structure will not; yet, the weak behavioural impairment produced by quisqualate lesions are accompanied by reductions in cholineacetyltransferase levels that are greater than that produced by IBO (Dunnett et al., 1987b). These experiments call into question the interpretation that the marked behavioural impairments produced by IBO-induced nucleus basalis lesions are the result of depletions in cholineacetyltransferase levels and suggest that the impairments in the IBO-lesioned animals are the result of destruction to another system (Sarter and Dudchenko, 1991).

The negative finding for hyperactivity in the QUIN-lesioned group appears to contrast with the report of hyperactivity in QUIN-lesioned animals by Sanberg and his colleagues (1989). However, our results are in fact quite compatible with those of Sanberg et al. : the hyperactivity described by these authors is considerably less than that expressed by our KA/IBO-lesioned animals, being restricted to only a few of the 20-odd paramaters measured and only one hour of the twelve hour testing period.

In light of the data from quisqualate lesions, the question arises as to whether rats with QUIN-induced striatal lesions demonstrate any significant behavioural impairments. Indeed, I have found that rats with QUIN-induced lesions of the medial striatum show significant impairments in tests of cognitive ability but no apparent motor deficits (refer to next chapter).

Nocturnal Hyperactivity: the role of the frontal cortex

Past research on the behavioural consequences of frontal cortex lesions may have implications for the study of nocturnal hyperactivity as produced through injections of kainic/ibotenic acid. A recent paper by Dunnett and his colleagues (1987a) has replicated and extended the findings of previous researchers (Lynch, 1970; Kolb, 1974) on a specific behavioural consequence of frontal cortex lesions: aspirative lesions of the frontal cortex (prefrontal cortex in Dunnett et al.'s paper) produce hyperactivity in rats that is remarkably similar to that demonstrated by rats with excitotoxic striatal lesions being most marked 1) at night and 2) under food deprivation. Thus, it may be suggested that nocturnal motor hyperactivity arises as a result of frontal cortex damage as well as striatal lesion. This would provide support for Rosvold and Swarczbart's (1964) idea that similar behavioural deficits may arise as a result of damage to either the frontal cortex or areas with which it is connected, one of which is the striatum. However, in light of this idea, the lack of hyperactivity from quinolinic-acid lesioned rats becomes difficult to interpret. If hyperactivity were indeed a common consequence of damage to both the frontal cortex and striatum, then one would have expected to see it in quinolinic acid lesioned rats. One might look at other differences between kainic/ibotenic and quinolinic acid-lesioned animals (i.e., size of lesion, magnitude of extrastriatal effects) in an attempt to interpret the experimental findings.

Conclusions

The present investigation has replicated the finding of spontaneous nocturnal hyperactivity in rats with KA/IBO-induced striatal lesions that has been demonstrated by others. In addition, an attenuation of this hyperactivity over prolonged periods of testing has been noted. Further, cohorts of QUIN-lesioned animals have failed to demonstrate significant hyperactivity, indicating that spontaneous nocturnal activity may not always provide an adequate index of striatal dysfunction.

CHAPTER 4

BEHAVIOURAL ANALYSIS OF QUINOLINIC ACID-INDUCED LESIONS IN RATS

II. An Examination of Cognitive and Motor Behaviours in Rats with Quinolinic Acid-Induced Lesions of the Medial Striatum

Introduction

Methods and Materials

Behavioural Tests: A. Tests of Cognitive Behaviour

I. Morris Water Maze

II. Delayed Alternation Task

Behavioural Tests: B. Tests of Motor Behaviour

I. Spontaneous Daytime Locomotor Activity

II. Spontaneous Nighttime Locomotor Activity

III. Food Manipulation

IV. Swim Posture

V. Tongue Extension

Histological Analysis

Results

Discussion

General Discussion

BEHAVIOURAL ANALYSIS OF QUINOLINIC ACID-INDUCED LESIONS IN RATS

II. An Examination of Cognitive and Motor Behaviours in Rats with Quinolinic Acid-Induced Lesions of the Medial Striatum

Introduction

The mammalian neostriatum, heterogeneous both in structure and function, is well-known to be involved in motor control (e.g., Pisa, 1988a, 1988b). However the clinical and experimental literature also suggest a role for the striatum in cognition. Many areas within clinical literature provide strong support for this notion. First, there is a marked neuropsychiatric impairment in patients suffering from Huntington's Disease, in which the striatum undergoes significant degeneration (Vonsattel et al., 1985). The various components of this neuropsychiatric disturbance are (as detailed previously) linguistic impairment (e.g., Illes, 1989; Ludlow et al., 1987), memory disorder (e.g., Butters et al., 1985; Heindel et al., 1989) , visuospatial impairment (Potegal, 1971; Fedio et al., 1979), affective disorder (Folstein et al., 1983a, 1983b) and deficits reminiscent of those with frontal lobe damage (Josiassen et al., 1983; Butters et al., 1985). Second, patients with Parkinson's Disease have been reported to be impaired in the learning of procedural information (Saint-Cyr et al., 1988). Third, patients with basal ganglia insult (e.g., stroke) also demonstrate an impairment which has been

described as resembling a "frontal lobe syndrome" (e.g., Laplane et al., 1989).

The rodent experimental literature has also suggested a role for the striatum in cognition. (e.g., Divac, Markowitsch and Pritzel, 1978; Packard and White, 1990). The problem associated with these studies is that the majority have lesioned the striatum using either electrolysis, which destroys fibres of passage as well as striatal tissue, or kainic acid, an excitotoxin which is known to produce extensive extrastriatal damage. Two recent studies, however, have lesioned the striatum using ibotenic acid (an excitotoxin believed to produce considerably less extrastriatal damage than kainic acid) and noted deficits in the processing of visuospatial information (Whishaw et al., 1987; Isacson et al., 1986), strenghthening the notion that the striatum may play an important role in cognitive function.

Recent research has also suggested a functional heterogeneity of the striatum. For example, Pisa (1988a, 1988b) has discovered that ibotenic acid-induced lesions of the lateral striatum in the rat produce chronic deficits in tongue and forelimb extension; such deficits are not associated with similar lesions of the medial striatum. Since motor behaviours associated with the striatum appear to be under the control of the lateral (and not the medial) striatum, it may be that cognitive behaviour may be similarly localized. One purpose of the present investigation was to further explore the role of the striatum in cognition, with a specific focus on the potential role of the medial striatum.

Further, a striatal lesion produced by the injection of quinolinic acid has been proposed as an animal model for HD. Despite the considerable research effort devoted to this model, behavioural study of this model is virtually non-existent; the majority of

research on this model has been devoted to neurochemical or neuroanatomical analyses. My work, described below, presents the first detailed behavioural study of rats with quinolinic acid-induced lesions of the striatum.

Rationale for the Choice of Behavioural Tests in the Evaluation of Rats with Quinolinic Acid-Induced Lesions of the Striatum.

When one is considering the behavioural evaluation of a particular animal model of a disease, one becomes concerned with the issues and problems of cross-species comparisons of behaviours and the generalization of behavioural test results. Sometimes, the same test may be used to examine both humans and animals, such as the use of the Piaget $A\overline{B}$ task in both human infants and primates (Diamond and Goldman-Rakic, 1989). More commonly, (particularly where rodents are concerned) the tests used in the evaluation of patients are too sophisticated to be used with animals. Thus, it is frequently necessary to use two different tests when examining animals and when examining human patients; it is presumed (not always correctly) that two tests measure the same behavioural capacity. Certain safeguards (detailed below) can be employed to reduce the risk of erroneous assumptions.

The tests I have employed in the behavioural evaluation of rats with quinolinic acid (QUIN)-induced striatal lesions (as well as the behavioural studies in the remainder of this thesis) have been chosen with a number of factors in mind. For example, I have chosen tests which are thought to examine those abilities believed to be impaired in HD. As detailed previously, there is a distinctive pattern of mental

disturbance in HD, consisting of cognitive decline (linguistic impairment, memory impairment, similarities to patients with frontal lobe damage), affective changes and perceptual impairments. For obvious reasons, linguistic impairment and affective changes cannot be reasonably studied in rodents. Thus, I have chosen tests which are thought to examine abilities known to be impaired in HD but which, at the same time, can be performed by rats; these include memory, visuospatial skills and abilities which have been attributed to the frontal lobes.

Secondly, I have employed multiple tests in order to reduce the possibility of erroneous generalizations from the results of a single test. The value of such an approach has been recognized by others (e.g., Kolb and Whishaw, 1983a; Kolb and Tees, 1990). Behavioural tests, especially where animals are concerned, are always presumed to measure some capacity; the experimenter can never know for certain whether or not these presumptions are correct. Further, the experimenter is rarely interested in the task itself, but in the more general ability presumed to be under examination. However, it is possible that a given test measures only a certain aspect of a particular ability, and that other tests measure other aspects. This point is particularly well illustrated by Kolb and Whishaw (1983a) in describing their attempts to analyze spatial behaviour in rats with medial frontal, orbital frontal and parietal cortical lesions. Because they were well aware of the potential pitfall of using a single test as a measure of spatial ability, they utilized three tests thought to examine spatial learning and memory: the Morris water maze, the radial arm maze and the Grice box. Extrapolating from the available human literature, it was expected that rats with

medial frontal and parietal lesions would be impaired on all three tasks, while those with orbital lesions would not be impaired on any task. Further, the experimenters hoped that the medial frontal and parietal lesion groups would show some differences in impairment as the human literature suggested that lesions in each of these cortical areas produced different types of impairments. This did not turn out to be the case, with each group demonstrating impairment on two tests (i.e., the medial frontal group was impaired in the performance of the Morris Water Maze and Grice box; the parietal group, in the performance of the radial arm maze and Grice box; the orbital frontal group, in the performance of the Morris Water Maze and radial arm maze). Had only one test been employed, the researchers may have concluded that those groups that had demonstrated impairment on the single test were demonstrating impaired spatial ability and that the groups which had demonstrated no impairment on the same test possessed normal spatial ability, when in fact such a broad generalization would have been incorrect.

Methods and Materials

Surgical Procedures

Sixteen male Sprague-Dawley rats (Charles-River, Québec) were used in the experiment; eight were used as controls, and the remaining eight were lesioned with quinolinic acid. The rats were between 225-250 grams at time of surgery. The animals were anaesthetized (sodium pentobarbital) and placed in a Kopf small animal stereotaxic apparatus (David Kopf, Tujunga, California). After the skull was exposed,

a Dremel drill was used to drill holes in the skull at the following stereotaxic co-ordinates (from the atlas of Paxinos and Watson, 1982): A/P +0.7, +1.7; M/L +/- 2.5; D/V -5.0 (from skull). Thus, four stereotaxic points were used for each animal, two in each striatum. A 10 ul, Hamilton syringe with a 30 gauge, blunt-tipped needle (Hamilton Company, Reno, Nevada), mounted on an electrode carrier, was used to deliver the drug solution. For the control animals (CONT), the needle was lowered to the appropriate co-ordinate, left in place for one minute, and retracted. For the experimental animals (QUIN), the needle was lowered to the proper co-ordinate, left in place for one minute, and then 0.5 ul of a 120 nm/ul solution of quinolinic acid (Sigma, St. Louis, Minnesota) was injected at a rate of 1 ul/minute (i.e., 60 nm of quinolinic acid in 0.5 ul of solution was injected over a period of 30 secondsinto each stereotaxic co-ordinate). After the injection was complete, the needle was left in place for two minutes (to minimize upward diffusion of the solution) prior to retraction of the needle. The scalp incision was closed with animal wound clips (Fisher Scientific, Unionville, Ontario), and the animal returned to its home cage.

Behavioural Tests: A. Tests of Cognitive Behaviour

I. Morris Water Maze

The Morris Water Maze (MWM) is a behavioural test of spatial learning and memory, devised in the early 1980s, in response to questions surrounding spatial learning and the hippocampus (Morris et al., 1982; Morris, 1984; Brandeis, Brandys and Yehuda, 1989). The basic procedure involves placing a rodent (typically rats,

although mice are also used) into a large pool of water containing a platform (which is either hidden or visible from the surface of the water, depending upon the paradigm used); several dependent variables assess the animal's ability to find the platform (i.e., escape from the water) and to remember the location of the platform over a period of testing. Since the time of its inception, the test has proven to be versatile and adaptable to a variety of situations (Brandeis, Brandys and Yehuda, 1989). The test is believed to examine spatial learning as it pertains to the use of distal cues (i.e., extramaze cues) in relationship to the platform (Morris et al., 1982; Morris, 1984; Whishaw et al., 1987; Brandeis, Brandys and Yehuda, 1989).

The MWM has many advantages over other, more traditional tests of spatial learning. First, it is quite easy to administer. Second, many dependent variables may be derived from the test (e.g., latency, path distance, time spent in the various quadrants, heading direction), thus providing the experimenter with many measures of spatial learning, all of which may be used to interpret the animal's performance of the task. Third, because a large pool of water is employed, and the intramaze environment is uniform, it is believed that intramaze cues (i.e., odor trails, environmental cues) are significantly reduced in comparison to tests such as the T-maze or the radial arm maze, where the presence of such cues may lead an animal to produce the correct response without utilizing spatial learning. Fourth, because a correct response in the MWM is not restricted to a fixed number of choices (i.e., 2 choices in the T-maze, 6-8 choices in the typical radial arm maze), the test is thought to place greater demands on the spatial localization system than traditional tests, and is therefore thought to be a

superior index of spatial learning when compared to the T-maze or radial arm maze (Morris et al., 1982; Morris, 1984; Brandeis, Brandys and Yehuda, 1989). However, in all fairness, the disadvantages of the MWM must be indicated. First, it is difficult to vary motivational level of the animals, and second, it is difficult to eliminate stress associated with immersing rodents in water. (Morris et al., 1982; Morris, 1984; Brandeis, Brandys and Yehuda, 1989). Yet, the advantages would seem to outweigh the disadvantages.

The version of the test used in my study is the reference memory, place version of the MWM test in which the platform is submerged just beneath the surface of the water in one of the pool quadrants, such that the platform is hidden. The rat is given four trials (i.e., one block) per day over a given period. Each trial consists of releasing the rat into the water at the edge of the pool at one of four compass points and then allowing the rat to swim in the pool. The starting point is varied randomly over the four trials such that no starting point recurs in one day. Varying the starting point in this way and placing the platform within one of the quadrants prevents the rat from finding the platform by using a particular sequence of movements (a praxis strategy) or using proximal cues associated with the goal (a taxis strategy). The use of praxis or taxis strategies would permit the rat to solve the maze by using nonmapping strategies; this is clearly not the desired objective (Brandeis, Brandys and Yehuda, 1989). It has been shown that the elimination of the extramaze environment (through the use of black curtains drawn around the pool) results in performance falling to chance levels, thus suggesting that a spatial mapping strategy is indeed being used by the animals

(Morris, 1984).

As described previously, neuropsychological studies have revealed that HD patients demonstrate deficits in the processing and recall of visuospatial information. Thus, one would want to determine whether or not deficits of a similar nature are also expressed in a given animal model-in this case, the QUIN model of HD. However, it is always difficult to compare animal and human behaviours, and tests of spatial learning and memory are no exception. First, the same tests can rarely be administered to both animal and human subjects. Second, as has been noted by others, spatial tests given to humans are usually sit-down, paper-and-pencil tests, whereas spatial tests given to rodents usually involve the animal moving from one point to another in an attempt to solve the task (Kolb and Whishaw, 1990). Both these points pose problems for interpretation of task results and the comparison of the animal model and the human pathological state. It is important to recognize these limitations, but it is important to characterize the animal model in as many ways as possible. Thus, the MWM was used in order to determine whether rats with QUIN-induced striatal lesions are impaired in visuospatial learning and memory, as is the case in patients with HD.

Apparatus The Morris Water maze apparatus consists of a circular tank, 1.83 metres in diametre, made of an opaque plastic material. The platform (30 cm high) is located in a constant position in one of the quadrants (training quadrant) during acquisition training. The location of the platform is marked on the bottom of the pool to ensure consistency of placement. A camera is suspended in a vertical position above

the pool and connected to a Sony Betamax video cassette recorder. The water maze is located in a large room with many other pieces of equipment (i.e., extramaze visual cues), the location of which are held constant during the entire testing period. A diagram of the apparatus appears in figure 4.1.

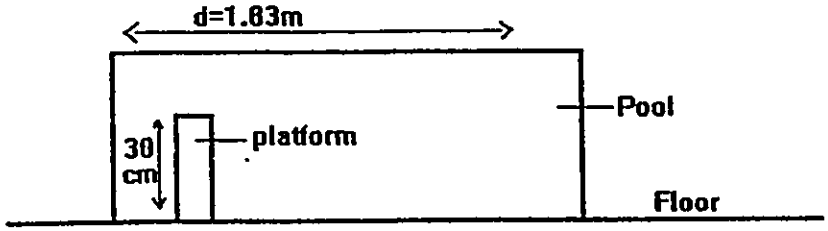
Testing Protocol The procedures used in this test were those involved in the place task version of the MWM, similar to those of Whishaw et al. (1987). The maze was filled with water (18 degrees Celsius) to one or two centimetres above the top of the platform. A layer of styrofoam obscured the location of the platform.

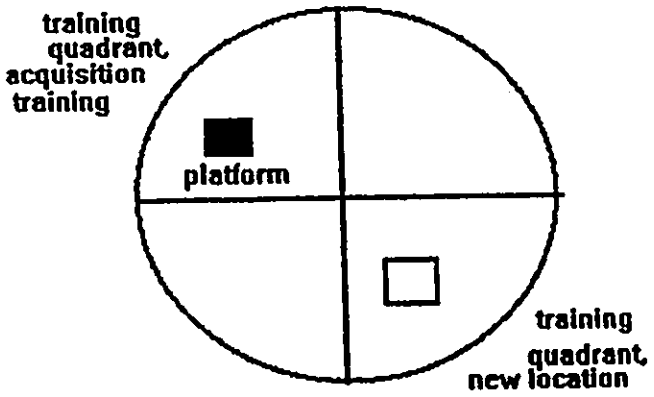
1. Acquisition Training. Four equidistant starting points were used. Each rat was gently placed in the water at a given starting point, facing the wall of the pool and allowed to swim for one minute, or until the platform was found (i.e., one trial). If the rat did not find the platform within one minute, it was taken out of the water and placed on the platform for twenty seconds. If the platform was found, the rat was allowed to remain on the platform for twenty seconds. At the completion of each trial, the rat was removed from the pool and put back in its cage. The time needed to find the platform was recorded by the experimenter (latency). Each rat was given four trials per day (i.e., one block), over ten consecutive days of acquisition training. The starting position of each trial was varied randomly such that no position recurred within a single day.

Platform Relocation. On the eleventh day, the platform was placed in the opposite

Figure 4.1 Diagram of the apparatus of the Morris Water Maze, side and superior views.


ceiling
camera


d=1.83m
Pool
30 cm
platform
Floor


training quadrant, acquisition training
platform
training quadrant, new location

quadrant, and four trials of training were given to each animal. Again, the latency of each animal was recorded.

2. Retesting. Five weeks after the first period of Acquisition Training, the animals in Experiment 2 were tested again in the MWM for a period of seven days. The platform was in its initial position. After this period of retesting, one day of testing was given where the top of the platform projected above the surface of the water and no styrofoam was in the pool; these modifications made the platform visible. After this, the rats were given three more testing days where the platform was again hidden.

3. New platform location. Three weeks after the end of the Retesting in Experiment 2, the two groups were again tested. The first day consisted of testing the rats at the old platform location; the next nine days consisted of testing the rats at a new platform location. On the next testing day, the platform was visible (i.e., no styrofoam, platform top projecting above the surface of the water). This was followed by two more days of testing in which the platform was hidden.

All water maze trials were videotaped and later analyzed for the time that each animal spent in the quadrant in which the platform was located (i.e., training quadrant) on each trial for each day. Analyses of variance (ANOVAs) were performed on the latency data as well as the time spent in the training quadrant for the all testing sessions.

4. Testing for memory of the platform location at progressively increasing intervals. One week after the last day of testing in the previous session, the animals in Experiment 2 were given four trials to test for memory of the platform location. Two

weeks after this date, the animals were again tested. Three weeks after the second week testing date, the animals were tested once again.

Results.

1. Acquisition Training. Latency. The ANOVA for the first period of acquisition (figure 4.2) revealed a significant effect for Group ($F(1,14)=23.367$, $p<0.01$), Days ($F(9,117)=34.302$, $p<0.01$), Trials ($F(3,117)=10.529$, $p<0.01$) and Day x Trials ($F(27,117)=2.504$, $p<0.01$). The most important effect is that of Group, which suggested that the QUIN group took significantly longer to find the hidden platform than the CONT group.

Percent Time in Training Quadrant. Refer to the graph in figure 4.3. For the first period of acquisition, the following effects were significant: Group ($F(1,14)=14.402$,$p<0.01$), Day ($F(9,126)=5.598$, $p<0.001$), Trial ($F(3,42)=3.343$,$p<0.05$), and Day x Trial ($F(27,378)=9.237$,$p<0.001$). The significant effect for Group indicated that the CONT group spent a significantly greater percentage of time in the training quadrant than the QUIN group.

2. Retesting. On the first day of this training, latency performance of the CONT group was similar to that on Day 10 of the first training period, while performance of the QUIN group was considerably slower (figure 4.4). The ANOVA for the second period of acquisition (latency data) revealed a significant effect for Group ($F(1,14)=9.910$, $p<0.01$), Day ($F(6,84)=13.419$, $p<0.01$), Trials ($F(3,42)=7.900$,

$p<0.01$), and Day x Trials ($F(18,252)=4.413$, $p<0.01$). Even with prolonged training, the QUIN group never achieved latency levels similar to the CONT group.

3. New platform location. The third period of training (new platform location) revealed significant effects (latency, figure 4.5) for Group($F(1,14)=16.679$, $p<0.01$), Day ($F(8,104)=22.821$, $p<0.001$), Trial ($F(3,39)=4.283$, $p<0.05$), Group by Trials ($F(3,39)=4.637$, $p<0.01$) and Day by Trials ($F(24,312)=3.005$, $p<0.001$). Again, the significant effect for Group indicated that the QUIN group took significantly more time to find the hidden platform, over the training period, than the CONT group.

A comparison of the control data (figure 4.6) from the first training period (acquisition training) compared to the third training period (new platform location) yielded a significant effect ($F(1,14)=10.975$, $p<0.01$); the same comparison for the QUIN group (figure 4.7) was not significant.

Comparisons of time spent in the old and new training quadrants revealed that the QUIN group spent a significantly greater percentage of swimming time in the old training quadrant (where the platform had been, figure 4.8) ($F(1,14)=6.702$, $p< 0.05$) than did the controls. A comparison of the time spent in the new training quadrant showed a pattern similar to that observed in acquisition training with the original platform location, whereby the QUIN group spent significantly less time in the new training quadrant than the CONT group (the new platform location, figure 4.9).

On Day 8 of the second period of acquisition (retesting), as well as Day 10 of the third period of acquisition (new platform location), the platform was visible. On this day, the performance of the CONT and QUIN group was almost exactly the same.

Figure 4.2 Mean latencies for original platform location.

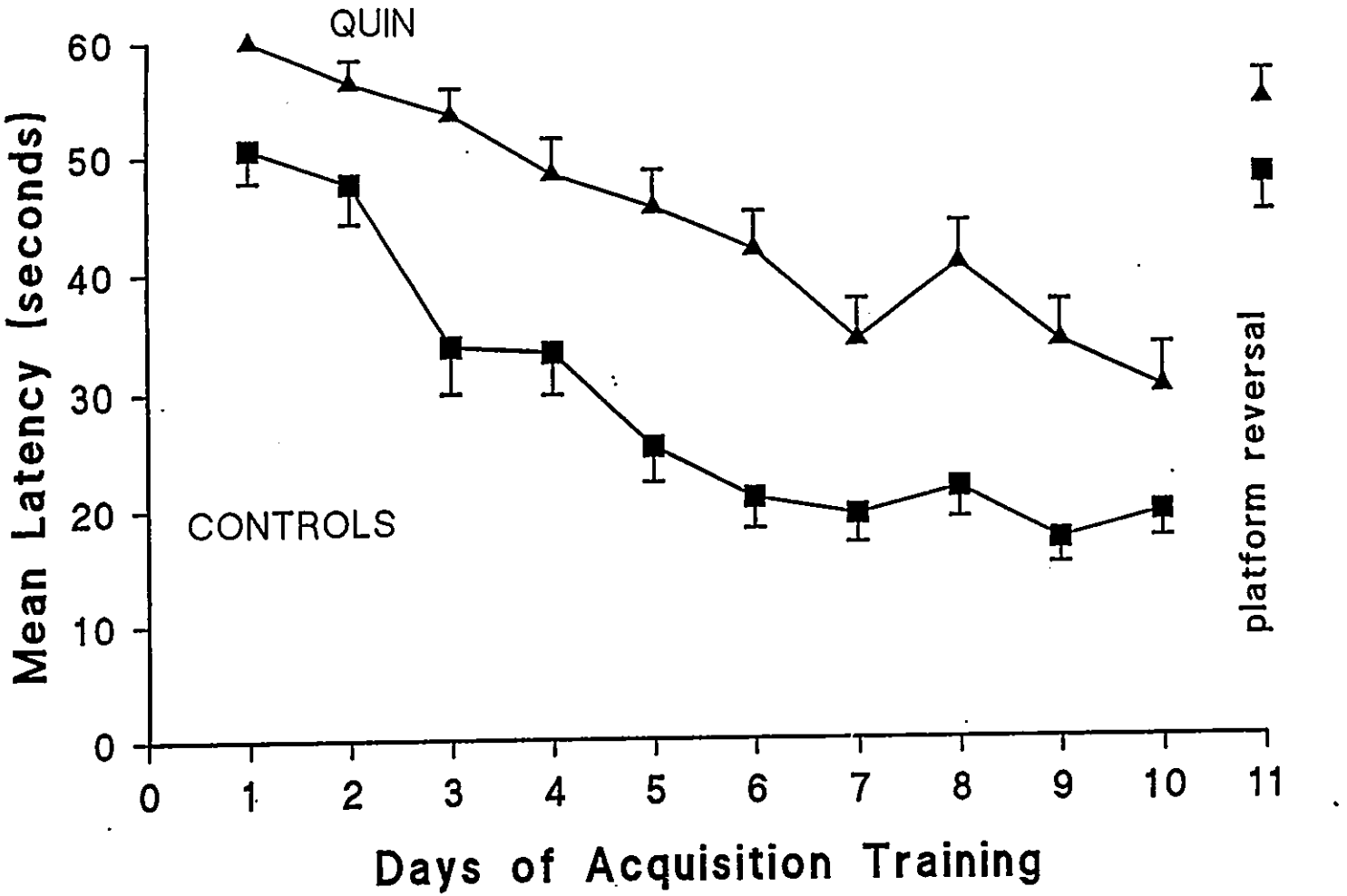

QUIN
CONTROLS
Mean Latency (seconds)
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
11
Days of Acquisition Training
platform reversal

Figure 4.3 Mean percent time spent in the training quadrant.

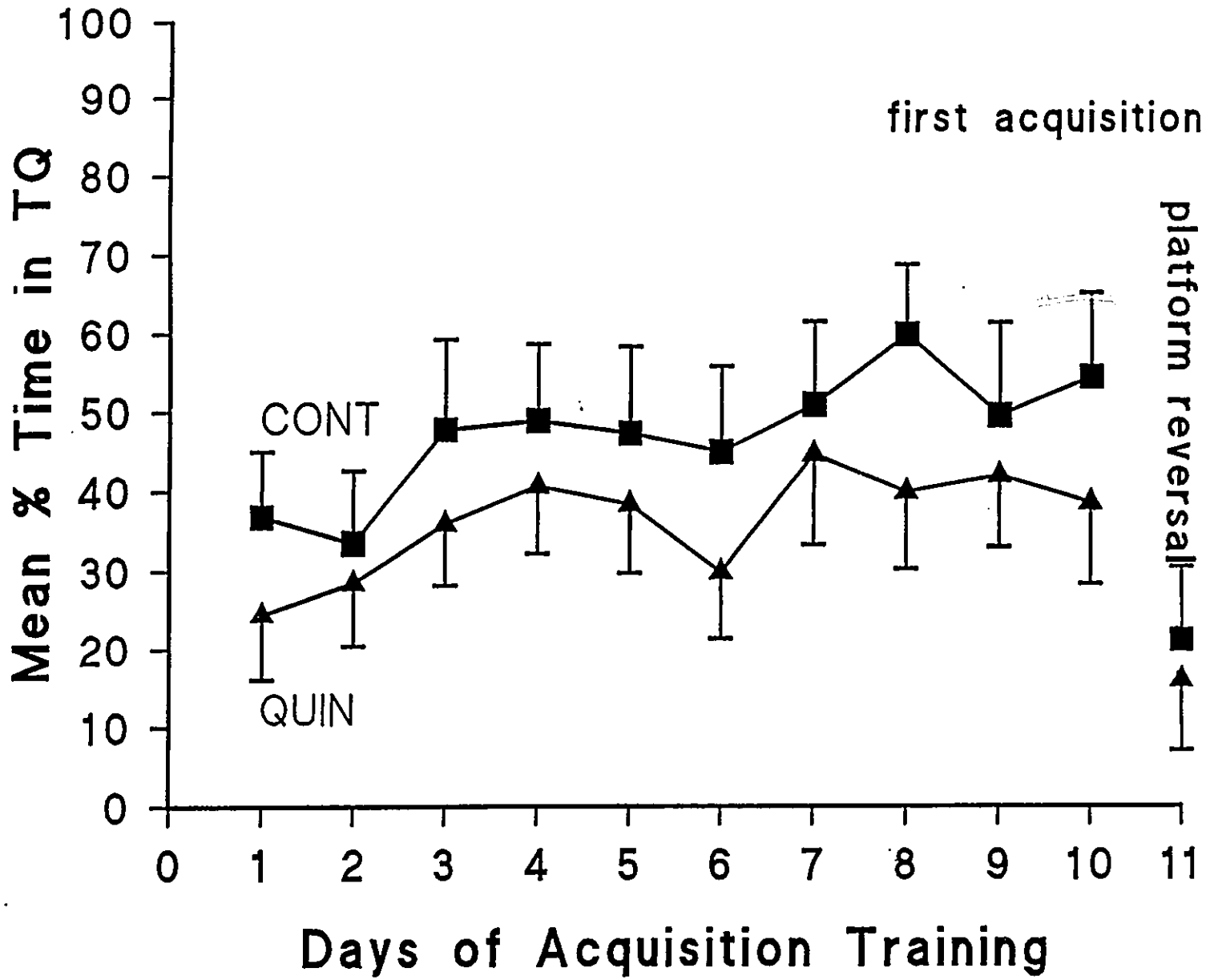

100
90
80
70
60
50
40
30
20
10
0
Mean % Time in TQ
first acquisition
platform reversal
CONT
QUIN
0
1
2
3
4
5
6
7
8
9
10
11
Days of Acquisition Training

Figure 4.4 Mean latencies for extension of training.

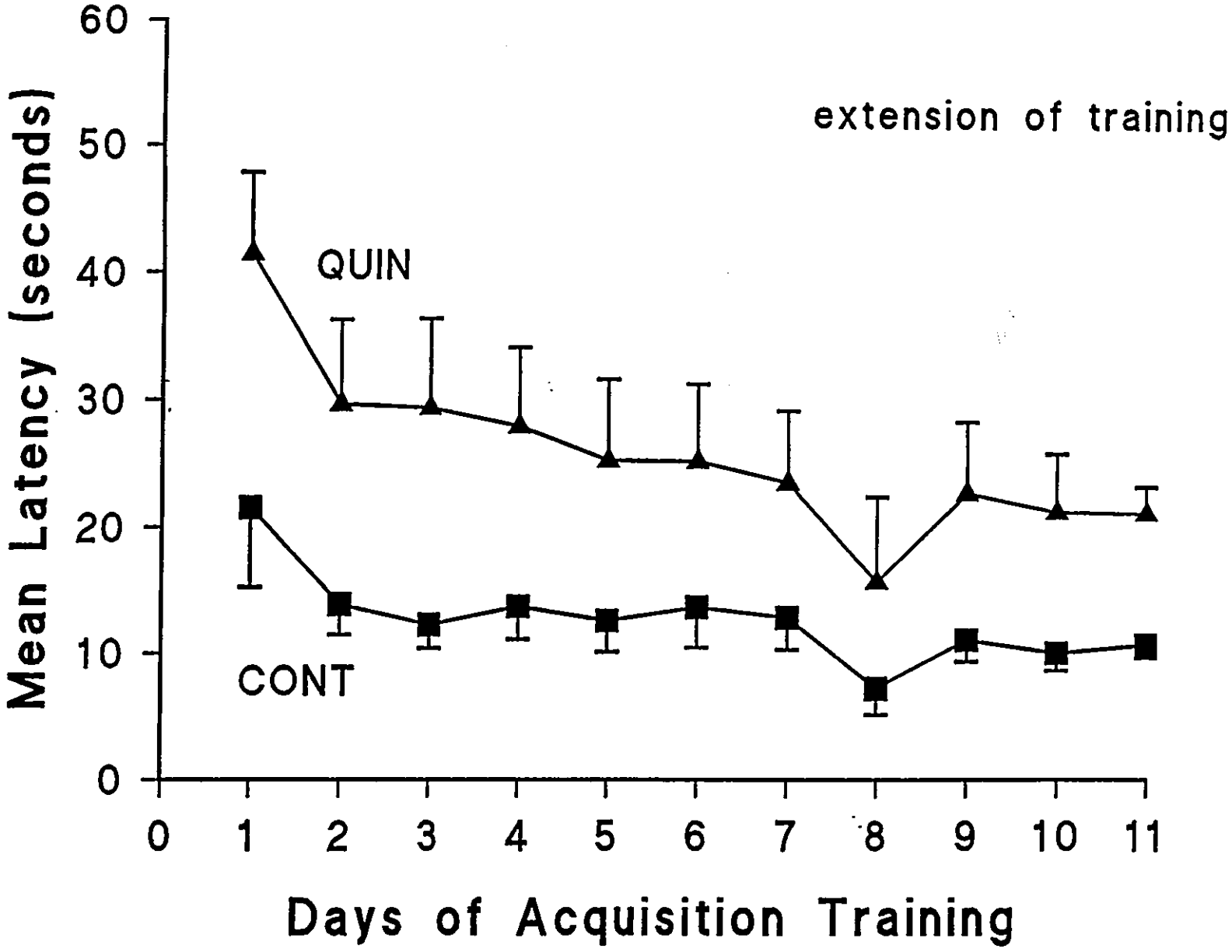

extension of training
QUIN
CONT
Mean Latency (seconds)
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11
Days of Acquisition Training

Figure 4.5 Mean latencies for the new platform location.

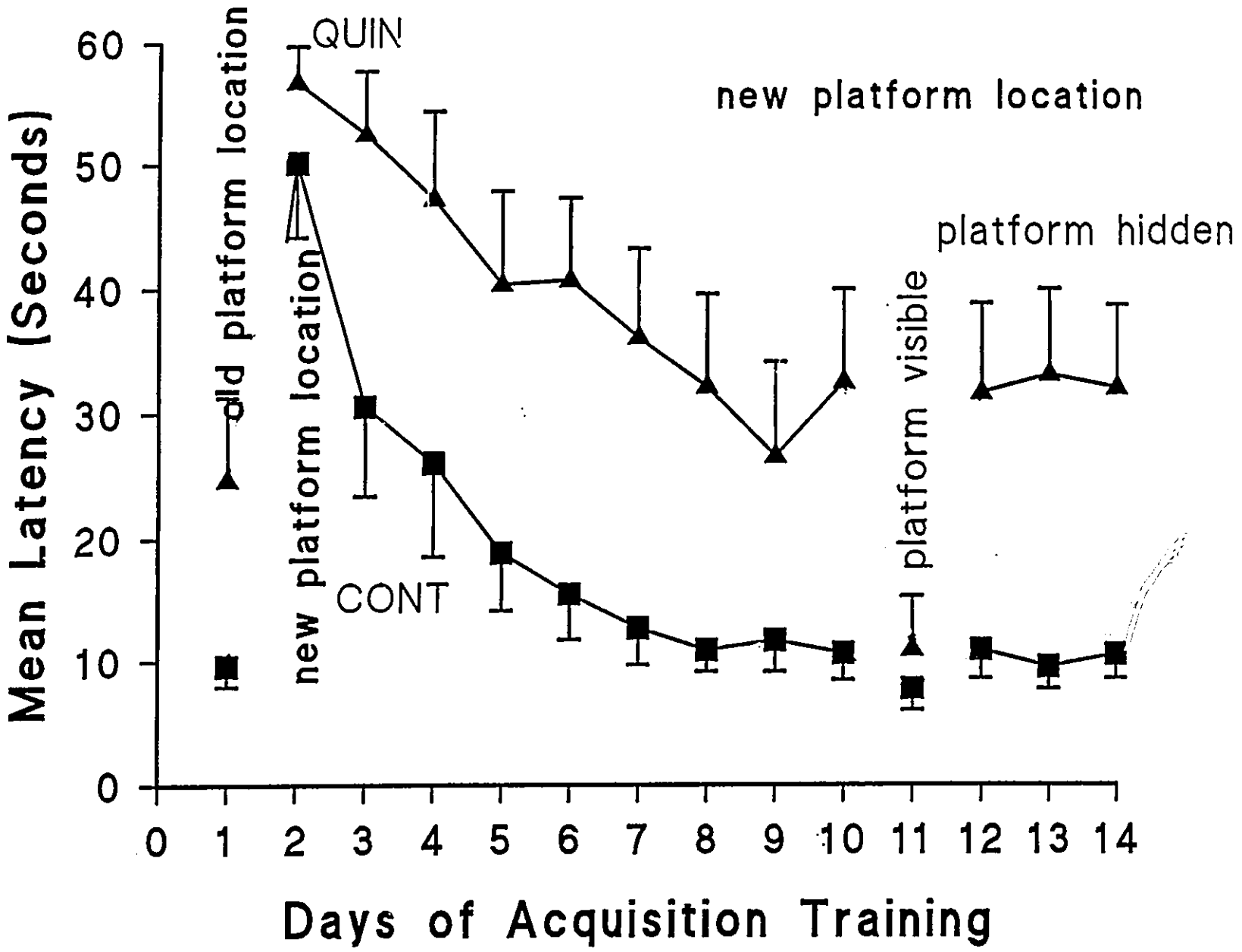

Mean Latency (Seconds)
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days of Acquisition Training
QUIN
CONT
old platform location
new platform location
new platform location
platform hidden
platform visible

Figure 4.6 Comparison of latency data for the original platform location and the new platform location, CONT group.

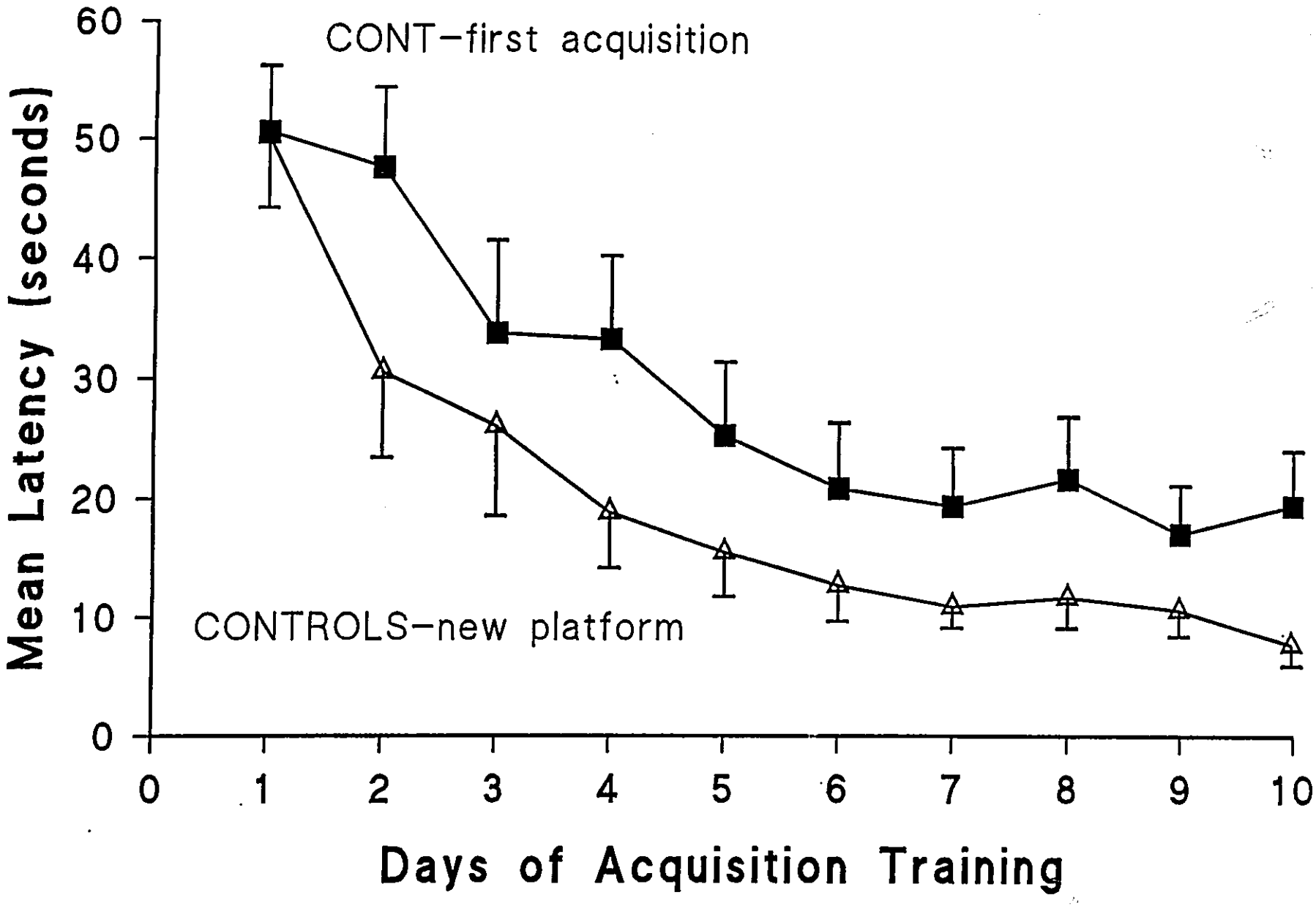

CONT–first acquisition
CONTROLS–new platform
Mean Latency (seconds)
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
Days of Acquisition Training

Figure 4.7 Comparison of latency data for the original platform location and the new platform location, QUIN group.

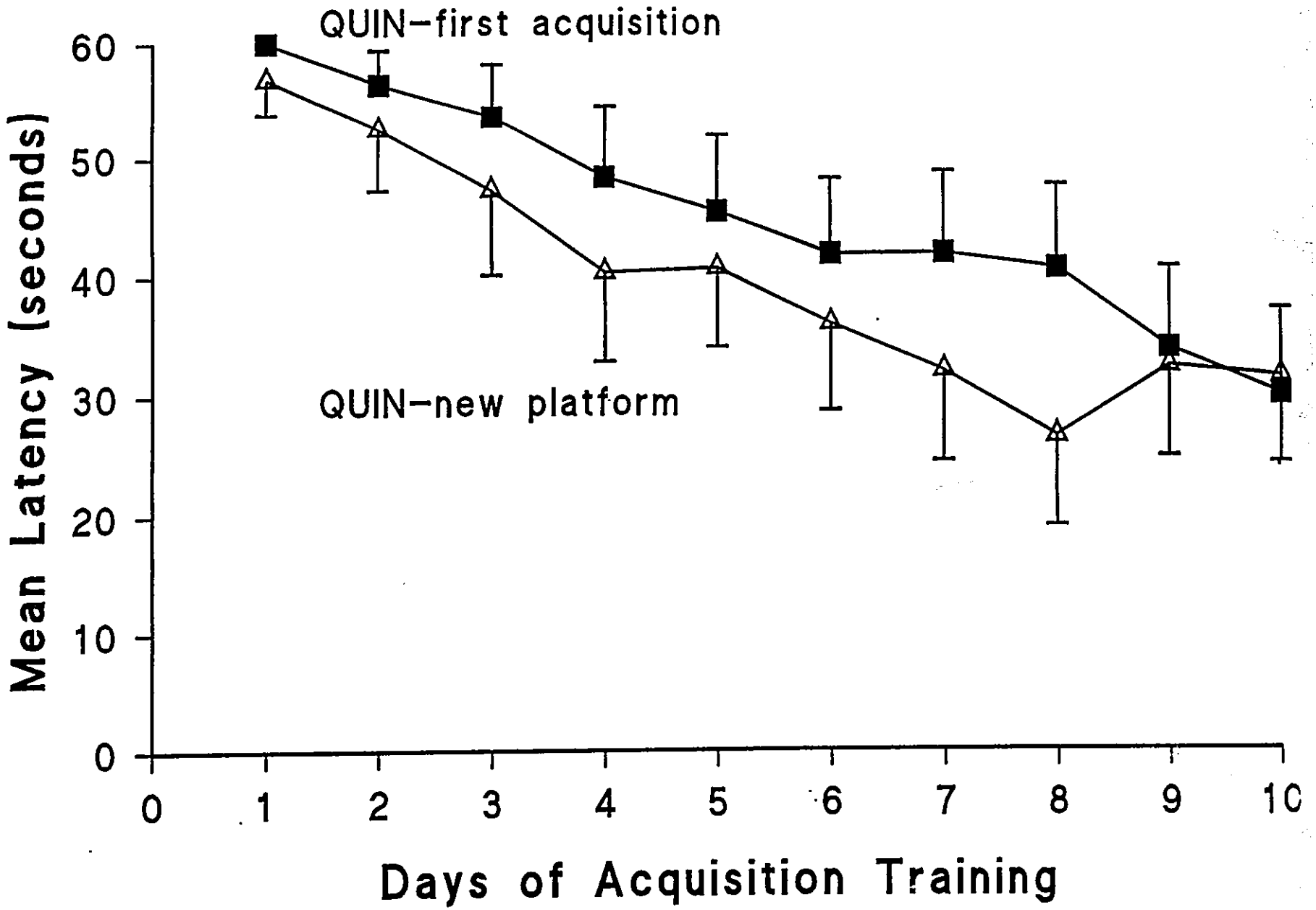

QUIN–first acquisition
QUIN–new platform
Mean Latency (seconds)
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
Days of Acquisition Training

Figure 4.8 Mean percent time spent in OLD training quadrant, new platform location

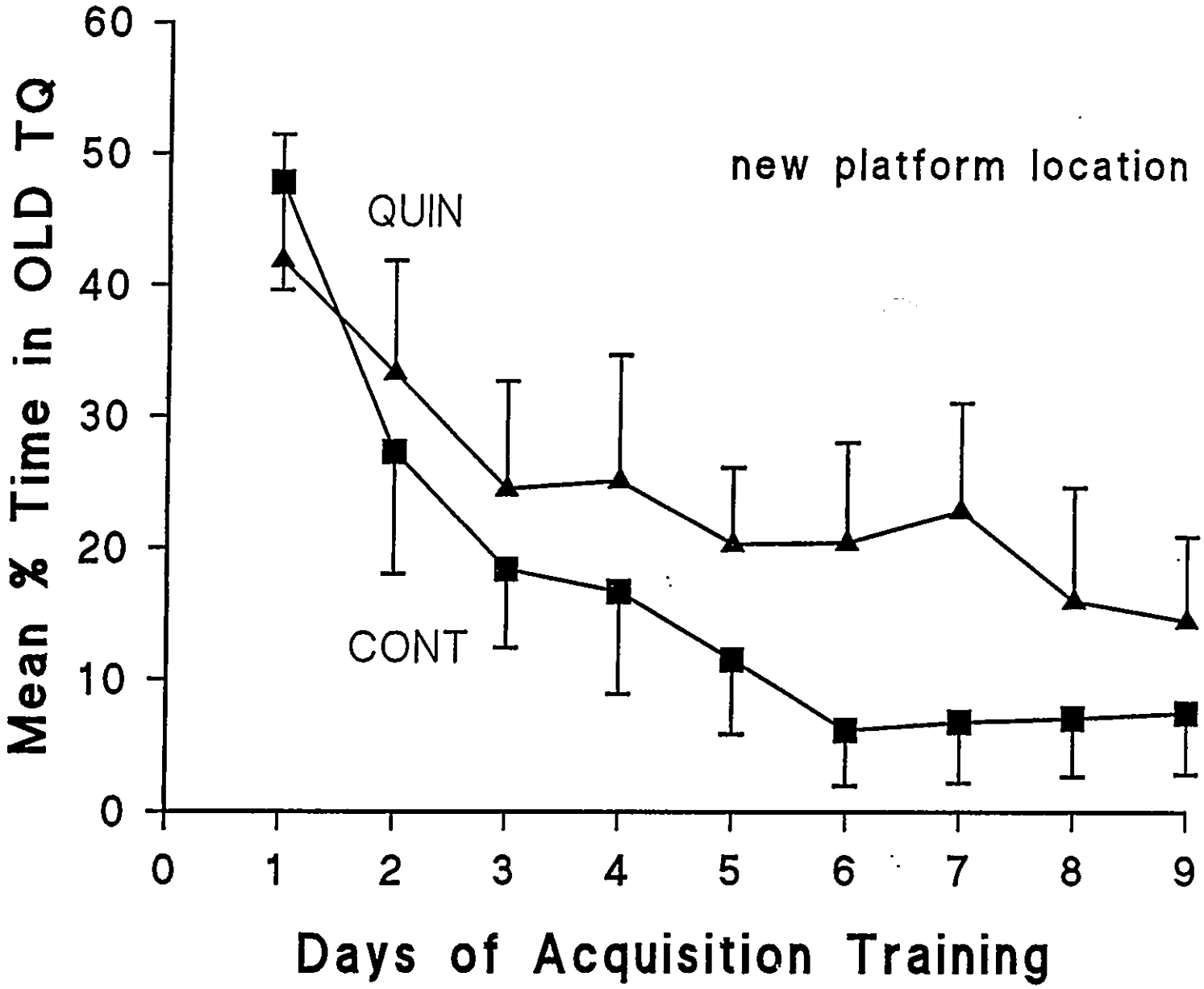

Mean % Time in OLD TQ
60
50
40
30
20
10
0
new platform location
QUIN
CONT
0
1
2
3
4
5
6
7
8
9
Days of Acquisition Training

Figure 4.9 Mean percent time spent in NEW training quadrant,new platform location.

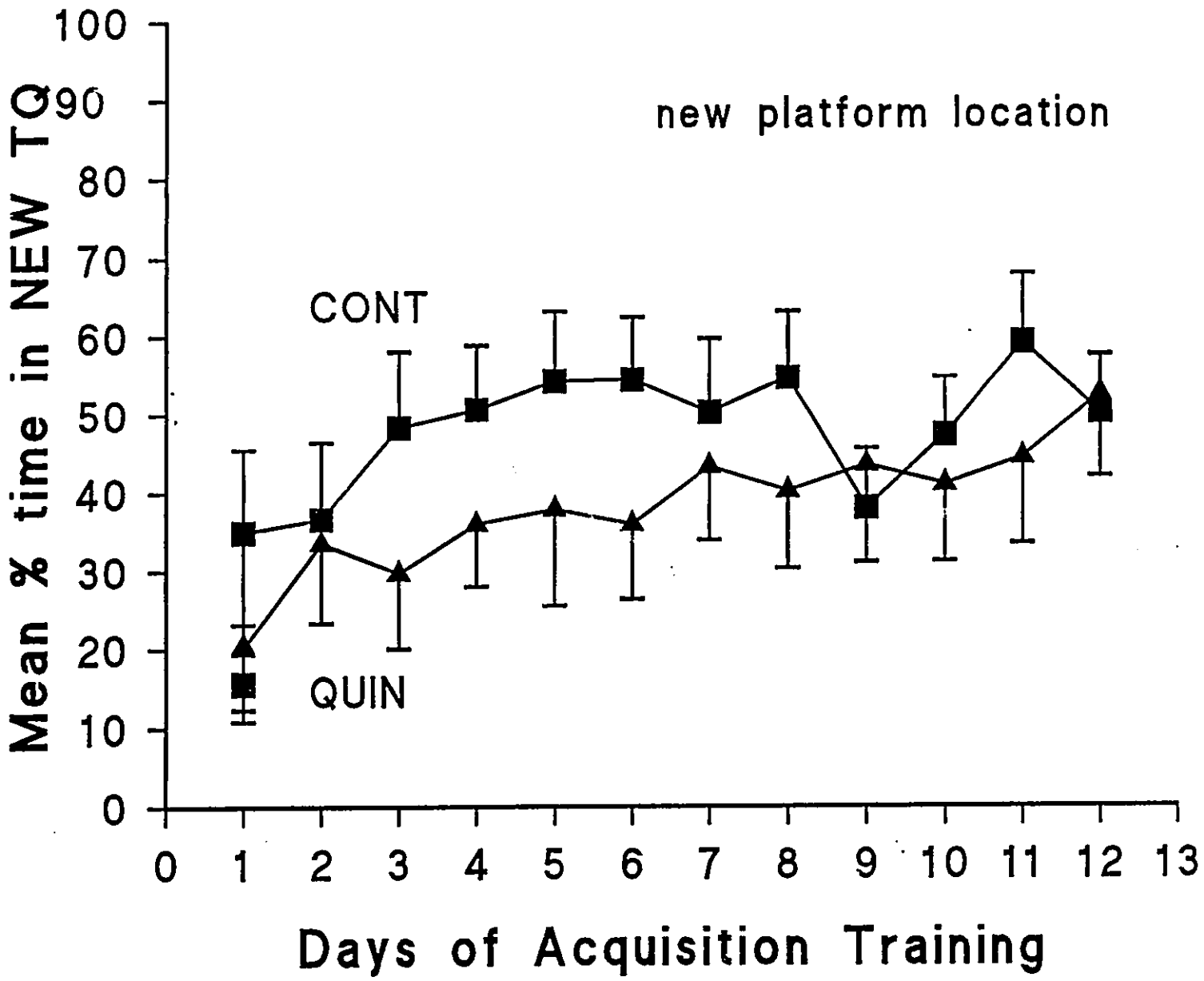
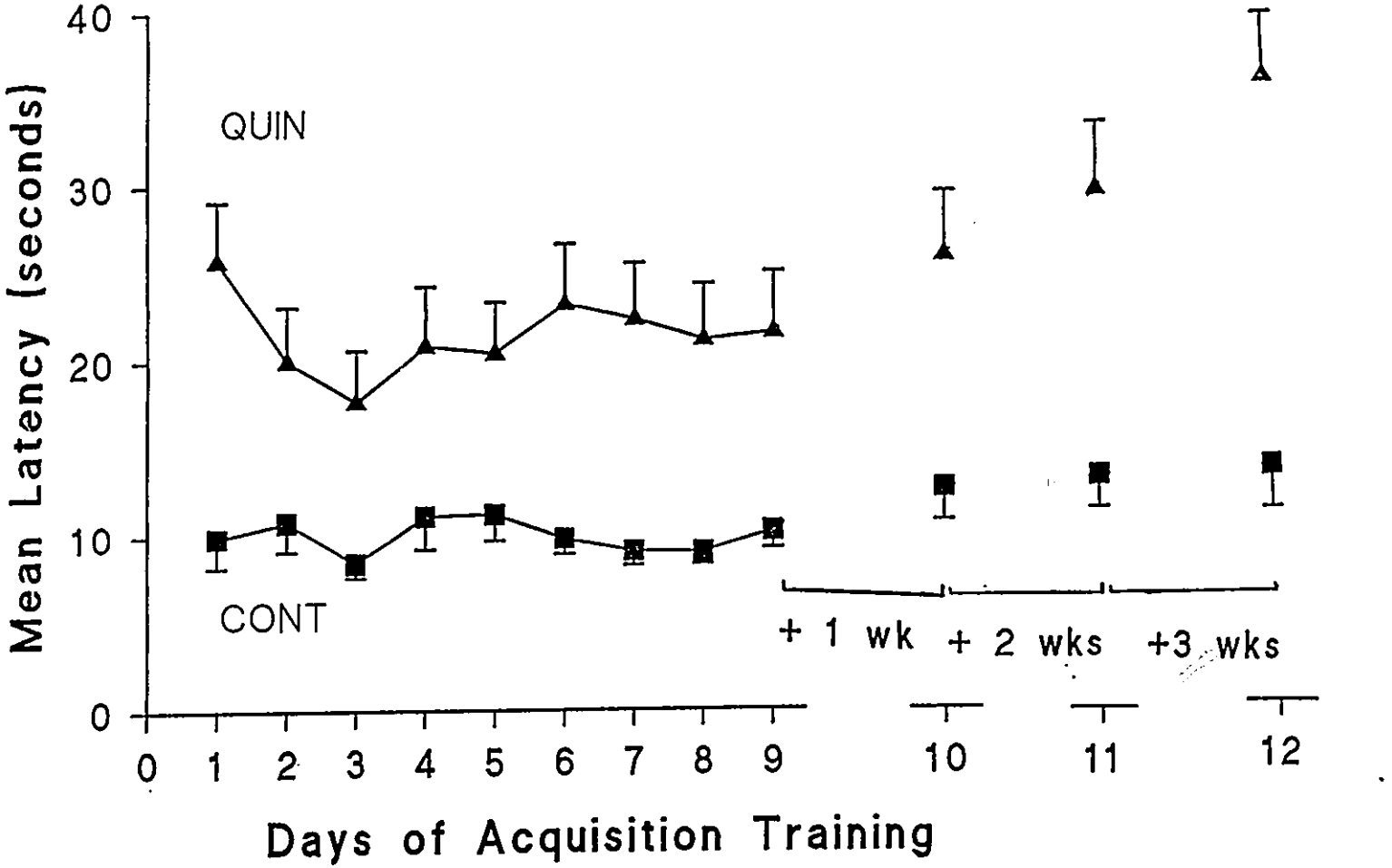

new platform location
CONT
QUIN
Mean % time in NEW TQ
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Days of Acquisition Training

Figure 4.10 Extension of training and memory recall for platform at progressively increasing intervals.


40
30
20
10
0
Mean Latency (seconds)
QUIN
CONT
+ 1 wk
+ 2 wks
+3 wks
0 1 2 3 4 5 6 7 8 9 10 11 12
Days of Acquisition Training

After this, performance returned to levels similar to Days 7 and 9, respectively. (The faster swimming speeds on Day 8 and 10 are due to the removal of the styrofoam from the pool.)

4. Testing for memory of the platform location at progressively increasing intervals. The control group maintained virtually the same level of performance for the entire fourth period of memory testing. In contrast, the QUIN group demonstrated a progressive deterioration of platform recall with increasing time intervals since the last testing session (figure 4.10).

Discussion

The use of the MWM in this study has revealed that rats with medial striatal lesions produced by quinolinic acid are impaired, relative to controls, in the time required to find the platform (latency) in the place task version of the MWM, as indicated by the significant effect for Group (latency data). Given sufficient training, the QUIN group can learn to find the platform within the tank, although their performance never becomes as good as that of controls (particularly given the data from the retesting period).

There was a significant difference between the two groups in when the percent time spent in the training quadrant was considered. Thus, not only did the QUIN group demonstrate increased latency for finding the platform, but they also spent a significantly lower percentage of time in the new training quadrant and a greater percentage of time in the old training quadrant than the CONT group.

The significant difference in the control group data when comparing the first and third period of acquisition, and the lack of a similar significant effect for the same comparison in the QUIN group (figures 4.8 and 4.9) suggested that previous experience was somehow facilitatory for learning a new platform location for the control group only, and not for the QUIN group.

Impaired recall memory in the QUIN group was suggested by the first day of Retesting. Whereas CONT performance on Day 1 of Retesting was similar to that at the end of the first training period five weeks earlier, QUIN performance was considerably worse. Impaired recall memory in the QUIN group was also evident in the fourth session (testing at progressively increasing intervals) by the apparent deterioration in memory for the platform location at progressively increasing intervals after the last testing session; this contrasted with the performance of the CONT group which demonstrated stable performance comparable to that of the last testing session.

When interpreting the results of a behavioural test, it is important to consider explanations other than those traditionally proposed. The MWM test is considered to be a test of spatial learning and memory, and impairments in the acquisition of the task are considered to be impairments in the ability to learn and retain a spatial test, but other factors may complicate this interpretation. For example, from the data presented above, it may be suggested that the impairment demonstrated may reflect one or more of the following on the part of the QUIN group: 1) an impaired visual system (McGeorge and Faull (1989) have described connections between the visual cortex and the striatum), 2) impaired locomotion, 3) lower level of motivation to find

the platform. In an attempt to evaluate and exclude these possibilities, the two days of experimentation in which the platform was visible are particularly useful. On these two days, the water was clear (i.e., no styrofoam) and the top of the platform projected above the surface of the water. As described previously, the performance (latency) of the CONT and QUIN groups were virtually identical, suggesting that the impairment demonstrated on the two acquisition periods may reflect impaired visuospatial processing rather than any of the alternate explanations suggested previously. Further, another test, to be described below, shows that the QUIN group is no different from the CONT group with respect to spontaneous daytime locomotor activity; thus, it is unlikely that the deficit demonstrated by the QUIN group on the MWM task is reflective of a locomotor abnormality.

Positive support for an interpretation of the deficit demonstrated by the QUIN group on the MWM as a deficit in visuospatial processing also comes from other researchers. Fibiger (1978) has reported that controls and rats with excitotoxin-induced striatal lesions are no different from each other where spontaneous locomotion is concerned. Pisa and Cyr (1990) have shown that rats with excitotoxin-induced medial striatal lesions are unimpaired in the performance of a visual discrimination task, but impaired in the performance of a position discrimination task, thus making an interpretation of impaired vision unlikely. Thus, it is likely that the impairment demonstrated by the present group of QUIN-lesioned animals is reflective of an impairment in the acquisition and retention of a task of spatial learning and memory. This is consonant with traditional interpretations of the MWM task.

The MWM task may also be considered a task of cognitive flexibility as the animals must learn to find the platform from four different locations; a single response will not satisfy the demands of the task. Thus, it may be suggested that the impairment demonstrated by the QUIN group may be reflect of an impairment of cognitive flexibility--where latency was concerned, the animals could not learn to adapt from each start position as quickly as the CONT group. Further suggestion of impaired cognitive flexibility comes from the third training session (new platform location) where the animals were required to learn a new platform location: the QUIN group spent a significantly smaller proportion of swimming time in the new training quadrant and more in the old, when compared with the CONT.

The impairment demonstrated on the MWM task is similar to that demonstrated by rats with ibotenic acid-induced lesions of the medial striatum (Whishaw et al., 1987) as well as lesions of the frontal cortex. (It is important to note that the present study is considerably more extensive than that of Whishaw et al., 1987). With respect to the latter, it has been found that adult rats receiving aspirative lesions of the entire frontal cortex, the orbital frontal cortex, or the medial frontal cortex, are impaired in the acquisition of the place version of the MWM task as far as latency is concerned (Kolb, Sutherland and Whishaw, 1983; Kolb et al., 1982; Sutherland et al., 1982; Kolb, 1990). Further, as part of a series of experiments beyond this thesis, I have also shown that rats with QUIN-induced lesions of the lateral striatum are also impaired in the acquisition of the MWM; this deficit has been accompanied by apparently normal daytime locomotion, both in and out of the water maze (Furtado and Mazurek, 1992).

How does the behavior on this task compare with that of patients with HD? As I have described, persons with HD have been found to be impaired on tests requiring the processing and recall of visuospatial information, whether these tests are pencil and paper tests (e.g., Money Road Map Test, the Tower puzzles, and subtests of the Performance scale of the WAIS-R (Block Design, Object Assembly) or whether they involve the subject moving through his environment (see Potegal, 1971). To the extent that the MWM task and these neuropsychological tasks both measure spatial skills, rats with quinolinic acid-induced lesions of the striatum and patients with HD may be described as having similar types of impairment. Further, if the MWM may also be considered a test of cognitive flexibility (as suggested previously), then the rats with QUIN lesions and patients with HD may again be considered similarly impaired. As I have described previously, HD patients have been found to be impaired on tests of cognitive flexibility, most notably the Wisconsin Card Sorting Test, as well as those tests mentioned above which are also thought to examine visuospatial skills (e.g., Money Road Map, Tower puzzles).

Behavioural Tests: A. Tests of Cognitive Behaviour

II. Delayed Alternation Task

The delayed alternation task (DA) is an older test, first used to study frontal lobe, and then striatal function first in primates and then in rodents (Warren, 1964; Larsen and Divac, 1978; Divac, Markowitsch and Pritzel, 1978). The task is thought to be a test of memory as well as spatial skills (Kolb, 1990); I suggest that, like the MWM, the delayed alternation test may also examine cognitive flexibility. The task is particularly relevant in the study of HD, as Oscar-Berman and her colleagues (Oscar-Berman et al., 1982) have shown that HD patients are impaired at the primate version of the delayed alternation task. Thus, it would be interesting to determine whether QUIN-lesioned rats are impaired in the performance of this task.

The rodent version of the test involves training rats in a T-maze. Using small (e.g., 45 mg) food pellets, rats on a limited diet are trained to choose a maze arm, at the end of which food may be found. At first, both arms are baited (spontaneous alternation), but with subsequent training, only one arm is baited (delayed alternation). For example, if the right arm was baited and the right arm was chosen, then on the next trial, the left arm is baited. To obtain the food, the rat must learn to make the opposite response-hence the use of the word "alternation". The use of the word "delay" refers to the interval between trials.

Apparatus The test was conducted in an elevated T-maze constructed of wood painted flat gray. Each of the three arms of the maze is 50 cm long and 13 cm wide;

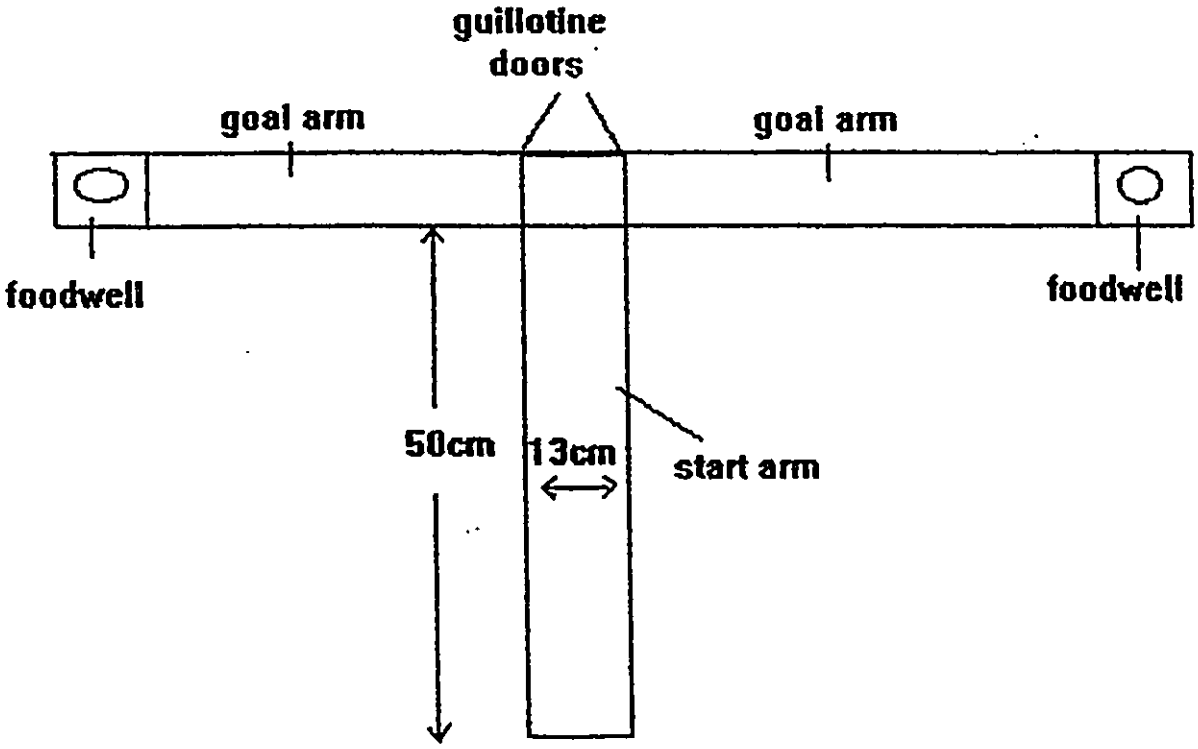
the walls of the maze are 15 cm high. The intersection of the three arms is a square, each side of which measures 13 cm. One arm serves as the start arm, and the remaining two arms serve as the goal arms; at the end of each goal arm is a foodwell, each of which is 3 cm in diametre and 2 cm deep. Guillotine doors at the beginning of each goal arm separates the goal arm from the rest of the maze. A diagram of the T-maze appears in figure 4.11.

Testing Protocol The protocol used in this experiment was derived from that used by others (Isacson et al., 1986; Dunnett et al.,1982). At the end of the MWM testing, the animals were placed on a limited diet of 10-15 grams of Purina Rat Chow per rat per day. Water was available ad libitum. The animals were placed on the restricted diet in order to bring their body weight down to 80-85% of the value at the beginning of the period of food restriction; this took approximately one week.

The first four days involved habituation to the maze. Each rat was placed in the maze and left there to consume the pellets of food distributed randomly throughout the maze and in the foodwells. (The pellets used in this experiment have been commonly used in similar behavioural experiments. Each pellet was 45 mg in weight (PJ Noyes Company, Technolab, Montreal, Quebec)). On the third and fourth days of habituation, wooden guillotine doors were introduced at the beginning of each goal arm, and if the rat entered a goal arm, the guillotine door for that arm was brought down, thus restricting the animal's movement to that particular arm.

1. Spontaneous Alternation Testing. Days 5 and 6 consisted of spontaneous

Figure 4.11 Diagram of maze for delayed alternation test, superior view.


guillotine
doors
goal arm
goal arm
foodwell
foodwell
50cm
13cm
start arm

alternation testing in which the food pellets were located only in the foodwells. Each rat was given ten trials per day; each trial consisted of placing the rat in the start arm and allowing it to choose one of the goal arms. Once the rat had entered the goal arm, the guillotine door was closed. After the rat had consumed the food pellets in the foodwell, the animal was removed from the maze, placed in a holding cage, and the side chosen was recorded. The intertrial interval was approximately 15 seconds. From the data, the number of left-right alternations made by each animal spontaneously for each day of testing was determined.

2. Delayed Alternation Testing. Days 7-12 consisted of delayed alternation testing. Eleven trials were administered to each animal per day. On the first trial, both arms were baited, then the animal was released into the maze and allowed to choose a goal arm. After the arm was chosen, the guillotine door was closed, and the animal was allowed to consume the food in the food well (if the baited arm had been chosen). The animal was then returned to the holding cage and the side chosen by the animal was recorded. On the second trial, the remaining arm was the only one baited. The trial was conducted as usual, and the side chosen was recorded. The intertrial interval was approximately 15 seconds. If the animal chose the baited arm, the opposite arm was baited for the next trial. If the animal did not choose the baited arm, the same arm was still baited on the next trial. The number of correct responses per day was counted for each animal.

Results.

Spontaneous Alternation. The results are graphed in figure 4.12. The ANOVA conducted on the number of spontaneous alternations in revealed a significant effect for Group $F(1,14)=126.477$, $p<0.0001$), indicating that the CONT group produced more alternations spontaneously than the QUIN group.

Delayed Alternation. The data from the DA testing may be analyzed in two ways. Following the traditional method, the results are graphed in figure 4.13. The ANOVA conducted on the delayed alternation data revealed a significant effect for significant effect for Group ($F(1,14)=239.464$, $p<0.0001$) and Days of Delayed Alternation Training ($F(5,65)=4.206$, $p<0.01$). This indicated that the 1) the CONT group made significantly more correct responses than the QUIN group and 2) that both groups of animals made more correct responses with progressive delayed alternation testing.

However, if one were to consider that the difference between the two groups in terms of absolute DA performance is influenced, at least in part, by the difference in the spontaneous alternation performance, then one might consider a different type of analysis. Alternatively, the SA data might be considered as a measure of baseline performance, and the DA data as changes from baseline performance. If a mean SA value (i.e., baseline) is calculated for each animal and this value is subtracted from each day's DA testing value, then it becomes possible to plot a change from baseline performance for each animal. These results appear in figure 4.14; the data do not suggest a difference between the two groups.

Figure 4.12 Spontaneous alternation

Spontaneous Alternation

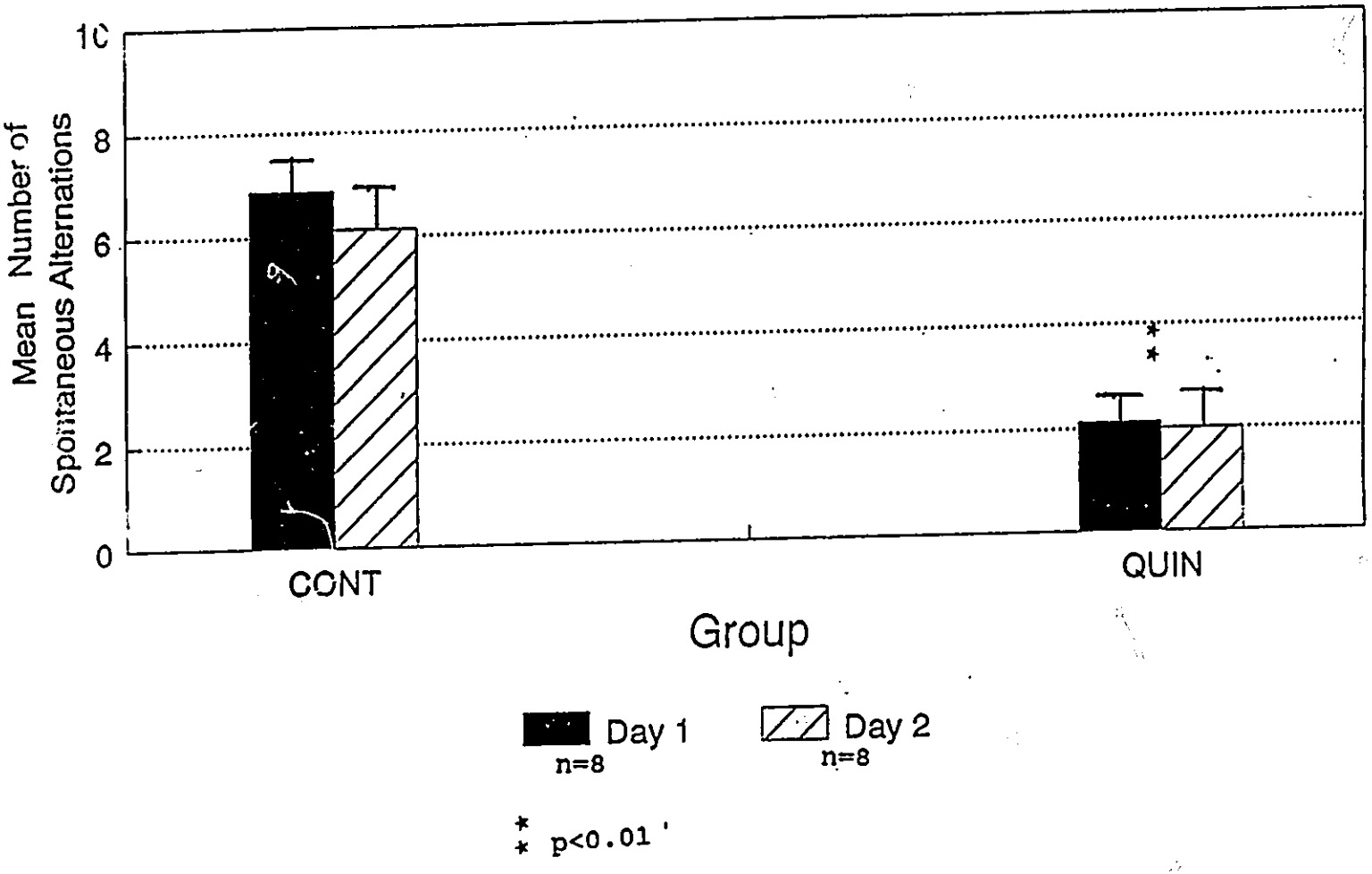


Figure 4.13 Delayed alternation

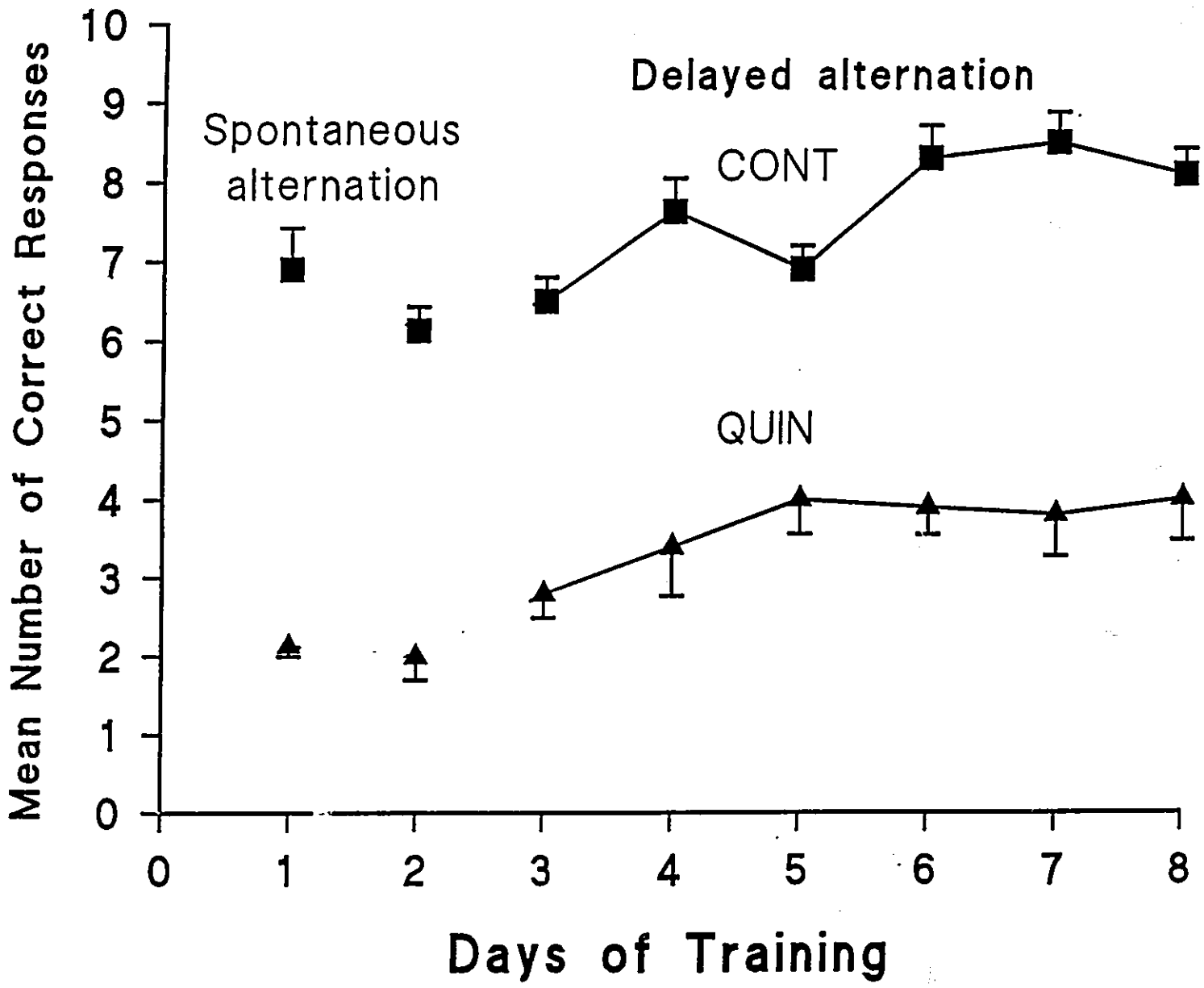
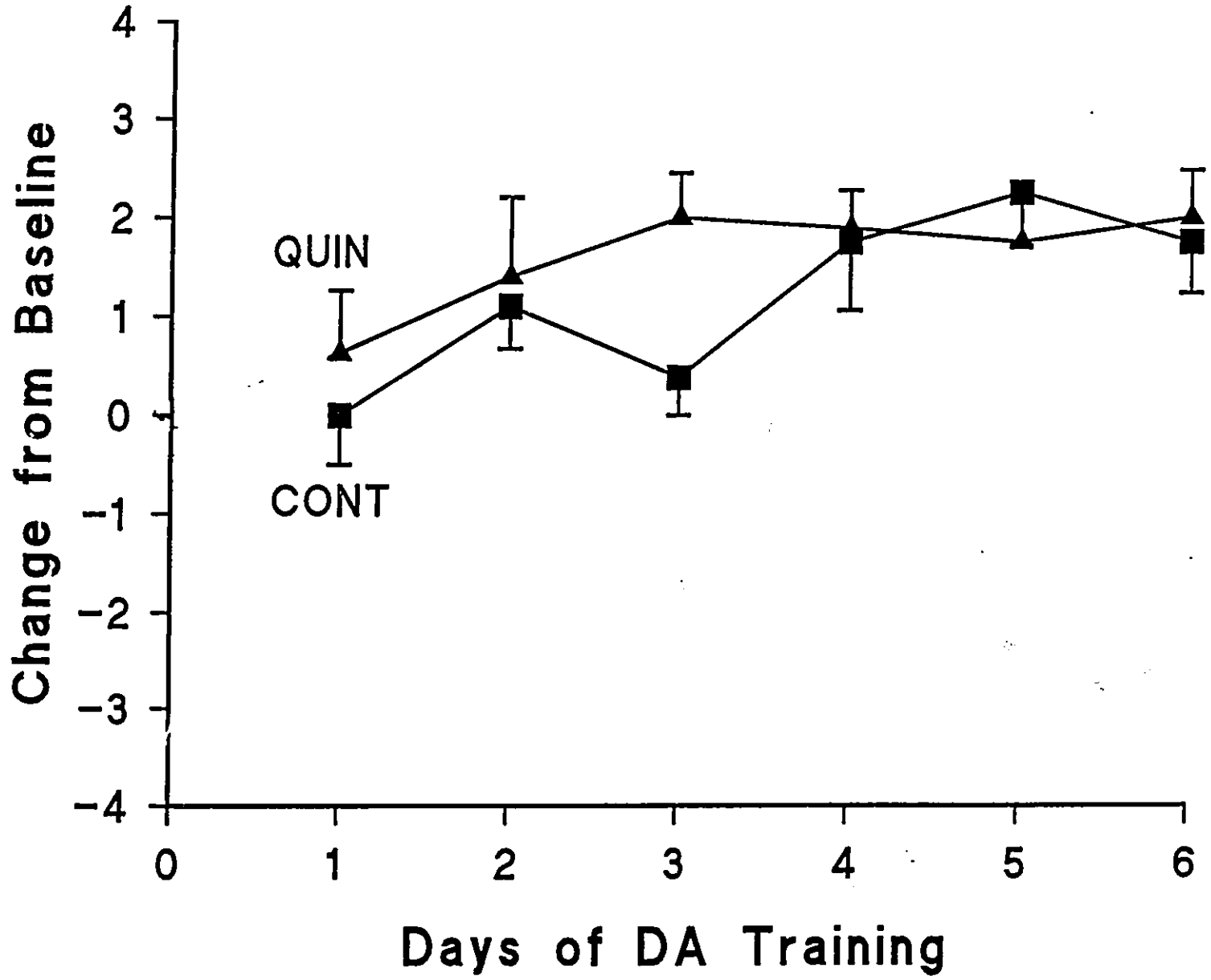

Mean Number of Correct Responses
10
9
8
7
6
5
4
3
2
1
0
Spontaneous alternation
Delayed alternation
CONT
QUIN
0
1
2
3
4
5
6
7
8
Days of Training

Figure 4.14 Change from baseline values, delayed alternation test.


Change from Baseline
4
3
2
1
0
−1
−2
−3
−4
QUIN
CONT
0
1
2
3
4
5
6
Days of DA Training

Discussion.

This test has shown that the QUIN group produces fewer spontaneous alternations than he control group. This difference may be suggestive of decreased motivation for exploration or possibly even perseverative tendencies in the QUIN group.

However, the DA data is somewhat more difficult to interpret. Given the traditional analytic method, the QUIN group is again significantly impaired, in relation to the control group, in the performance of the delayed alternation task. Since the DA task is considered to be a test of spatial learning and memory, as well as a test of cognitive flexibility, it may be suggested that the QUIN group is impaired in these abilities. However, using the second analytic method, there does not appear to be any difference between the two groups where DA performance is concerned.

This pattern of SA and DA performance parallels that found with rats with kainic acid- or ibotenic acid-induced striatal lesions where similar impairments were found (Divac, Markowitsch and Pritzel, 1978; Isacson, Dunnett and Björklund, 1986). This finding also parallels that of rats with frontal lesions (Larsen and Divac, 1978; Divac, Wikmark and Gade, 1975; Kesslak et al., 1986; Labbe et al.,1983; Dunnett et al., 1987b).

Behavioural Tests: B. Tests of Motor Behaviour

The motor tests were selected for three reasons. First, as discussed previously, they were part of the larger battery of tests chosen to reduce the possibility of making erroneous generalizations from the results of a single test or from tests of a single kind (i.e., tests of cognition). Second, all the tests (cognitive and motor) were chosen because of their relevance to HD: it is known that HD patients demonstrate cognitive impairment similar to that demonstrated by patients with frontal lobe damage. The tests chosen are those in which rats with lesions of the frontal cortex have been found to be impaired. Thus, the motor tests would provide a contrast with the learned tests of behaviour (e.g., MWM, delayed alternation). Finally, the motor tests were performed in order to exclude the possibility that the impairment demonstrated on the two cognitive tests was not due to impaired motor abilities.

For all tests described below, the animals were under the limited food regimen described for the delayed alternation task. These tests were conducted largely in parallel with the delayed alternation task.

I. Spontaneous Daytime Locomotor Activity. Spontaneous daytime activity of rats on the limited diet was measured in the Digiscan Animal Activity Monitors, using methods described previously (see chapter on nocturnal activity). Procedure. The rats were placed in the Digiscan Animal Activity Monitors (Omnitech, Columbus, Ohio) and their spontaneous activity recorded from 1300h-1500h, in the light part of the day/night cycle. The two hour period was broken up into eight samples of 15 minutes,

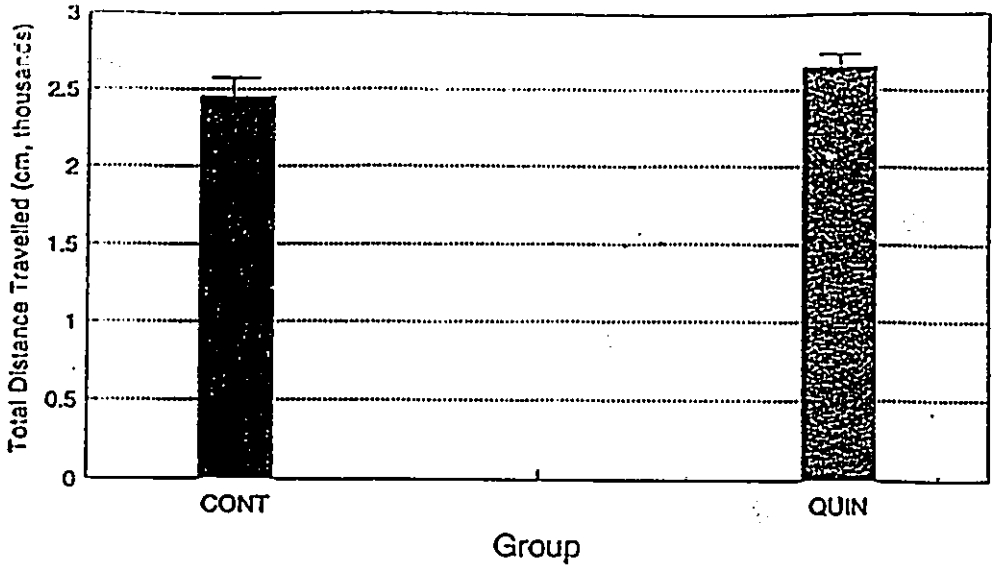
each. As described before, the Digiscan Animal Activity Monitors are designed to routinely gather data for a large number of dependent variables; the variables total distance travelled, number of movements, average speed, movement time and rest time were chosen for analysis.

Results The data from each sample were totalled and the means are presented in figures 4.15 and 4.16. The groups were not significantly different from each other on any of the variables assessed.

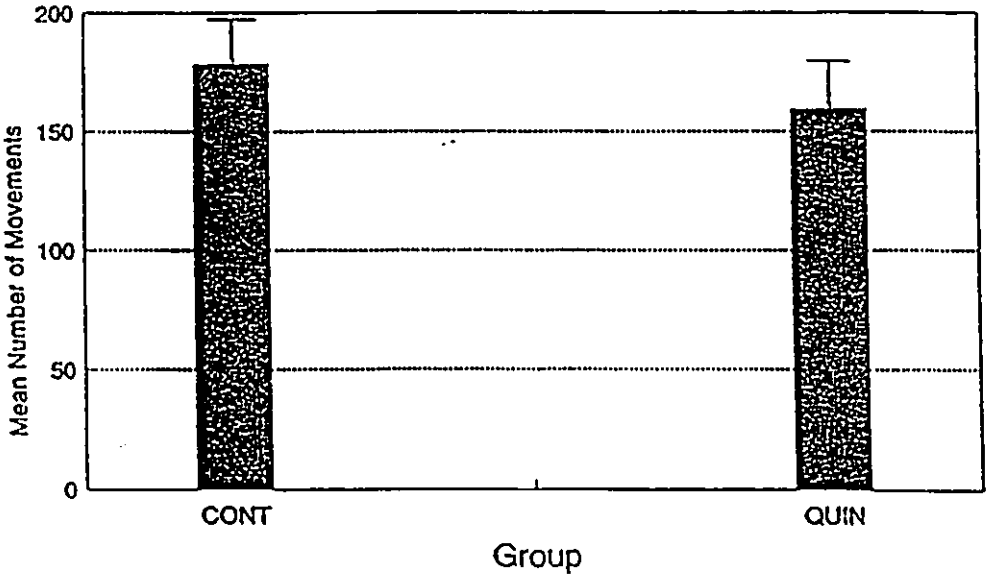
Discussion. Fibiger (1978) has reported that rats with kainic acid-induced striatal lesions are no different from controls where spontaneous daytime activity is concerned; my data shows a similar finding for QUIN-induced striatal lesions. The results from this test may also be helpful in elucidating the explanation for the impairment found in the QUIN group in the Morris Water Maze and the Delayed Alternation Task. One potential criticism of my interpretation of the results from the MWM may be that the longer latency demonstrated by the QUIN group in the MWM is reflective of general motor abnormalities on the part of the QUIN group. The results from the assessment of spontaneous daytime locomotor activity demonstrate that the QUIN animals were no different from the CONT animals, as assessed by the Activity Monitors, thus strengthening the interpretation of an impairment in cognitive function on the part of the QUIN group.

Figures 4.15-4.16 Spontaneous daytime activity: total distance travelled, number of movements, average speed, movement time and rest time.

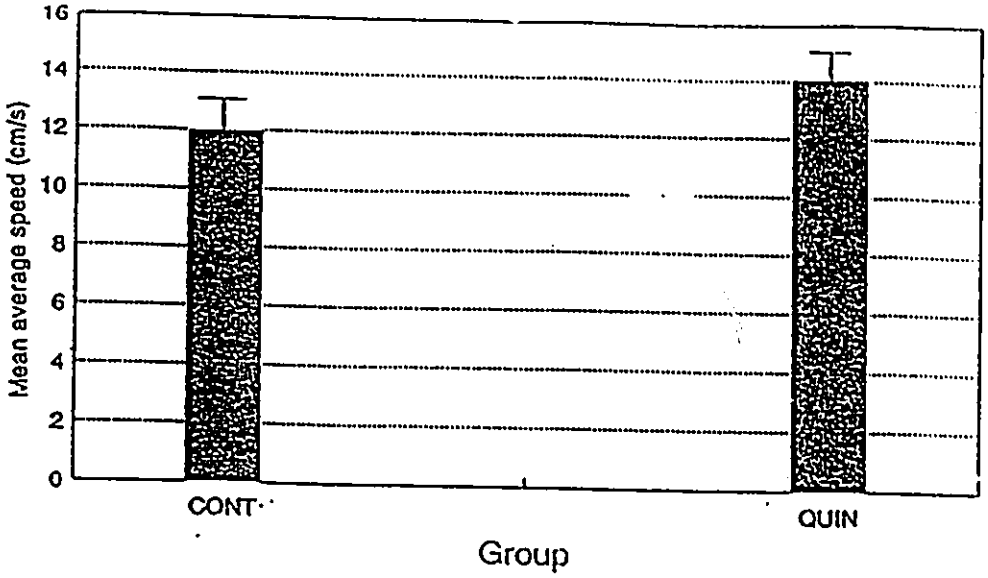
Total Distance Travelled



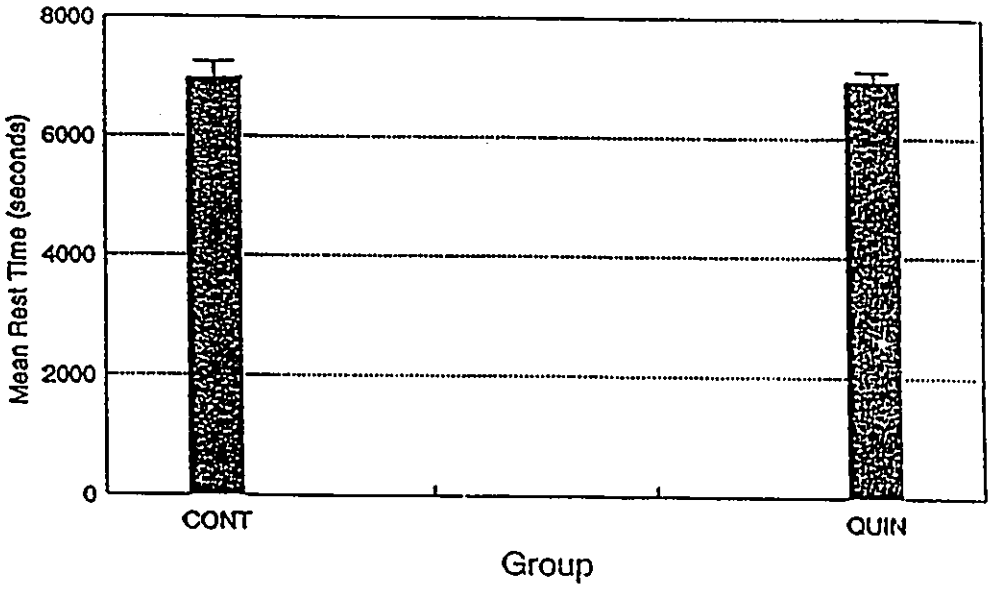
Number of Movements



Average Speed



Rest Time (seconds)



II. Spontaneous Nighttime Locomotor Activity. Locomotor activity was measured overnight, from 1600h-800h (the last twelve hours were in the dark).

Procedure Animals were food deprived for 24 hours prior to the commencement of testing. The apparatus was the same as that used in the testing of daytime activity. Each sample was 30 minutes long, 32 samples were gathered, and variables similar to those chosen in daytime activity were used.

Results No differences were found between the CONT and QUIN groups.

Discussion These results are in agreement with my previous finding (refer to Chapter 3) of no difference between QUIN- and CONT animals where nocturnal activity is concerned.

III. Food Manipulation The test of food manipulation has proven to be sensitive to damage in certain areas of cortex and striatum.

Procedure. It has been noted that if an animal is to be tested within a cage, then this cage should either be the home cage or a cage to which the animal has habituated (Whishaw et al., 1983). Thus, rats were first habituated to the cage used in this experiment by placing each animal in the cage to be used for 10-15 minute periods for four to five days preceding testing. For the test itself, each animal was tested individually. The animal was placed in the testing cage and one Purina Rat Chow food pellet was introduced onto the floor of the cage. Each animal was observed for a ten-minute period, or until the entire pellet was consumed, whichever came first. At the

end of the observation period, a score was assigned to each animal, based on a scale used by other researchers (Kolb and Holmes, 1983):

0: unable to manipulate pellet with forepaws

1: held pellet against floor while eating

2: held pellet sometimes on floor, sometimes in paws

3: picked up pellet in forepaws and ate, but dropped prior to completion

4: picked up pellet in forepaws and ate until complete.

The test was conducted twice, about one week apart, and again, three months after surgery.

Results. The data are presented in Table 4.1. Since the animals had been habituated to the cage and were on a limited diet, they found and started to consume the food pellet quickly. Since the data is ordinal in nature, the Friedman ANOVA by Ranks was conducted. The differences between the groups were not significant, indicating that the QUIN rats were not impaired, in relation to the CONT rats, where food manipulation is concerned.

Discussion. Pisa (1988b) has found that rats with ibotenic acid-induced lesions of the lateral, but not the medial, striatum demonstrate deficits in the manipulation of food pellets. Similarly, in another set of experiments, I have shown that rats with QUIN-induced lesions of the lateral striatum are significantly impaired in the food manipulation test; in contrast, the rats with QUIN-induced medial striatal lesions in this experiment failed to show a deficit (Furtado and Mazurek, 1992). This would seem to provide further support for the result found in the present study as well as

emphasizing the validity and sensitivity of this test.

Others have found that rats with aspirative lesions of the medial frontal and motor cortex demonstrate a significant impairment where food manipulation was concerned (Kolb and Holmes, 1983; Kolb and Whishaw, 1983b); in those studies, no deficit was demonstrated by cohorts of controls, or those with orbital frontal, or parietal cortical lesions.

Thus, the available literature on striatal lesions would seem to be in agreement with my finding, namely that rats with QUIN-induced lesions of the medial striatum are not impaired, in relation to controls, where food manipulation is concerned. The more interesting issue arises from the available literature on cortical lesions, and has implications for the evaluation of this animal model. This will be discussed later.

IV. Swim Posture. The test of swim posture has been shown to be sensitive to lesions in various cortical areas.

Procedure. Each animal was tested individually. Rats were placed in a pool of clear water, at 20 degrees, Celsius, and allowed to swim for approximately one minute. The posture of the forelimbs during swimming was observed, and at the end of the testing period, a rating was given to each animal according to the following scale (Kolb and Holmes, 1983):

0: no forepaw inhibition

1: one forepaw inhibited for at least one stroke

2: both forepaws inhibited for at least one stroke

3: both forepaws inhibited for a number of strokes

4: both forepaw inhibited for most of the swim.

If the animal did not move a given forepaw during the swim, this was defined as "inhibition" of the forepaw. The animals were also tested in water at 30 degrees, Celsius, since it has been found that different water temperatures may produces different responses, according to the locus of the lesion (Whishaw et al., 1983).

Results. The data for the swim posture test for are presented in Table 4.2. Again, as the data was ordinal in nature, a nonparametric test was required for statistical analysis, and so the Kruskal-Wallis H test was conducted. The differences between the groups were not significant, indicating that the QUIN group was no different from the CONT group, where swim posture is concerned.

Discussion. To the best of the author's knowledge, there is no data within the literature concerning the swim posture tests and rats with striatal lesions. My own experiments with QUIN-induced lesions cf the lateral or medial striatum have not shown group differences (Furtado and Mazurek, 1992). However, it is worth noting that rats with aspirative ablations of the medial and orbital frontal, motor and parietal cortex in adulthood have been found to be significantly impaired, in relation to controls, in this test (Kolb and Holmes, 1983; Kolb and Whishaw, 1983b). The implications for this difference will be discussed.

V. Tongue Extension. This test was carried out in order to determine whether the two groups were different from each other with respect to tongue extension.

Apparatus. Each animal was tested individually. The test was carried out in boxes with three metal sides and one perspex side and perspex roof. One of the metal sides had various slots that could be opened and closed by the experimenter. In order to prepare the rats for testing in these boxes, each animal was habituated to the box for a period of 15-20 minutes for five consecutive days.

Procedure. Once the animals were habituated, they were trained to eat cookie mash from a spatula through one of the slots (5 cm x 3 cm, located 6 cm above the floor of the cage) in one of the metal cage sides. At first, the spatula with mash was inserted into the cage. Later on, the spatula was withdrawn and held so that the narrow edges were oriented vertically and the tip touched the outside of the cage. The slot was too small to permit the use of the paws, and therefore, the animal was forced to use its tongue. On the first day of mash presentation, the rat was given mash for a period of 10 minutes via the 5 cm x 3 cm slot. On the second day of mash presentation, the size of the slot was decreased to 1.5 cm x 1.5 cm by moving a piece of perspex across most of the opening. This reduced the opportunity for the animal to stick its nose through the slot, thereby forcing the animal to use its tongue to a greater extent to obtain the mash. The third and fourth days comprised the testing sessions. For each trial, the end of a stainless steel ruler (approximately two centimetres or so) was covered with a thin layer of cookie mash and presented to the animal. As in the training sessions, the tip of the ruler just touched the front of the cage at the location

of the slot and did not extend into the cage; further the narrow edge of the ruler was oriented vertically. Each animal was given three trials of 15 seconds each per day, for two days. At the end of each trial, the area cleared of mash on the ruler was recorded to the nearest millimetre.

Results. The results are presented in Table 4.3. The two groups did not differ from each other in the test of tongue extension.

Discussion. Another author (Pisa, 1988b) has reported tongue extension to be impaired only in rats with lateral, and not medial, striatal lesions. Again, in another set of experiments, I have confirmed that rats with QUIN-induced lesions of the lateral striatum are grossly impaired in the tongue extension test, while cohorts of animals with medial striatal lesions are unimpaired on the same test, thus providing additional support for the present study (Furtado and Mazurek, 1992).

Further, rats with lesions of the orbital frontal and motor (Kolb and Holmes, 1983; Kolb and Whishaw, 1983b) cortex have also been found to be impaired in tongue extension when compared to controls. Thus, the animals in this study were different both from rats with lateral striatal lesions and also rats with frontal cortex lesions.

Histology. Six months post-surgery, the animals were sacrificed. The rats in both groups were overdosed with sodium pentobarbital, perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. The brains were removed and post-fixed overnight in 4% paraformaldehyde at 4 degrees Celsius. The next morning, the tissue

Table 4.1 Mean ratings for food manipulation

Mean Ratings for Food Manipulation

	First testing	Second Testing
CONT	3.00	2.63
QUIN	2.63	2.63

Table 4.2 Mean ratings for swim posture

Mean Rating for Swim Posture

Water Temperature=20 degrees, Celsius

CONT	3.875
QUIN	3.75

Water Temperature=30 degrees, Celsius

CONT	3.75
QUIN	3.75

Table 4.3 Mean tongue extension

Tongue extension-Mean area cleared from the ruler (mm)

	First testing	Second testing
CONT	7.1, SE=0.3	6.0, SE=0.19
QUIN	6.5, SE=0.32	6.1, SE=0.21

was rinsed in 0.1 M phosphate buffer and the tissue then transferred to 30% sucrose in 0.1 M phosphate buffer. After the brains sank (2-3 days), each brain was flash-frozen in 2-methylbutane cooled to -55 degrees Celsius and cut at a thickness of 40 um in a cryostat (Reichert). Every section from the frontal pole through the striatum was collected on chrom-alum subbed slides. The tissue was subsequently stained with cresyl violet, dehydrated and coverslipped out of xylene.

Histological Analysis

Sections at the following levels of Paxinos and Watson (1982) (relative to bregma) were selected for analysis: +2.7, +1.7, +1.2, 0.7, -0.3. These sections were expanded and traced using a Bausch and Lomb projector. The area of the striatum and neocortex was assessed in these planes, according to the boundaries defined in figures 4.17 and 4.18.

Area was assessed using a Bioquant System IV software package (R and M Biometrics, Nashville, Tennessee). The individual tracings were placed on a a Hipad Digitizer software tablet (Houston Instruments, Austin, Texas) connected to an IBM Personal Computer. Area was measured using a cursor (Houston Instruments) attached to the software tablet. For each individual structure, two measurements were taken and the mean of the two was calculated.

Results Representative sections for the CONT and QUIN groups are presented in figures 4.19-4.22. The means for cortical area and striatal area appear in Tables 4.4 and 4.5. This data is presented diagrammatically in figures 4.23-4.26. The data were analyzed for side (right hemisphere, left hemisphere), coronal plane (2.7, 1.7, 1.2,

0.7, -0.3), and group (QUIN, CONT). Two separate analyses were conducted for the striatum and the neocortex.

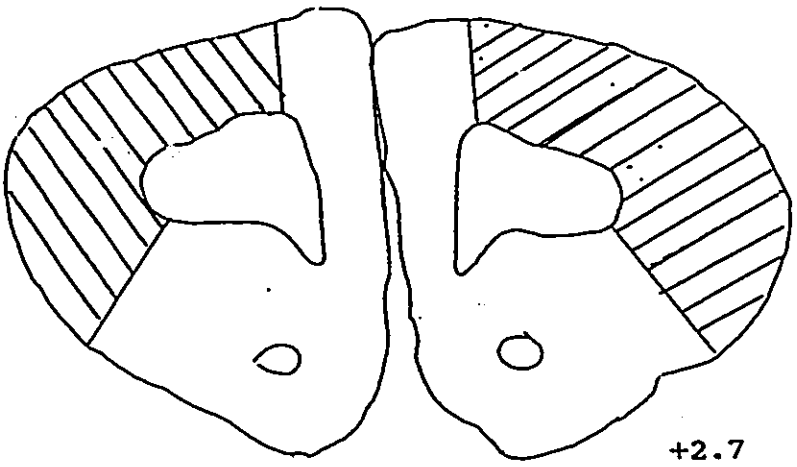
Striatal area in the QUIN group was considerably smaller than that in the CONT group, as indicated by the significant effect for Group ($F(1,80)=188.267$, $p<0.00001$). The effect of Coronal Plane was also significant ($F(3,80)=13.199$, $p<0.0001$), indicating that striatal area increased with rostro-caudal progression; the effect Group x Coronal Plane was significant ($F(3,80)=7.151$, $p<0.001$), indicating that the striatum was considerably smaller in the QUIN than the CONT group at all planes examined.

The analysis for cortical area did not reveal any significant effects, except that for Coronal Plane ($F(4,98)=3.983$, $p<0.01$), indicating an increase in cortical area with rostro-caudal progression.

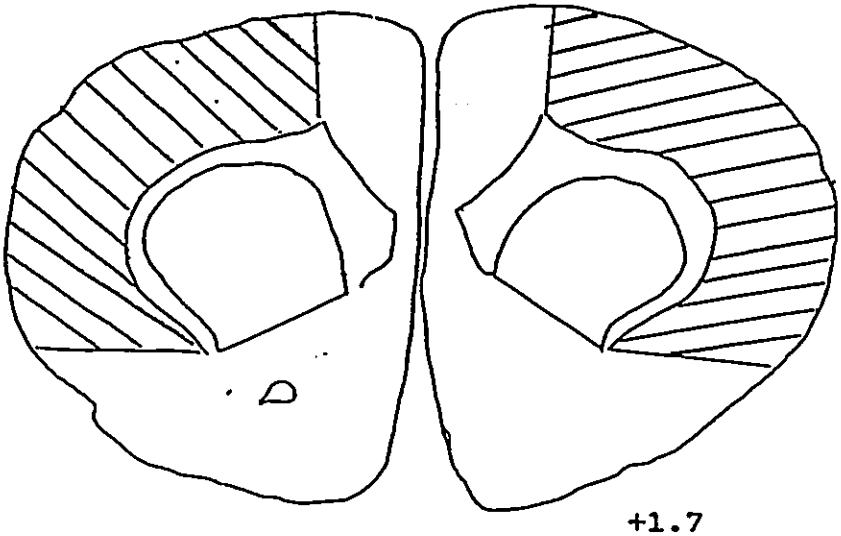
Discussion The analysis for striatal area indicated significantly less striatal tissue in the QUIN group than the CONT group, reflecting the effect of the intrastriatal injection of quinolinic acid in the QUIN group. The analysis for cortical area did not reveal any significant effects between groups. Thus, this has demonstrated that the intrastriatal injection of quinolinic acid will produce significant striatal atrophy, but no evidence (on a gross level) of degeneration of the cortical ribbon. Having noted this result, it is important to note that neurochemical analysis or cell counts may reveal degenerative effects in the neocortex that are not reflected at a macroscopic level. Further, it may well be the case that even longer post-surgery survival times will reveal degenerative effects in the frontal cortex.

No significant asymmetry was noted in cortical area in the CONT group. This is

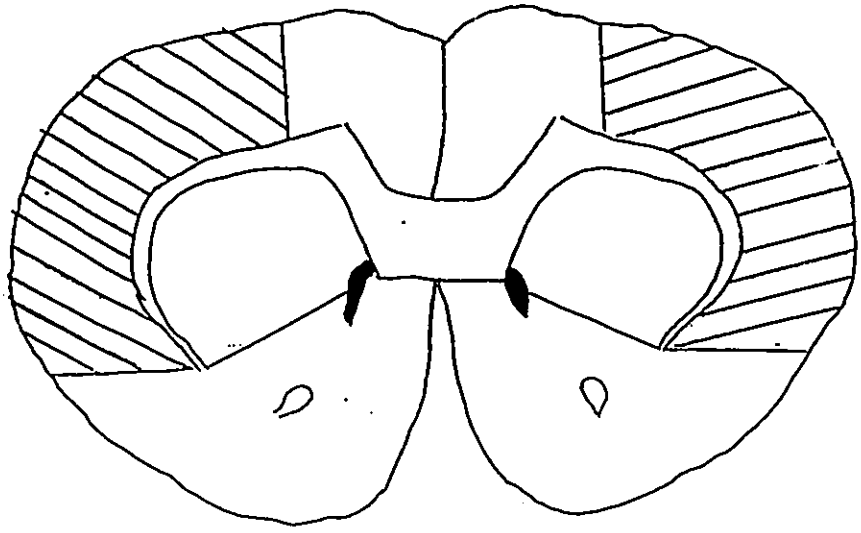
Figure 4.17 Controls. Delineation of cortex and striatum boundaries for areal measurements.



+2.7



+1.7



+1.2

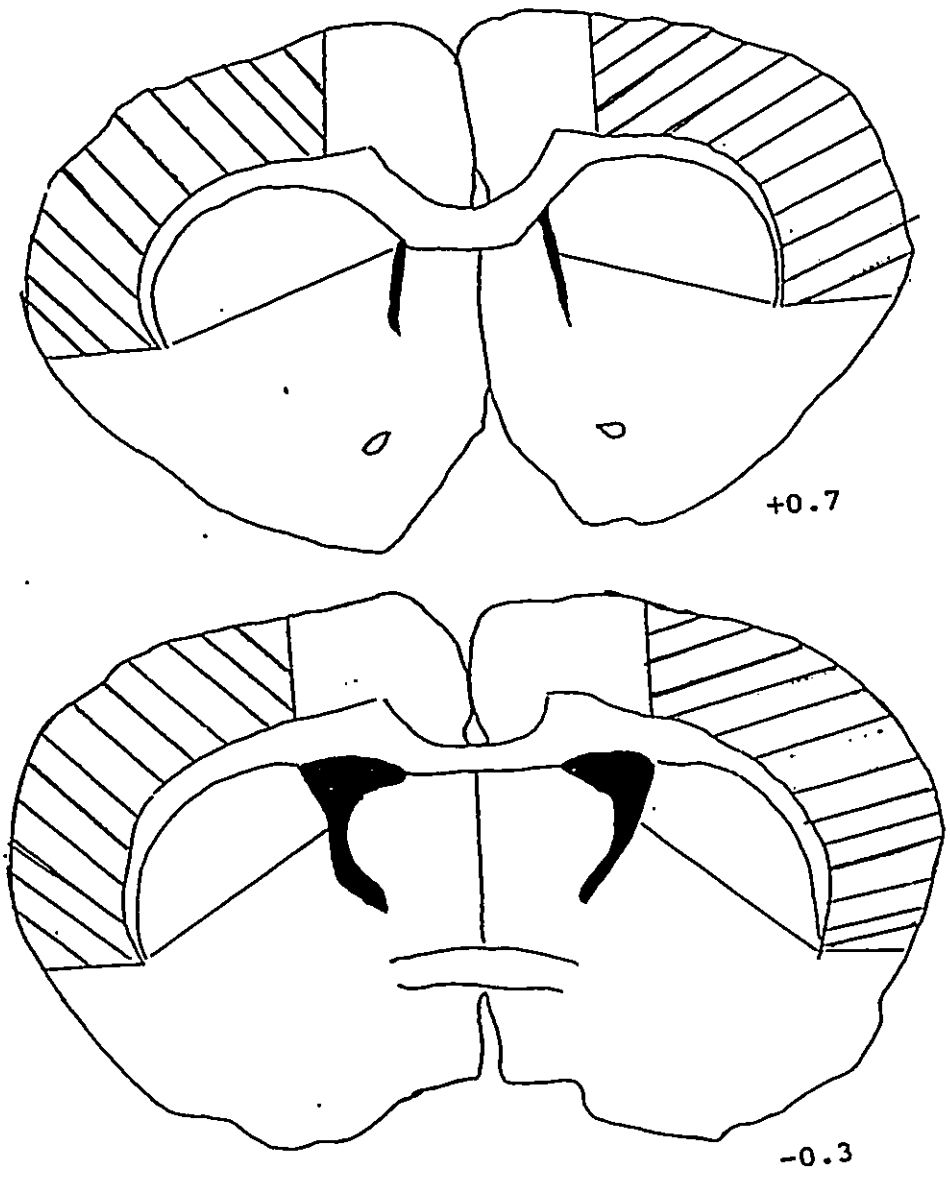
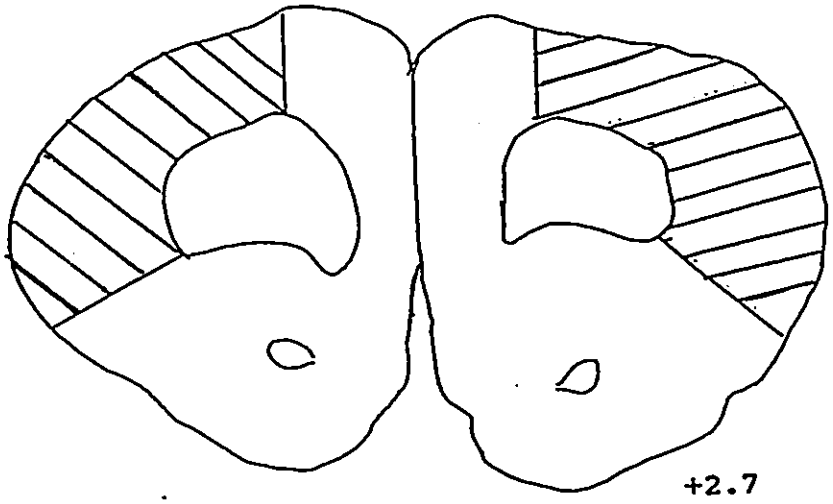
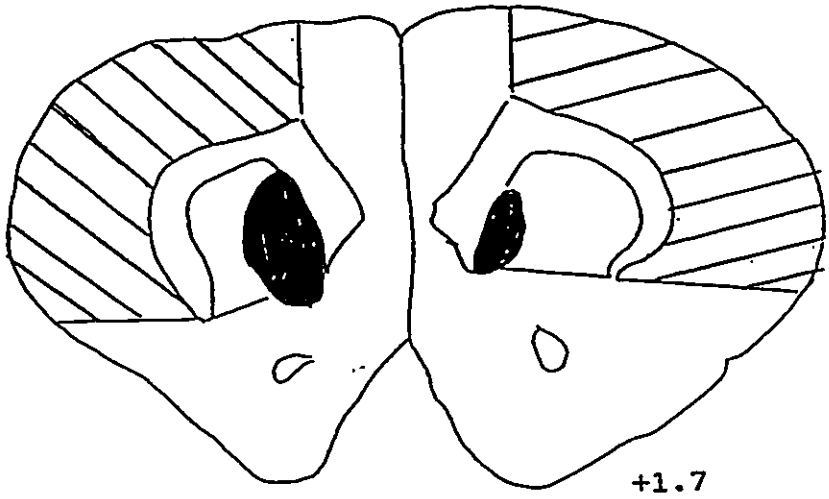

+0.7
-0.3

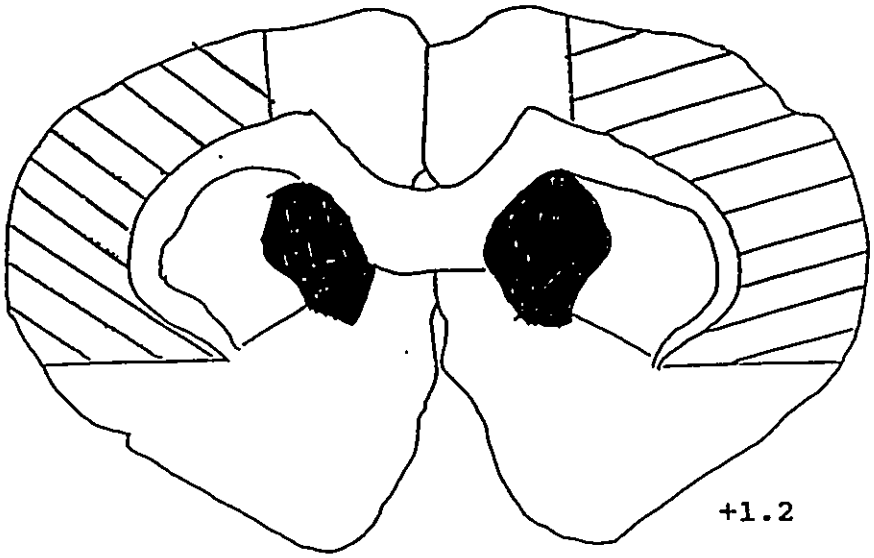
Figure 4.18 QUIN-induced striatal lesions. Delineation of cortex and striatum boundaries for areal measurements.



+2.7



+1.7



+1.2

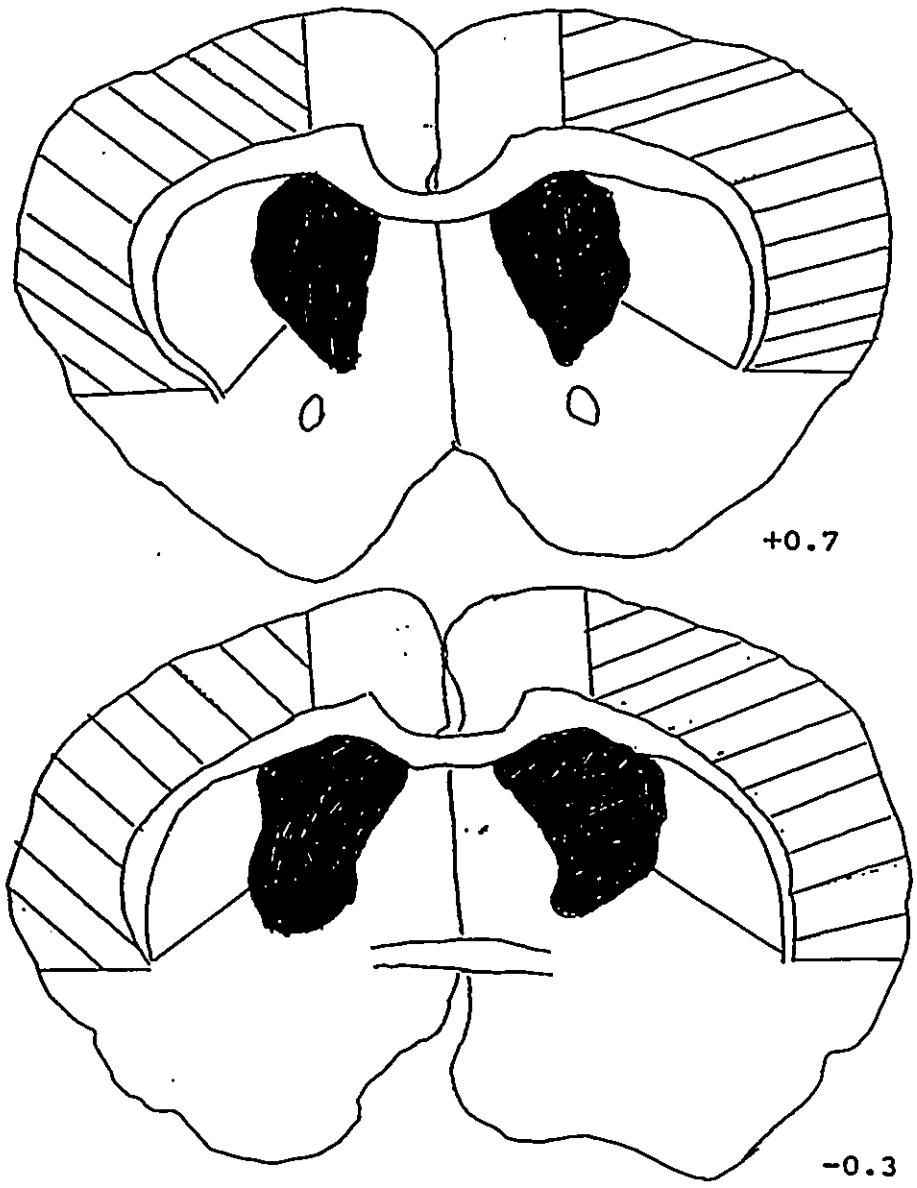
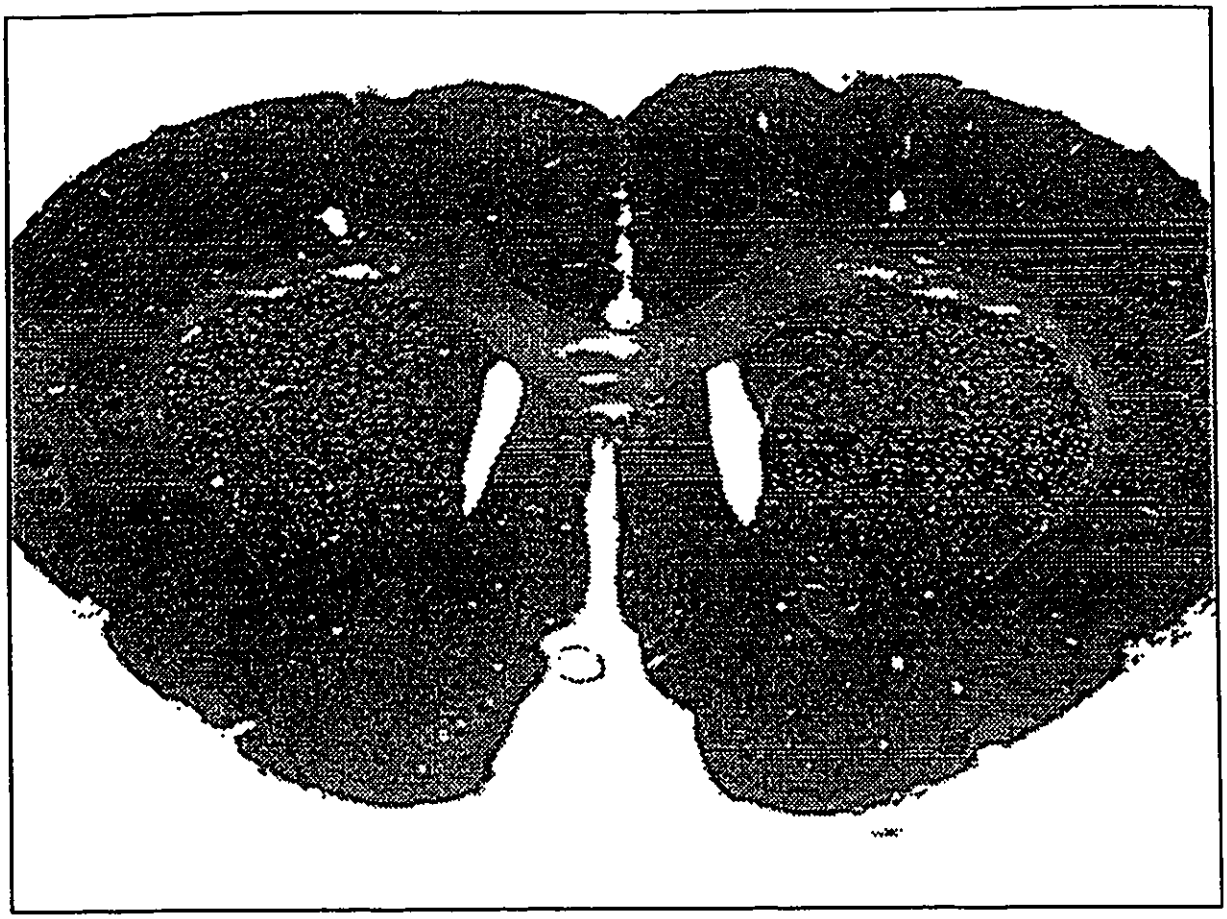
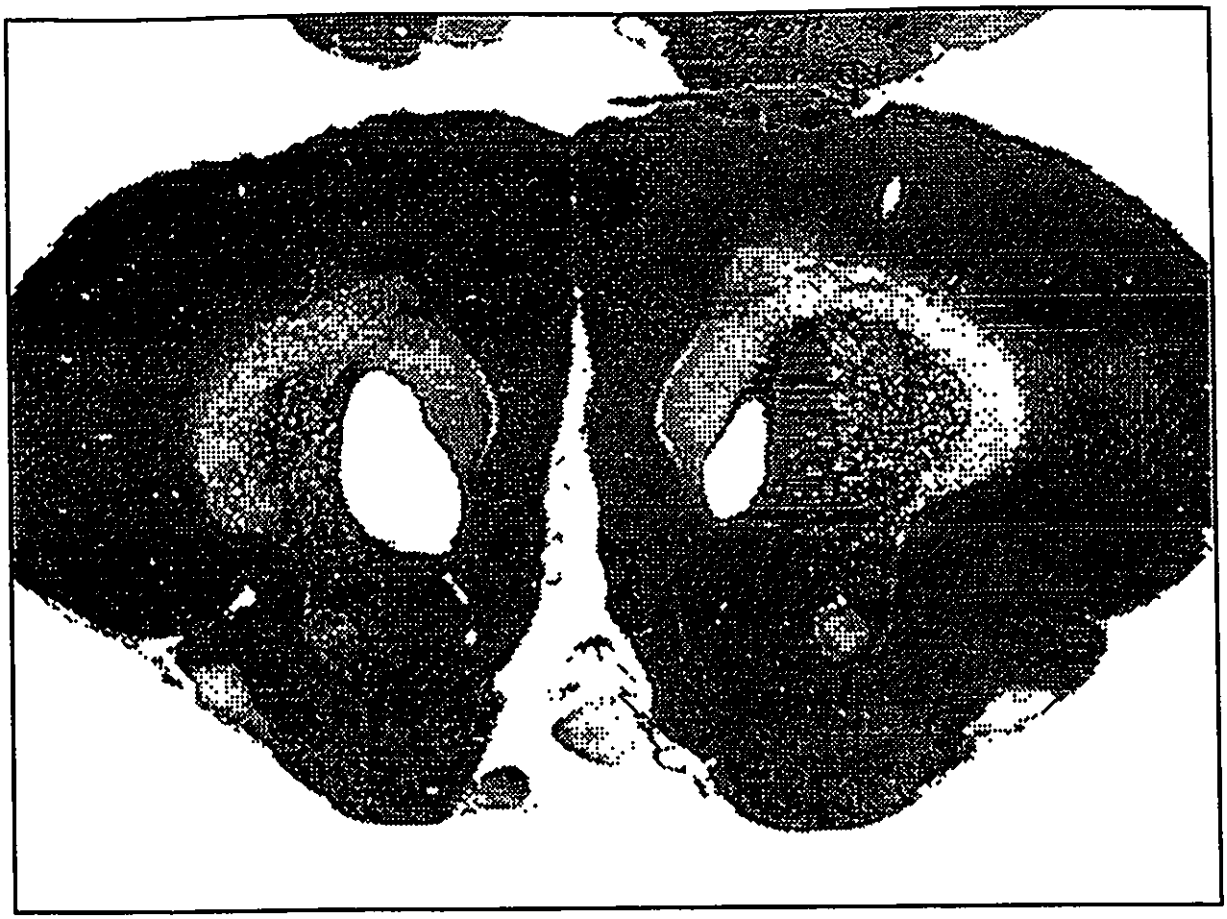
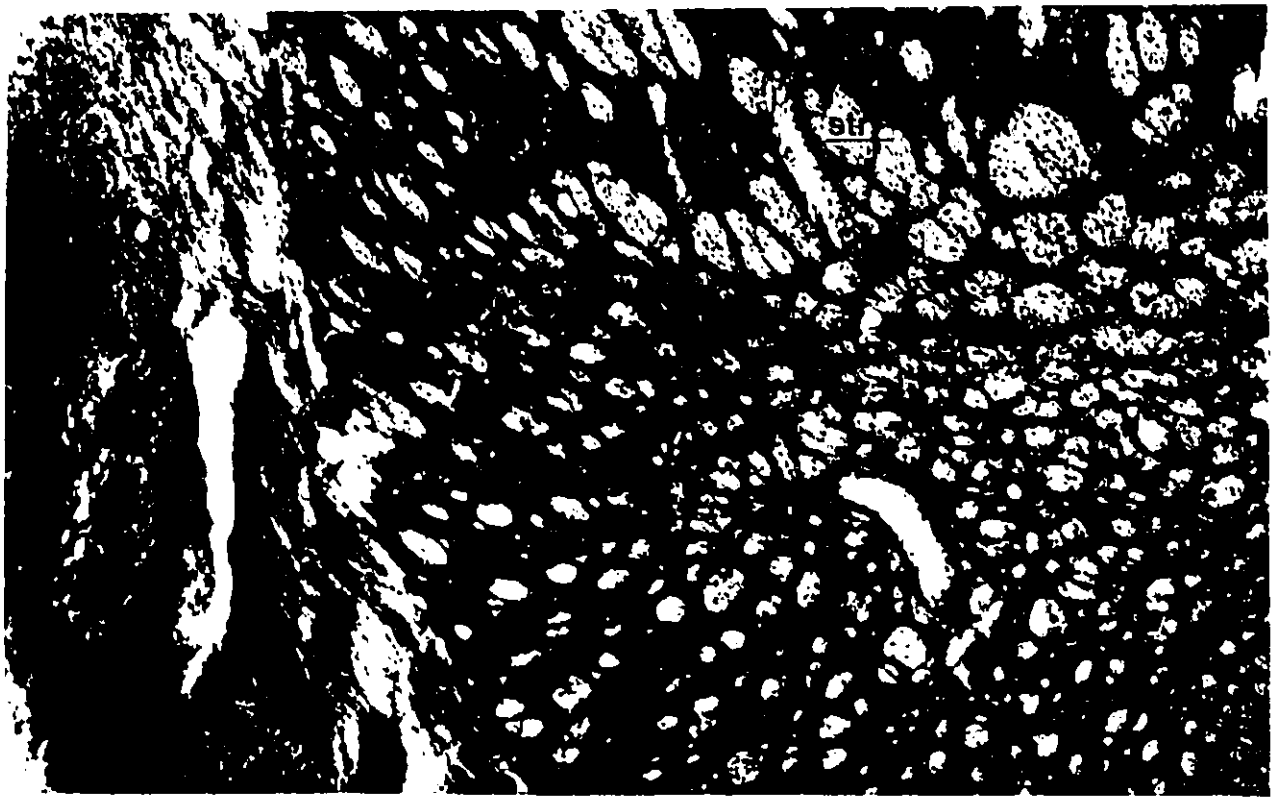

+0.7
-0.3

Figure 4.19 Medial striatum, CONT group; Nissl stain. Representative section.



Figures 4.20-4.22 Medial striatum, QUIN group; Nissl stain. Representative sections.
Figure 4.20 A/P +1.7 Figure 4.21 A/P +1.7, X 4; striatum in the area of the lesion.
Figure 4.22 A/P +0.7




ste

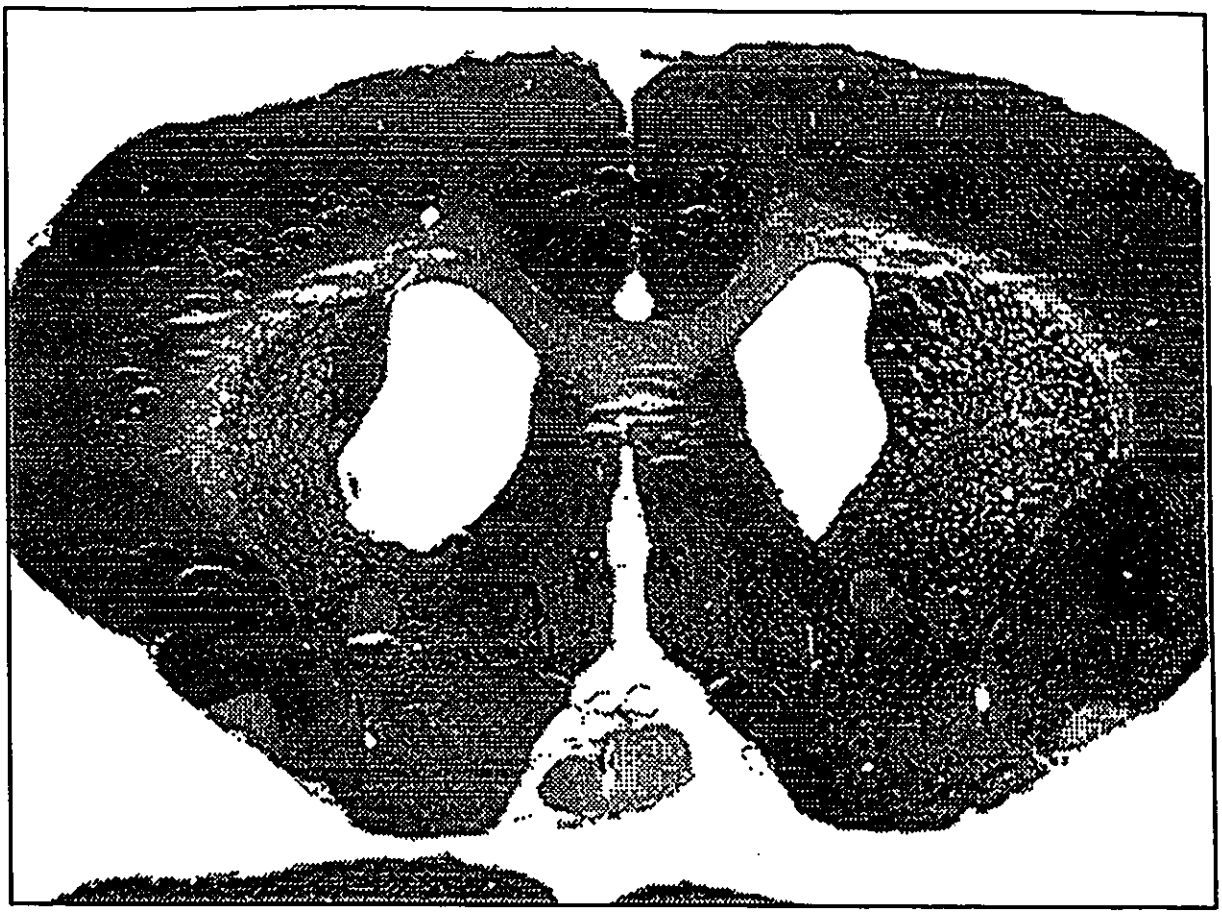


Table 4.4 Areal data for cortex and striatum, CONT.

Mean area (mm2) and associated SEs

Group

Controls

Plane	Cortical area Hemisphere left	Cortical area Hemisphere right	Striatal area Hemisphere left	Striatal area Hemisphere right
2.7	1994.2	2042.3		
	84.5	76.1		
1.7	2040.5	1983.6	873.9	804.5
	61.6	112.4	48.7	52.2
1.2	1967.1	1905.6	1167.4	1155.3
	76	89.5	79.7	78.5
0.7	1946.8	1942.2	1114	1048.3
	80.2	96.7	64.9	52.1
-0.3	2093.6	2135.1	908.9	850.4
	79.8	93.4	50.8	54.1

Table 4.5. Areal data for cortex and striatum, QUIN.

Mean area (mm2) and associated SEs

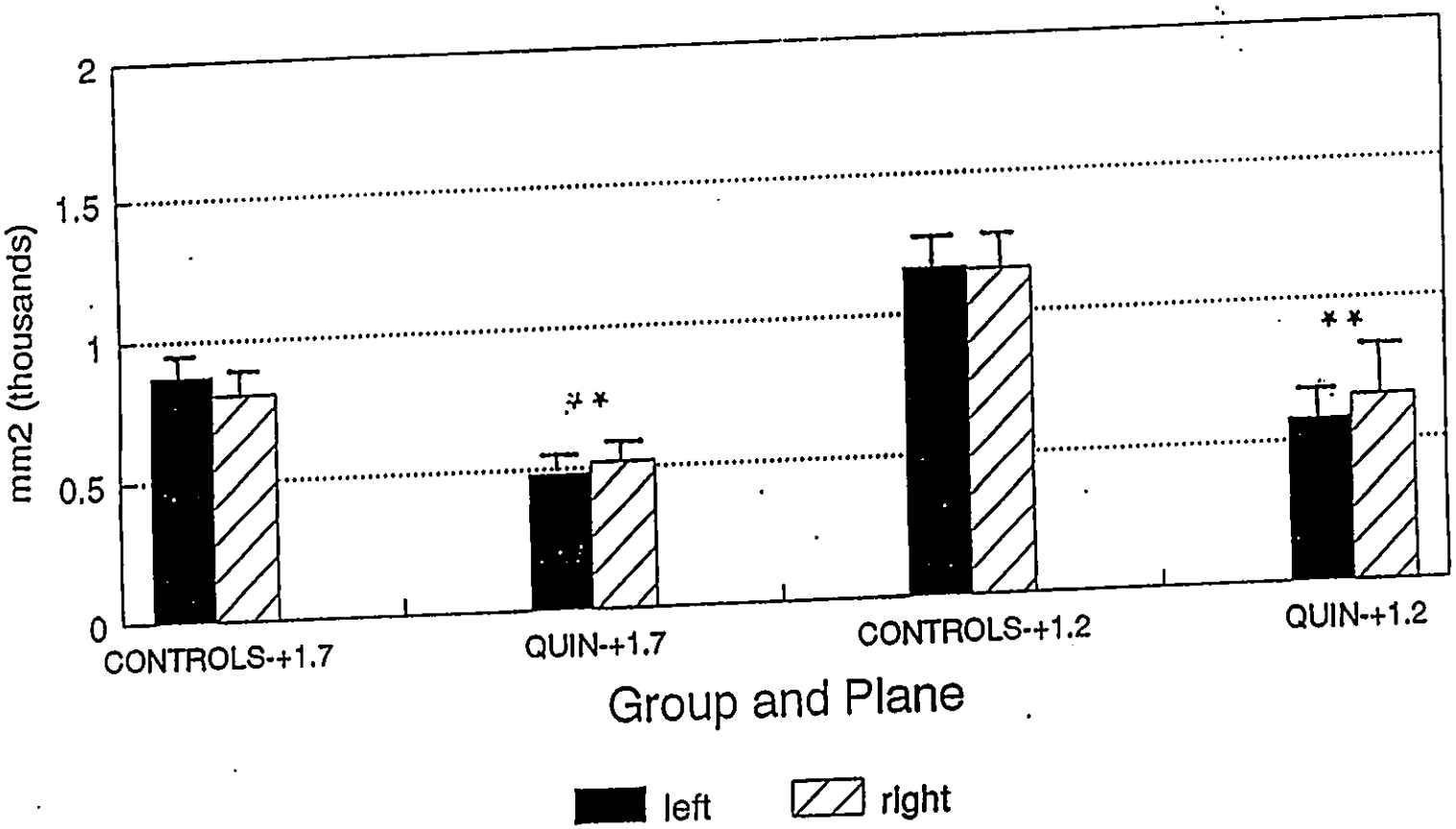
Group

QUIN

Plane	Cortical area Hemisphere left	Cortical area Hemisphere right	Striatal area Hemisphere left	Striatal area Hemisphere right
2.7	1717.2 129.3	1874.1 68.9		
1.7	2094.1 91.7	1907.4 71.9	484.4 54.9	520.5 34.4
1.2	1901.2 78.2	580.4 93.7	655.9 66	685.8 48
0.7	2005.9 85.6	2039.8 118.7	599.7 48.4	709.8 33.4
-0.3	2115.3 89.5	2241.5 50.1	680.9 46.2	685.8 38.2

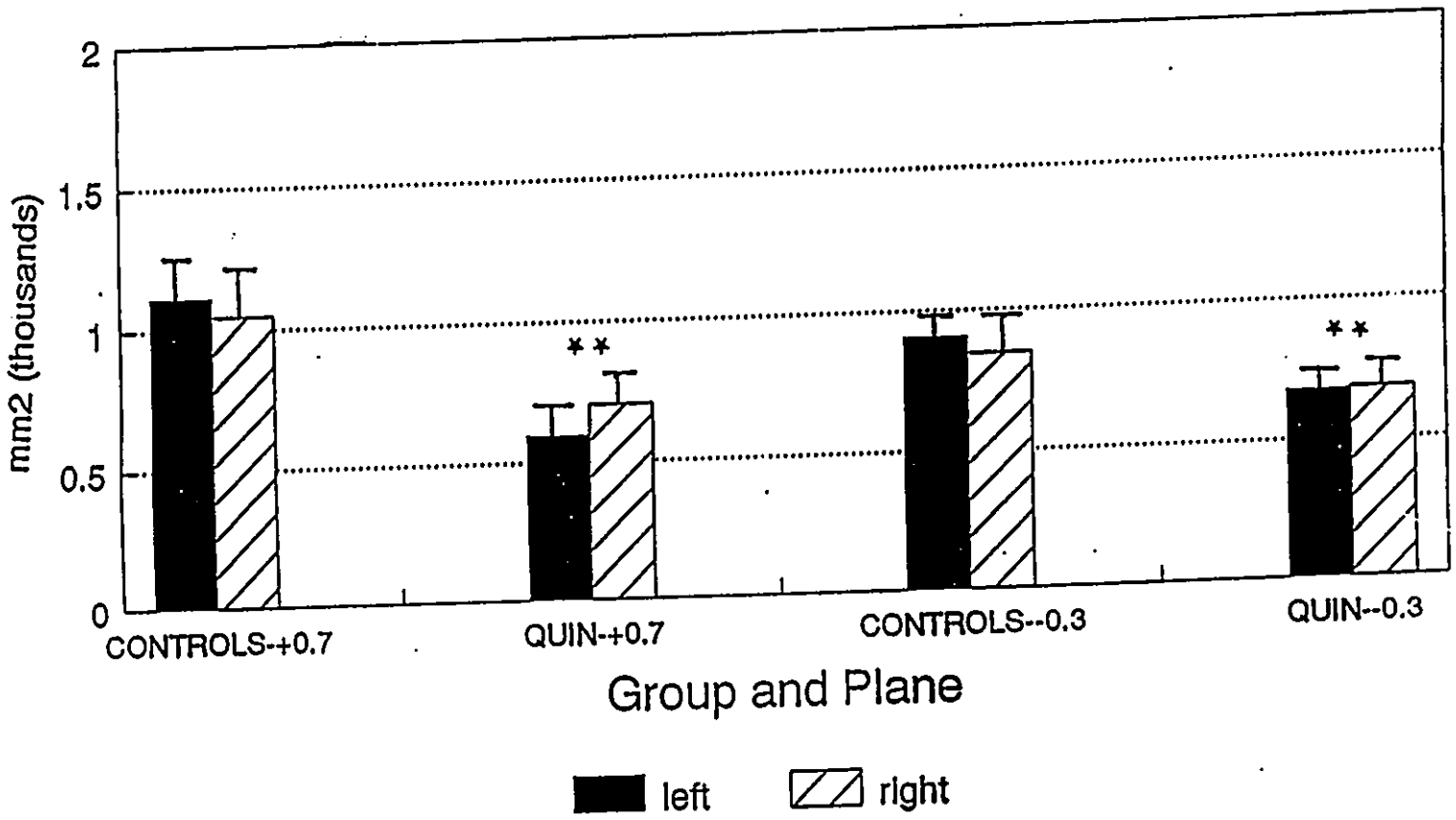
Figures 4.23-4.26 Mean cortical and striatal area, CONT and QUIN

Morphometry: Striatum



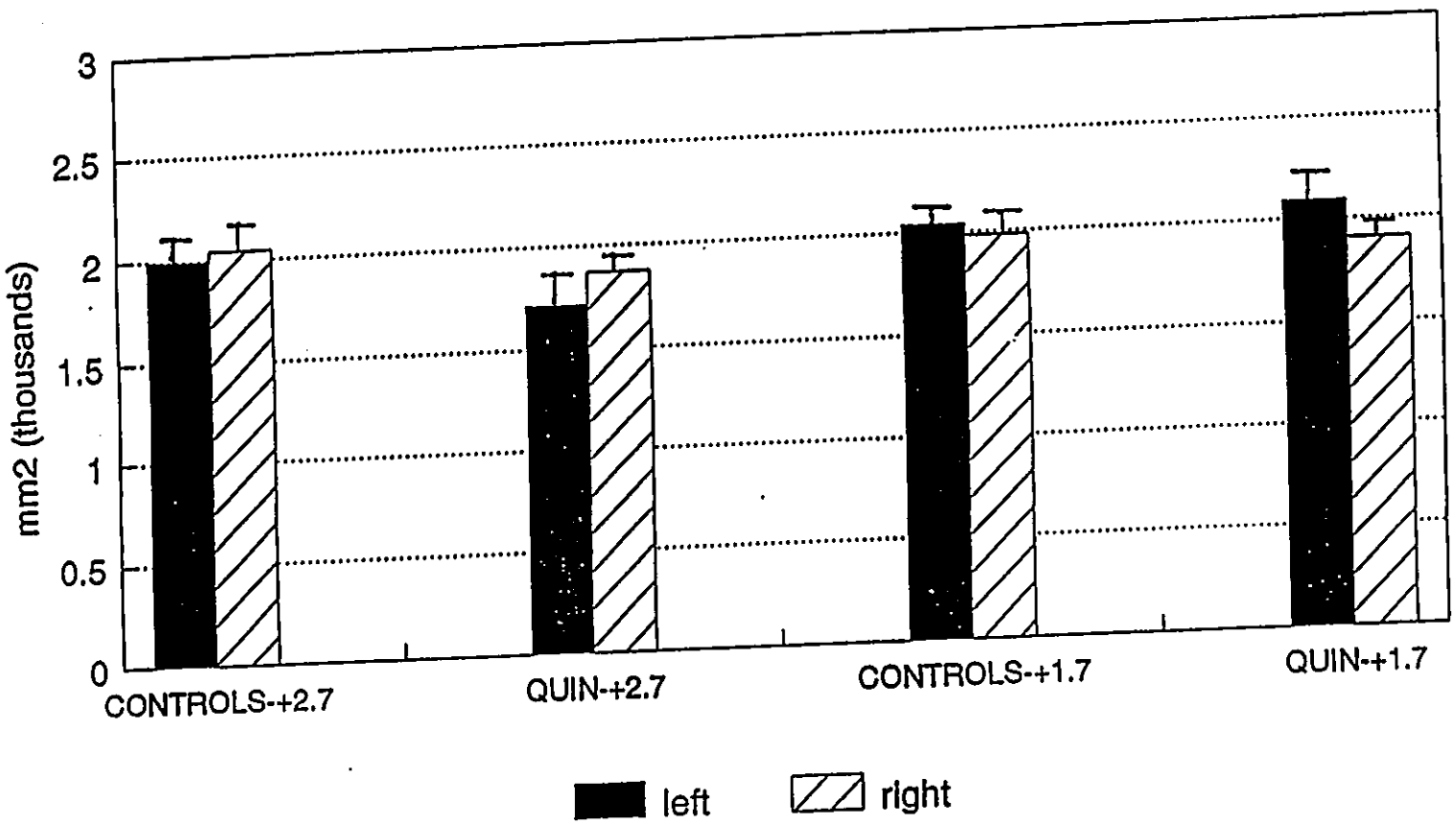
** p<0.01

Morphometry: Striatum

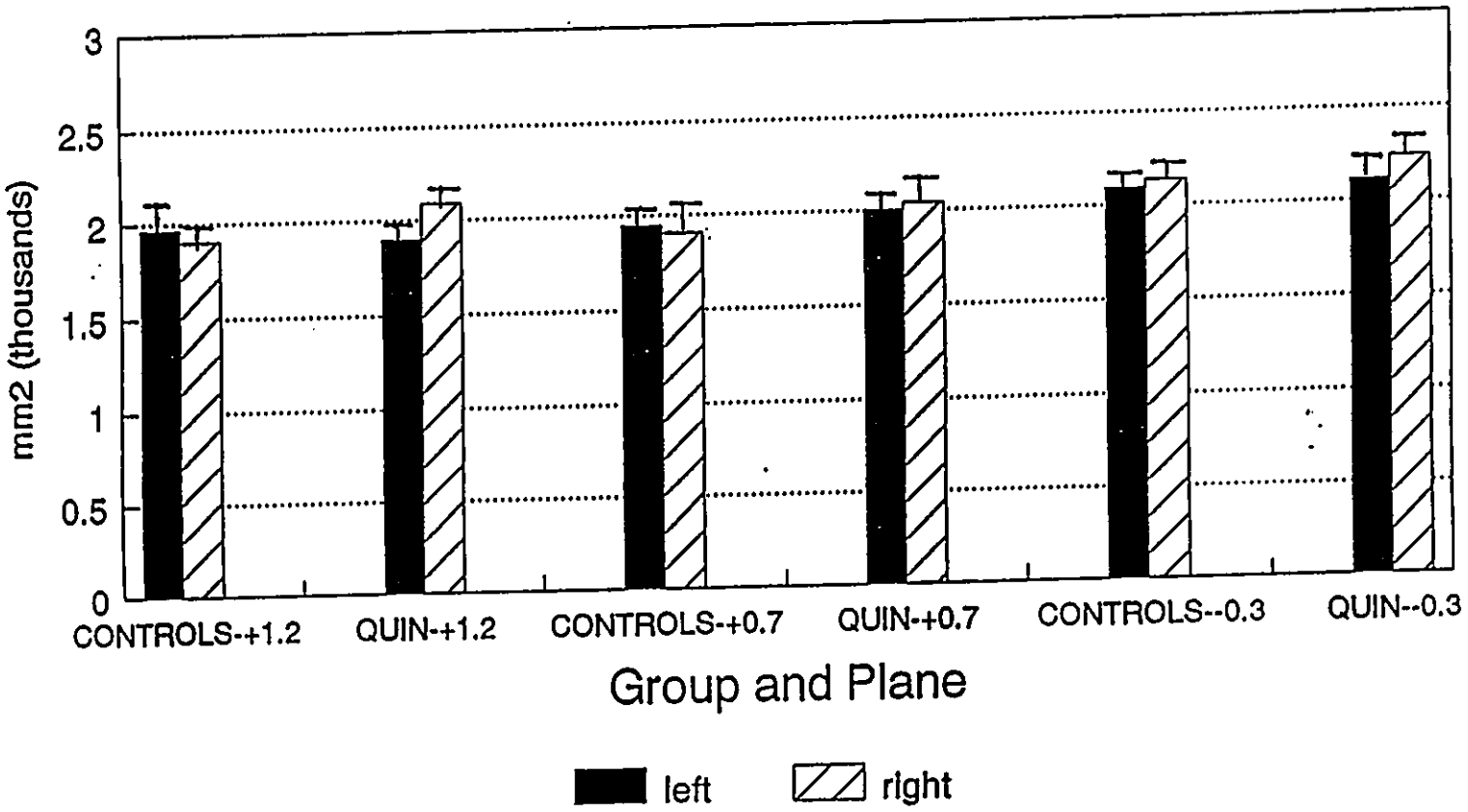


** $p<0.01$

Morphometric Analysis: Cortex



Morphometry: Cortex



contrary to the research of others who have noted a significantly thicker right neocortex than left neocortex in control animals (Kolb, 1987; Stewart and Kolb, 1988; Van Eden, Uylings and Van Pelt, 1984). However, as noted by these researchers themselves, cortical asymmetry, whether for cortical thickness or cortical area, is a variable and fragile phenomenon, seemingly subject to such factors as age, prenatal stress, and sex hormones. Thus, it is perhaps not of any great consequence that no cortical asymmetry was found in the control group in either experiment.

General Discussion

The behavioural analysis presented in these two experiments has shown that rats with QUIN-induced lesions of the medial striatum are impaired in the performance of the MWM and SA (the interpretation of the DA data has proven to be more problematic), but seemingly unimpaired on a number of motor tests. Quantitative histological analysis at the macroscopic level revealed significant bilateral striatal degeneration in the QUIN group when compared to the CONT group but no measurable difference in frontal cortical area at different planes. Thus, keeping in mind the possibility of changes at the cellular level that are not reflected at the macroscopic level, or changes in brain areas not examined in this study, it may be reasonable to attribute the behavioural abnormalities observed in this study to quinolinic acid-induced damage of the striatum.

The cognitive impairments exhibited by the QUIN-lesioned animals in this study are similar to those observed in rats with aspirative lesions of the frontal cortex. In the

clinical literature similar cognitive impairments are found in patients with HD and those with frontal lobe lesions.However, no group differences were found on a host of motor tests; by contrast, previous researchers have found rats with medial frontal cortex lesions to be impaired in these motor tests. Taken together, these studies suggest that the striatal degeneration of HD may be sufficient to account for the observed cognitive abnormalities in HD. Secondly, it lends support to the idea that certain areas of the cortex, basal ganglia and thalamus are connected in a series of distinct circuits such that damage to a certain area of basal ganglia may result in the same impairments as damage to the connected area of cortex or thalamus (Alexander et al., 1990).

It has been suggested that the MWM is a test of spatial learning and memory, so from the results of these tests, it may be suggested that the QUIN-induced medial striatal lesions may produce impairment of spatial learning, memory, and cognitive flexibility. The results from the SA test may also be interpreted as reflective of an impairment of cognitive flexibility in the QUIN group. The implementation of various motor tests and the negative findings in each of the motor tests has been extremely useful in supporting the idea that the deficits found in the cognitive tests in rats with QUIN-induced lesions of the medial striatum can indeed be attributed to cognitive impairment, rather than any other explanation such as impaired motor function. Also, as I have already discussed, analogies can be drawn between the deficits found in the QUIN group and those found in HD patients.

Although other researchers have found that rats with kainic acid- and ibotenic

acid-induced striatal lesions demonstrate cognitive impairments, the widespread extrastriatal effects of kainic acid make it difficult to reliably ascribe the abnormal behaviour to striatal lesion.; As for ibotenic acid-induced lesions, my behavioural analysis with QUIN-induced lesions of the medial striatum considerably extends the findings from studies of ibotenic acid-lesioned animals. Specifically, this study establishes the following novel findings: rats with lesions of the medial striatum 1) are impaired not only in the rate of acquisition in the MWM but also in the ultimate extent of acquisition, as they are always slower than the controls, even with extended testing; 2) demonstrate behaviour (perseveration, etc) that may be interpreted as impaired cognitive flexibility; 3) exhibit progressive memory deterioration with increasing testing intervals.

CHAPTER 5

BEHAVIOURAL ANALYSIS OF QUINOLINIC ACID-INDUCED LESIONS IN RATS

III. Quinolinic acid-induced lesions of the medial prefrontal cortex in rats: behaviour and anatomy

Introduction

Methods

Behavioural Assessment: Morris Water Maze, Motor Tests

Results

Discussion

BEHAVIOURAL ANALYSIS OF QUINOLINIC ACID-INDUCED LESIONS IN RATS

III. Quinolinic acid-induced lesions of the medial prefrontal cortex in rats: behaviour and anatomy

Introduction

I have shown that rats with quinolinic acid-induced lesions of the medial striatum demonstrate behavioural abnormalities that appear to be attributable to impairments in cognitive abilities and not motor function (refer to previous chapter). Histological analysis of these animals with chronic striatal lesions revealed lesions that appeared to be confined to the striatum; further, morphometric analysis of the cortex failed to reveal cortical pathology. In recognition of the frontal cortex pathology present in HD, it would be interesting to determine whether QUIN-induced lesions of the frontal cortex produce analogous impairments. Further, in recognition of the intimate afferent and efferent connections between the cortex and striatum, it is important to ask whether the cortical pathology of HD is primary or secondary to the striatal pathology. The answer to this problem is relevant to the identification of the neural substrate of the neuropsychiatric disturbance in HD, and will have implications regarding strategies for treatment of the disease. Yet, the answer to this question is not likely to come from studies of the human disease state (where both processes occur simultaneously) but from the study of animal models of the disease, where the experimenter is able to selectively damage the striatum or the cortex and characterize the effects of each type of lesion. Further, considering the nature of the pathology in HD and the recent excitotoxic theories of pathogenesis, it may be suggested that the well-known animal

model of frontal cortex damage produced by aspirative lesions of the frontal cortex in rats is more relevant to the study of cortical excision in humans, not, necessarily, HD. A better model for the study of cortex pathology in HD may be provided by the use of excitotoxins to lesion the cortex. There is virtually no behavioural literature on this topic. Further, a recent study has suggested that QUIN-induced lesions of the frontal cortex may produce a neurochemical profile similar to that of HD (Beal et al., 1991a). Thus, the following study is concerned with the behavioural and neuroanatomical characterization of frontal cortex lesions induced by QUIN.

Methods

Surgery. Male Sprague-Dawley rats (Charles-River, Quebec) were anaesthetized with sodium pentobarbital and placed in a Kopf small animal stereotaxic. The skull was exposed and gradually worn away by using a Dremel drill in order to expose the medial frontal cortex. The posterior boundary was bregma, the anterior boundary was the frontal suture and the lateral boundary was the zygomatic bone. The frontal cortex was exposed on both sides; a strip of bone was left intact in the midsaggital area in order to protect the underlying sinus.

In the case of control animals, the wound was closed at this point. In the case of the experimental animals, two mg of dry QUIN (Sigma) were weighed and carefully powdered onto the exposed dura. According to methods used by others (Sofroniew and Pearson, 1985; Sofroniew, Isacson and Bjorklund, 1986), five minutes were allowed to elapse for abosorption of the excitotoxin. The wound was subsequently closed with animal wound clips (Fisher Scientific, Unionville, ON) and the animal returned to its

home cage. One week of recovery was allowed prior to the commencement of behavioural analyses.

Behavioural Assessment

A. Morris Water Maze. The version of the MWM that was used was again the place task, reference memory version of the task, similar to that used by Whishaw et al. (1987) and which has been described previously (refer to section on QUIN-induced striatal lesions, Acquisition of Training in the water maze), except that a new platform of Perspex replaced the platform previously used, and thus, the styrofoam became unnecessary.

B. Motor Tests. Motor testing was similar to that used with the striatal animals and included food manipulation, swim posture, spontaneous daytime and nighttime locomotion. Refer to Chapter 4 for a detailed description of the procedures used.

Histological Analysis

Three to three and one half months post-surgery the rats were sacrificed. They were overdosed with sodium pentobarbital, perfused with 0.9% saline followed by 4% paraformaldehyde, and the brains were removed. The brains were fixed in 4% paraformaldehyde at 4 degrees Celsius, overnight. The next day, the tissue was placed in 30% sucrose and two days later, cut at 40 um on a cryostat. Prior to sectioning the whole brains were photographed.

After sectioning, the brains were stained with cresyl violet (0.5%), and selected sections were expanded and traced using a Bausch and Lomb slide projector.

Results

A. Morris Water Maze Acquisition of the Morris Water maze task was significantly impaired ($F(1,14)=14.8$, $p<0.01$) in the animals with QUIN-induced cortical lesions of the medial prefrontal cortex (figure 5.1). A platform visible test revealed no differences between the two groups.

B. Motor Tests

Food manipulation The test of food manipulation revealed no differences between the two groups.

Swim posture Similarly, the test of swim posture revealed no differences between the two groups.

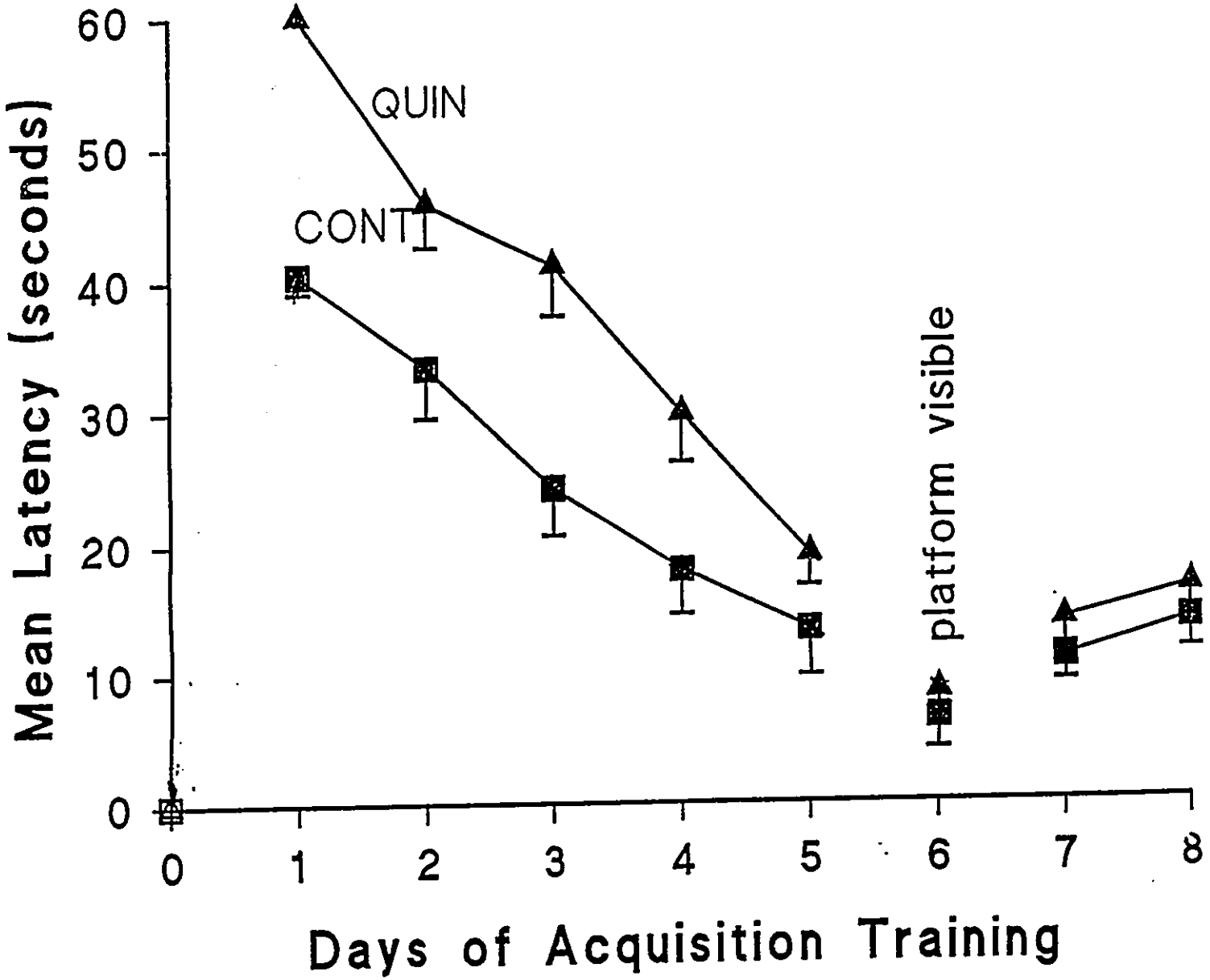
Spontaneous Day and Night Locomotion Again, these tests revealed no group differences.

Histological Analysis Examination of the cortex in the lesioned animals revealed significant cortical degeneration in the area of the intended lesion, with no extensive cortical degeneration outside the lesion area readily apparent. Within the area of the lesion, damage included both ablation of tissue as well as necrosis of remaining tissue (figures 5.2-5.9, shading represents areas of cortical damage).

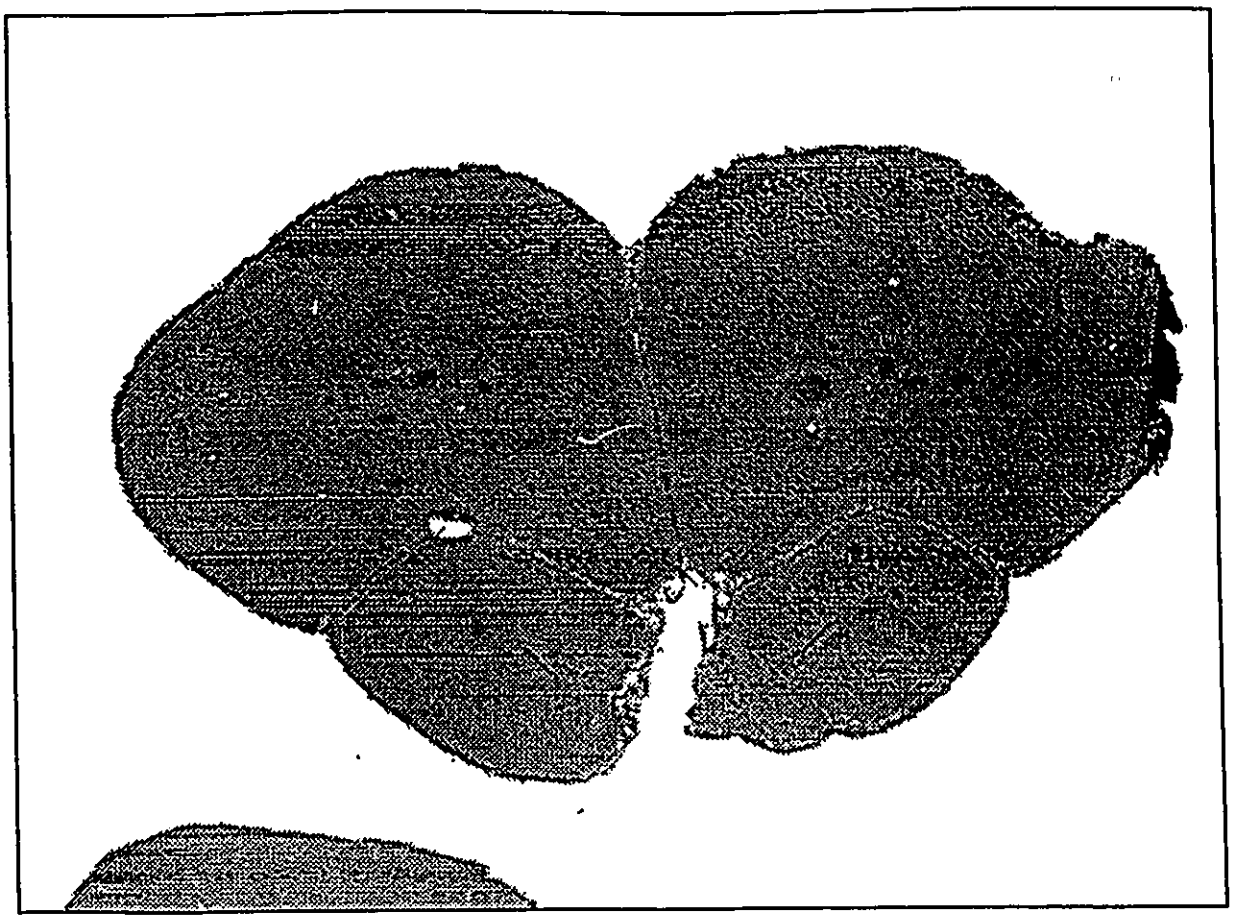
Interestingly, striking ventricular enlargement (figures 5.6, 5.7, 5.9) was present in six out of eight in lesioned animals. No comparable effect was visible in the control group.

Discussion The present study indicates that rats with quinolinic acid-induced lesions of the medial prefrontal cortex demonstrate impairments in the performance of

Figure 5.1 Mean latencies for acquisition, QUIN and CONT.


Mean Latency (seconds)
60
50
40
30
20
10
0
QUIN
CONT
platform visible
0
1
2
3
4
5
6
7
8
Days of Acquisition Training

Figures 5.2-5.3 Control animals. Representative section.



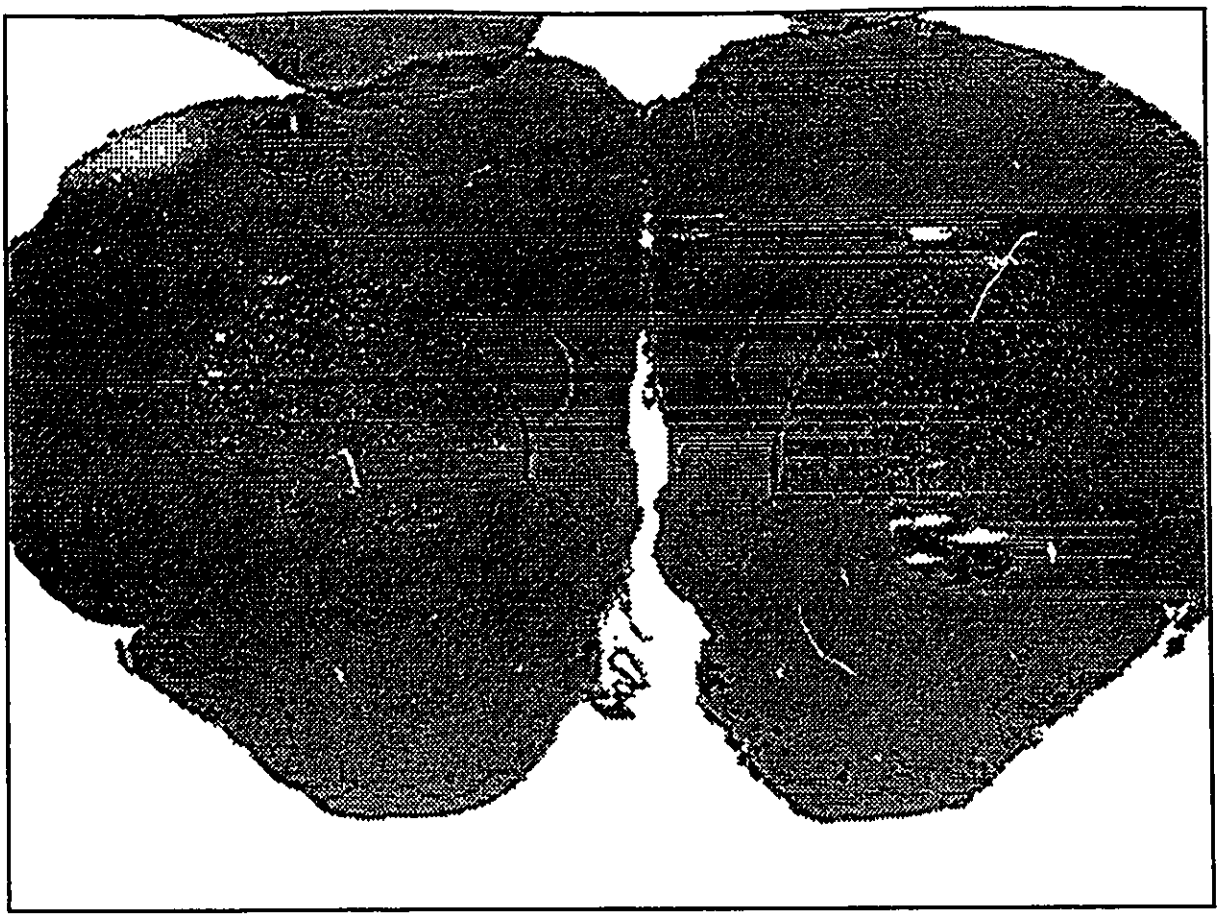
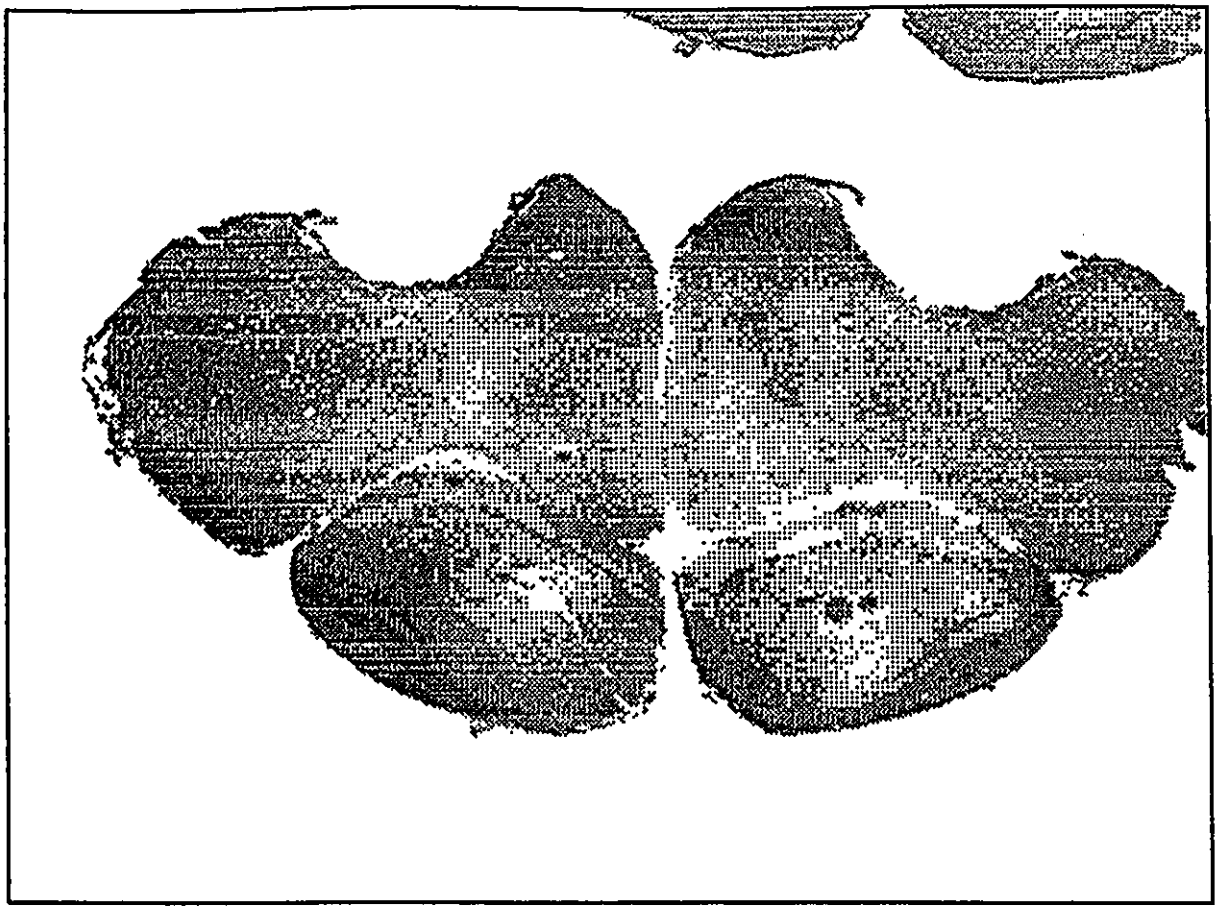
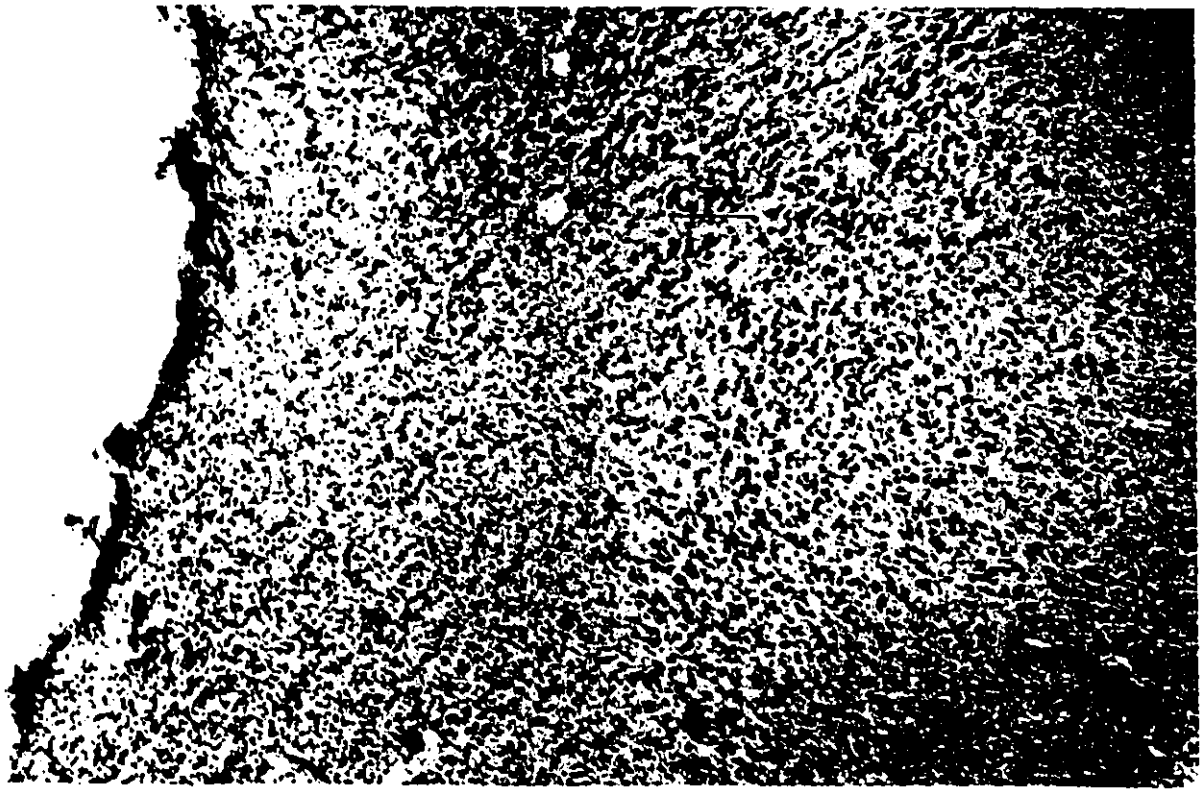
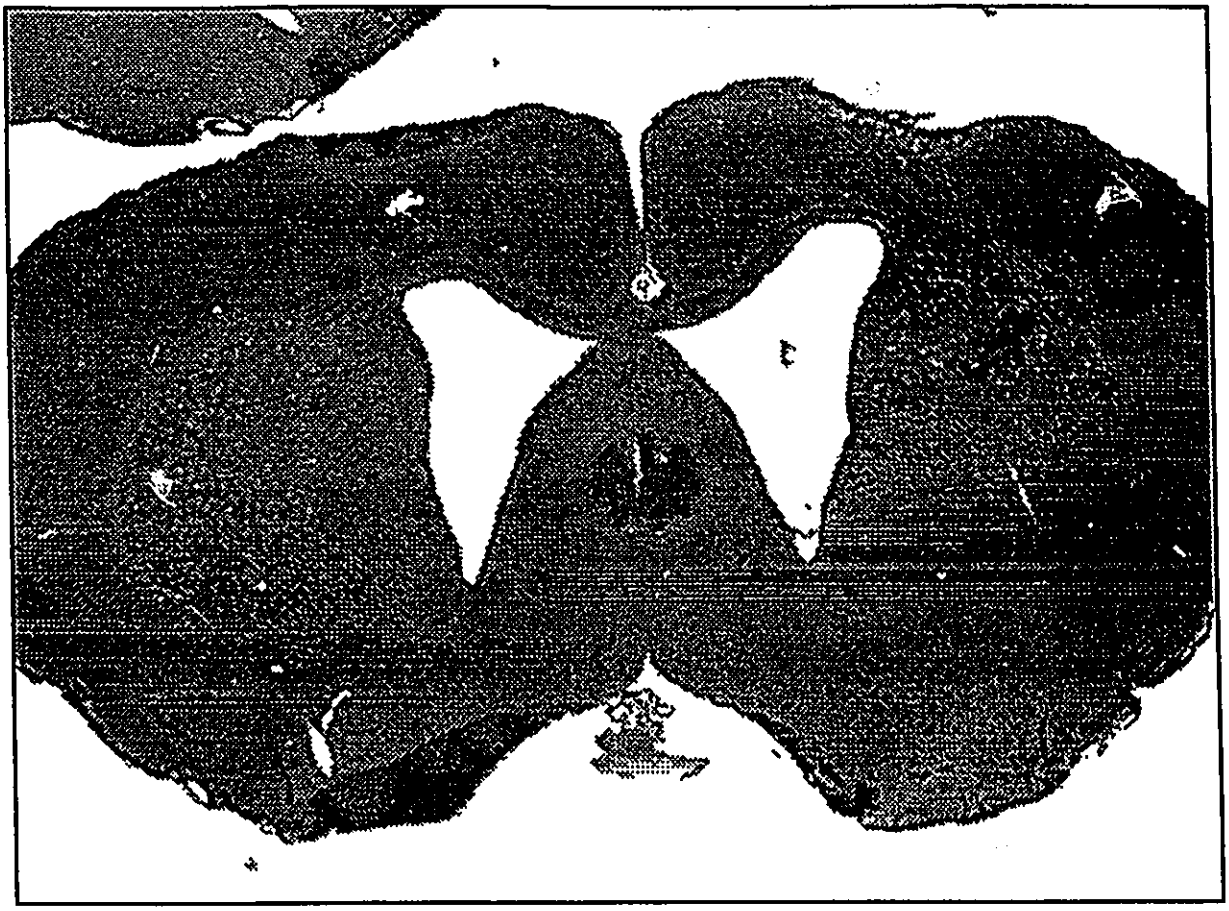


Figure 5.4 QUIN group, frontal cortex lesion, representative section.

Figure 5.5 QUIN group, frontal cortex lesion, x4. Representative section depicting cortex in the area of the lesion. Ctx=cortex.







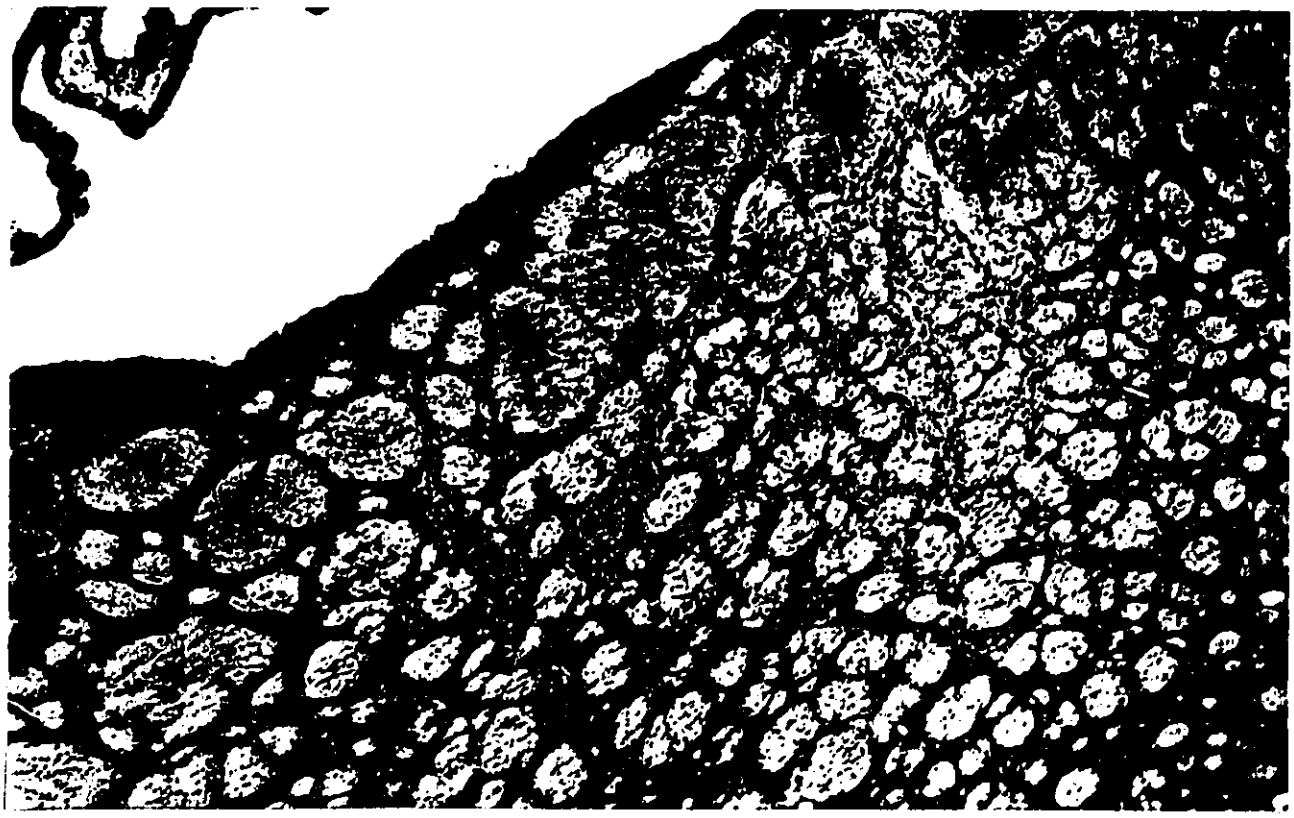
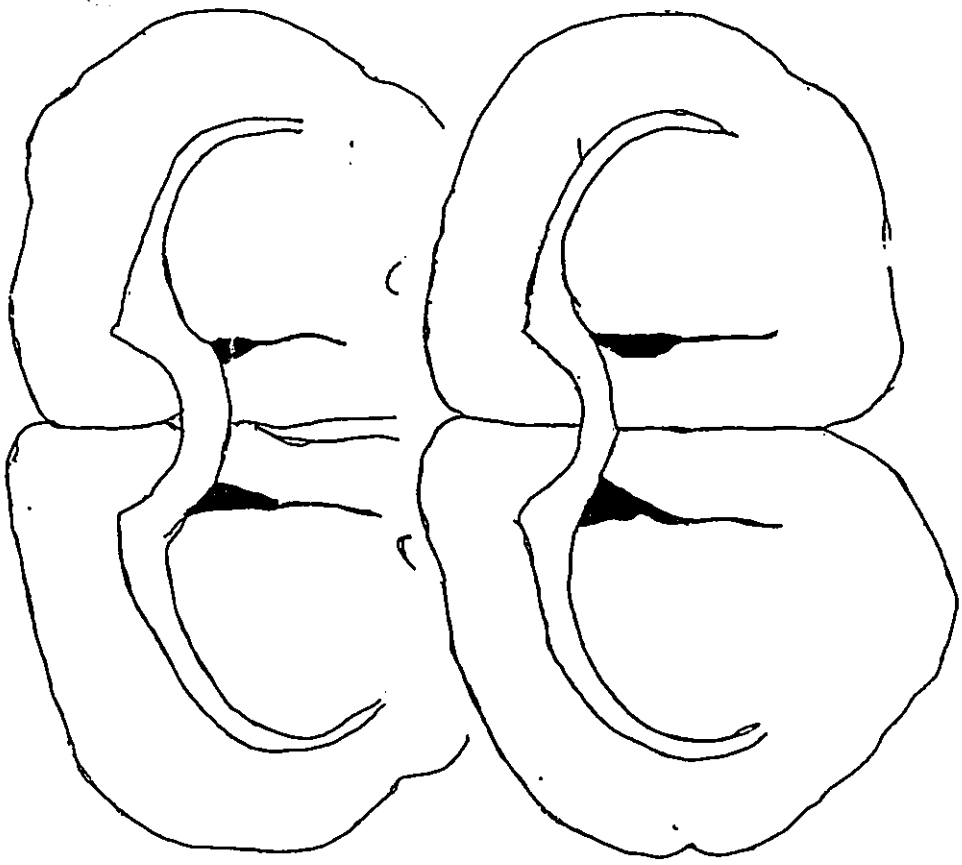


Figure 5.8 Tracings of coronal sections, controls.



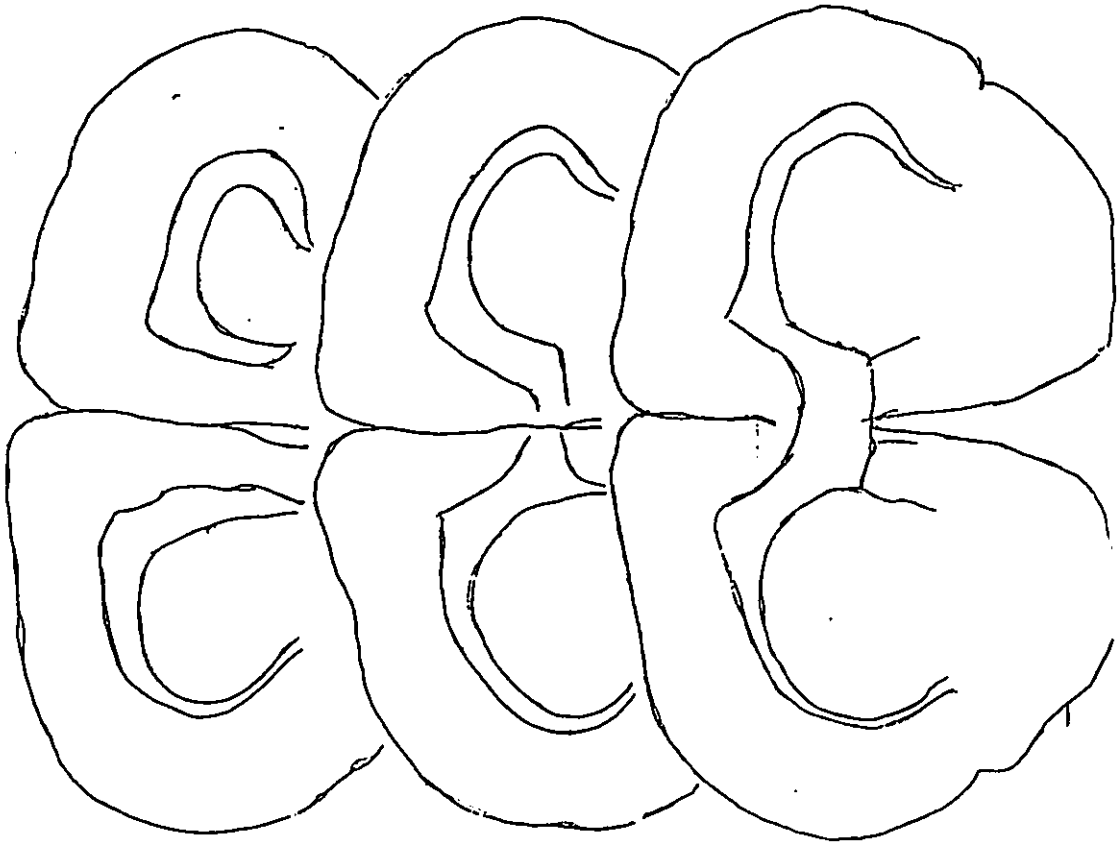
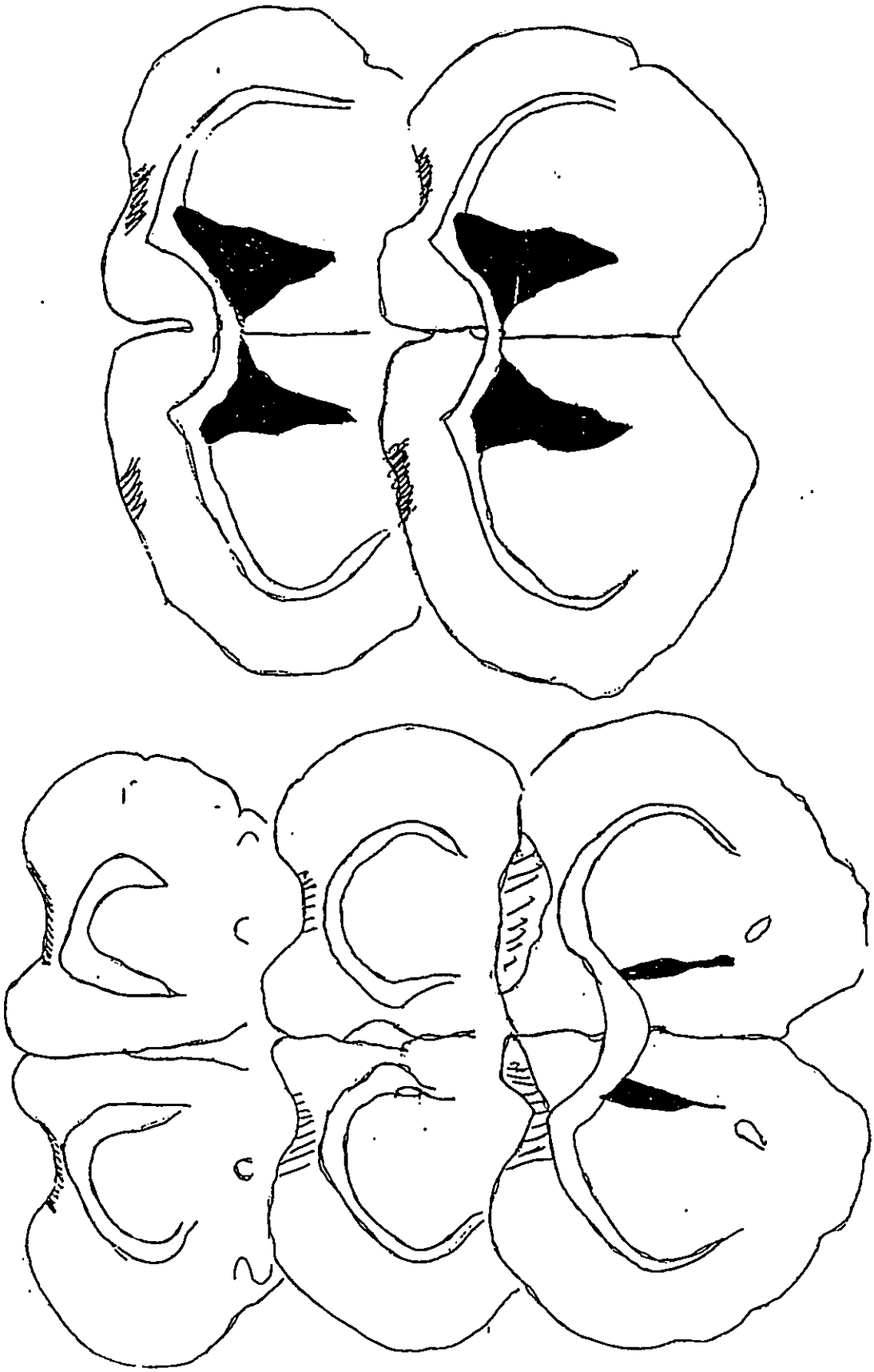


Figure 5.9 Tracings of coronal sections, QUIN.



the MWM, but no apparent motor deficits. However, it may not be possible to attribute the impairments observed solely to the cortical damage as histological analysis also suggested the presence of striatal damage. However, this pattern of cognitive impairment may be considered analogous to certain aspects of the behavioural impairment demonstrated in HD (i.e., visuospatial impairment, memory deficits).

Interestingly, there were variations between the behavioural profile of my QUIN lesioned groups and that which has been described in the literature for animals with aspirative lesions of the medial prefrontal cortex (e.g., Kolb, 1987). While both experimental groups (QUIN-lesioned and aspiration-lesioned animals) are impaired in the performance of the MWM, the animals with aspirative lesions were reported to be impaired in the motor tests (e.g., food manipulation, swim posture, spontaneous locomotion) while the QUIN-lesioned animals in this study were not. The reasons for this difference are unclear at this time, but may be related to the extent of damage produced by each technique.

Perhaps one of the most interesting outcomes of this set of experiments was the presence of gross ventricular degeneration (possibly reflective of striatal degeneration of some sort) in some of the lesioned animals. A variety of neural mechanisms may be proposed to account for this effect. For example, it may be that when QUIN is powdered onto the cortex, there is selective destruction of the cortical cell bodies that project to the striatum via the corticostriatal tract, thus resulting in degeneration of both these terminals and the neurons upon which they synapse. Alternatively,

application of QUIN to the cortex may result in depolarization of the corticostriatal terminals and sufficient glutamate release in the striatum, thus producing excitotoxic cell death within the striatum. Regardless, the presence of striatal degeneration in these animals suggests that it may be impossible to lesion the frontal cortex with excitotoxins without accompanying damage to the striatum. This result, will, of course, need to be carefully confirmed. Should the effect prove to be reliable and valid, however, this model may well prove to be the best model of HD available to date.

It is to be acknowledged that much room remains for confirmation and refinement of this model. Questions remain concerning the extent of the behavioural impairment, and how this behavioural profile differs from rats with aspirative lesions of the frontal cortex. Investigation of a dose-response curve may prove to be useful. The cortex damage in these experiments was quite severe--this is not the case in HD. In light of this, can minimal, but chronic, QUIN-induced damage to the cortex, also produce behavioural deficits? In terms of selectivity of the destruction of cell populations are there any advantages in using QUIN? What is the nature of the gross ventricular dilation seen in many of the experimental animals? Is this indeed reflective of damage to the striatum and is this damage selective in some way? Can any sort of manipulation, pharmacological or other, attenuate the damage? The primary purpose of this study was not to provide answers for these detailed and extensive questions, but to begin to establish, in a set of preliminary experiments, whether or not behavioural impairments can be produced following QUIN-induced lesions of the cortex.

In conclusion, it appears to be possible to produce a similar pattern of cognitive

(but not motor) impairment after quinolinic acid-induced lesions of both the frontal cortex and striatum. As far as HD is concerned, given that no cortical pathology appears to be present after chronic quinolinic acid-induced striatal lesions, it may be that the cortical pathology in HD represents an independent process and that therapeutic strategies for HD will have to be directed at both the cortex and striatum.

CHAPTER 6

LONG-TERM CONSEQUENCES OF EXCITOTOXIN INJECTION INTO RAT STRIATUM:

Morphometry and Neurochemistry

LONG-TERM CONSEQUENCES OF EXCITOTOXIN INJECTION INTO RAT STRIATUM:

Morphometry and Neurochemistry

Introduction

The histological analysis from both experiments of the behavioural analysis of animals with QUIN-induced lesions of the medial striatum (refer to Chapter 4) showed striatal degeneration without apparent cortical degeneration (analyzed through morphometric techniques) when the rats were sacrificed six months post-lesion. This is unlike the situation known to exist in HD where cortical degeneration is known to be part of the pathological picture. It is possible, however, that even longer post-surgery survival time would have revealed degeneration in the cortex, as well as other structures. The purpose of the present study was to determine whether a chronic striatal lesion in the rat could produce extrastriatal pathology similar to that reported in HD. At issue is the question of whether or not the extrastriatal pathology observed in HD is secondary to striatal degeneration. The analytic methods used included morphometry and neurochemistry. With regards to the former, two studies were conducted, one in which the animals were sacrificed at one year post-surgery, and a second in which the animals were sacrificed at five months post-surgery.

I. Morphometric Analysis of Striatal and Extrastriatal Structures in Rats with Chronic QUIN-induced Striatal Lesions

A. Histochemical Stains: Nissl and Acetylcholinesterase

Methods

Surgery. The animals used in this experiment received unilateral striatal lesions created via the injection of one of three excitotoxins (quinolinic acid, kainic acid, ibotenic acid) or saline (controls). These animals are the same as those in Experiment 2, of the spontaneous activity study.

Animals were injected unilaterally with one ul of vehicle or one of three excitotoxins at the following co-ordinates: A/P: +0.2; M/L 2.7; D/V 6.0. 0.9% saline, 10 nm of KAIN, 240 nm of QUIN or 190 nm of ibotenic acid (IBO) were delivered at a rate of one ul per minute.

Histology. Twelve months after surgery the three groups of rats lesioned unilaterally with one of three excitotoxins and the accompanying saline-injected control group were deeply anaesthetized with sodium pentobarbital, and perfused with 0.9% saline followed by 4% paraformaldehyde. The brains were removed and placed in the same fixative overnight at 4 degrees, Celsius. The following day, the tissue was rinsed with 0.1 M phosphate buffer, and the tissue subsequently stored in a solution of 30% sucrose in 0.1 M phosphate buffer at 4 degrees, Celsius until the tissue sank (approximately 2 days). Care was taken to ensure that the brain tissue from the different groups was treated in the same way.

Each brain was blocked and frozen in a cryostat. The tissue was attached to

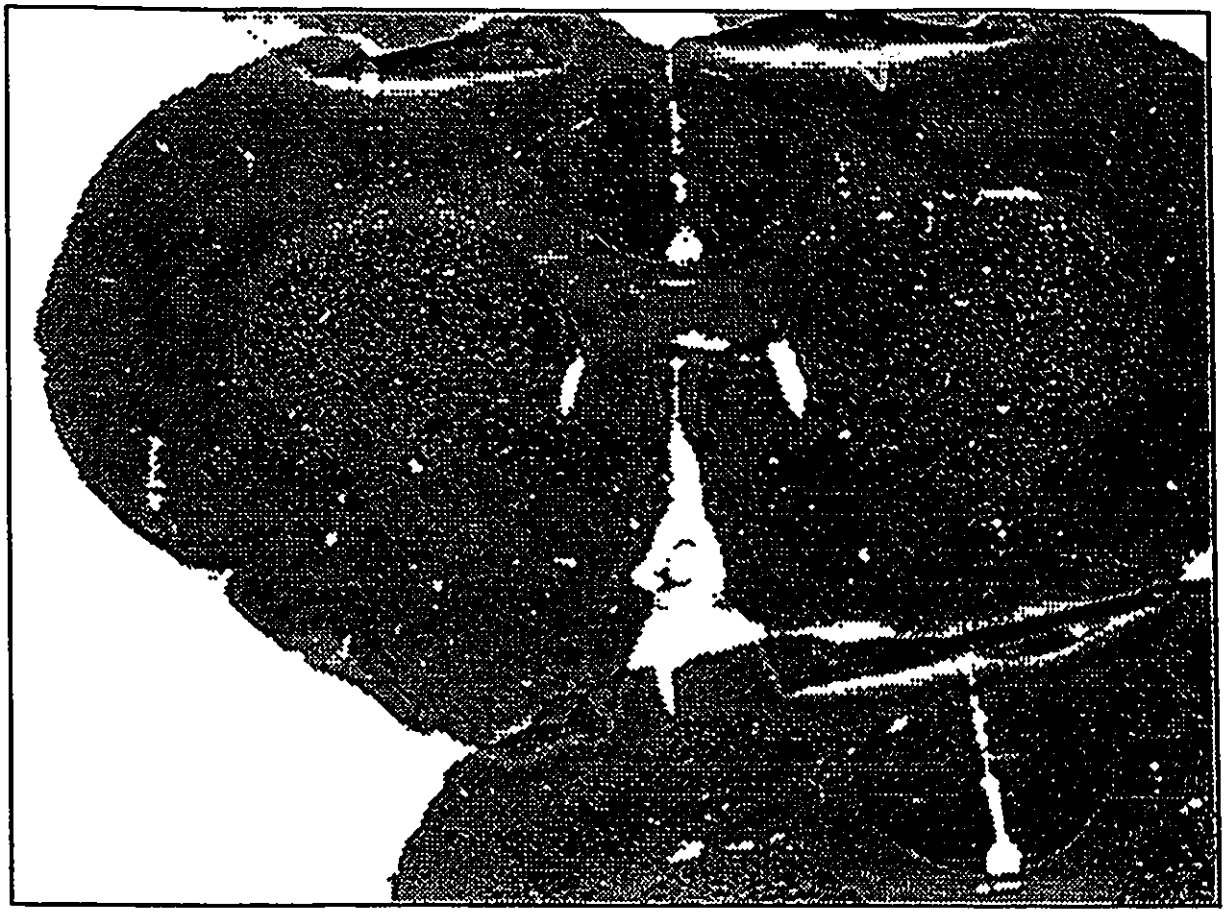
cryostat chucks using Histoprep (Fisher Scientific), and the brains were subsequently sectioned in the coronal plane at a thickness of 40 um. Sections were collected on chrom-alum subbed slides.

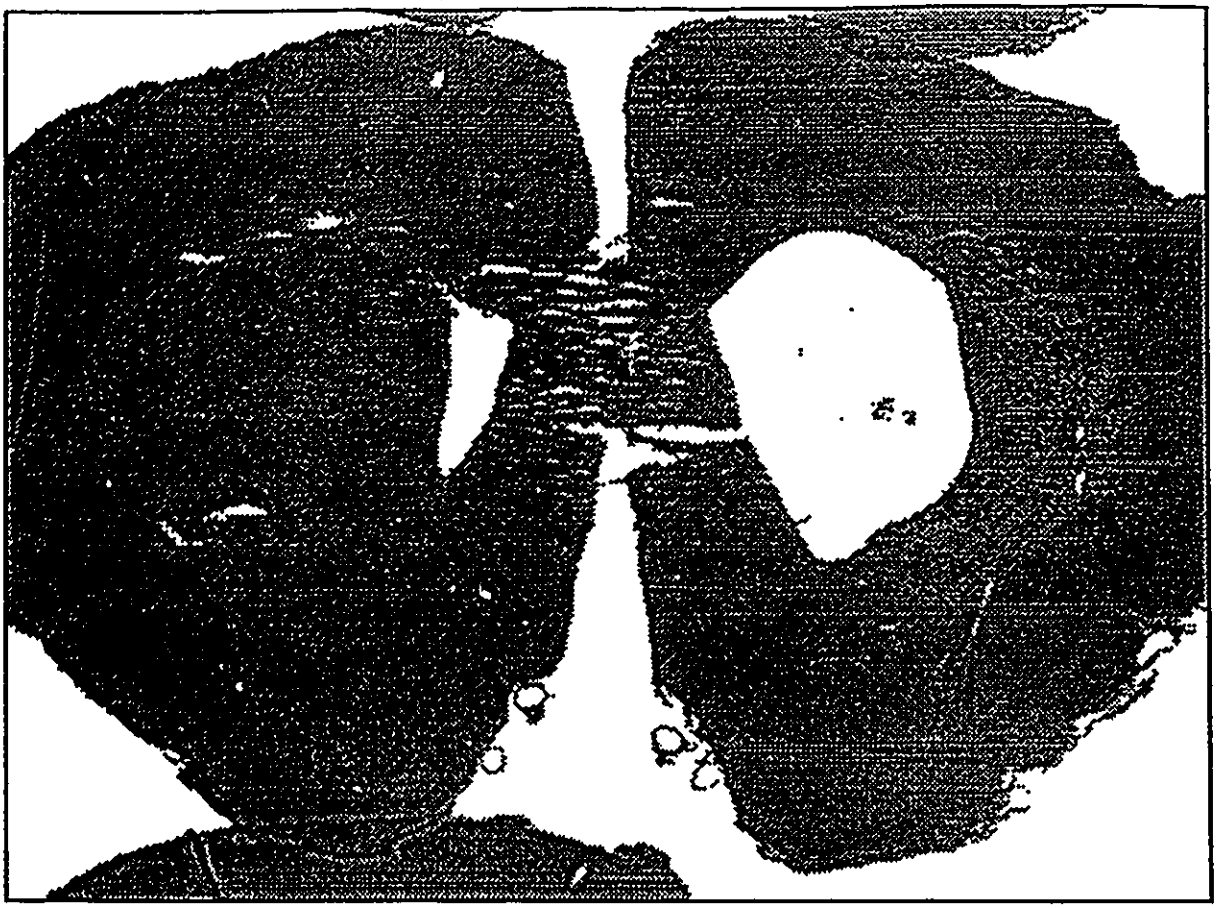
The sections from the anterior half of each brain were stained with cresyl violet (Nissl stain) while the sections from the posterior half were stained with acetylcholinesterase (AChE) using the method of Koelle. Briefly, tissue was incubated in AChE buffer stock containing S-acetylthiocholine iodide (substrate) and ethopropazine (inhibitor of non-specific cholinesterases) overnight at 4 degrees, Celsius. The next morning, the tissue was developed, dehydrated through an ascending series of alcohols and mounted out of xylene. Elimination of the substrate did not result in staining.

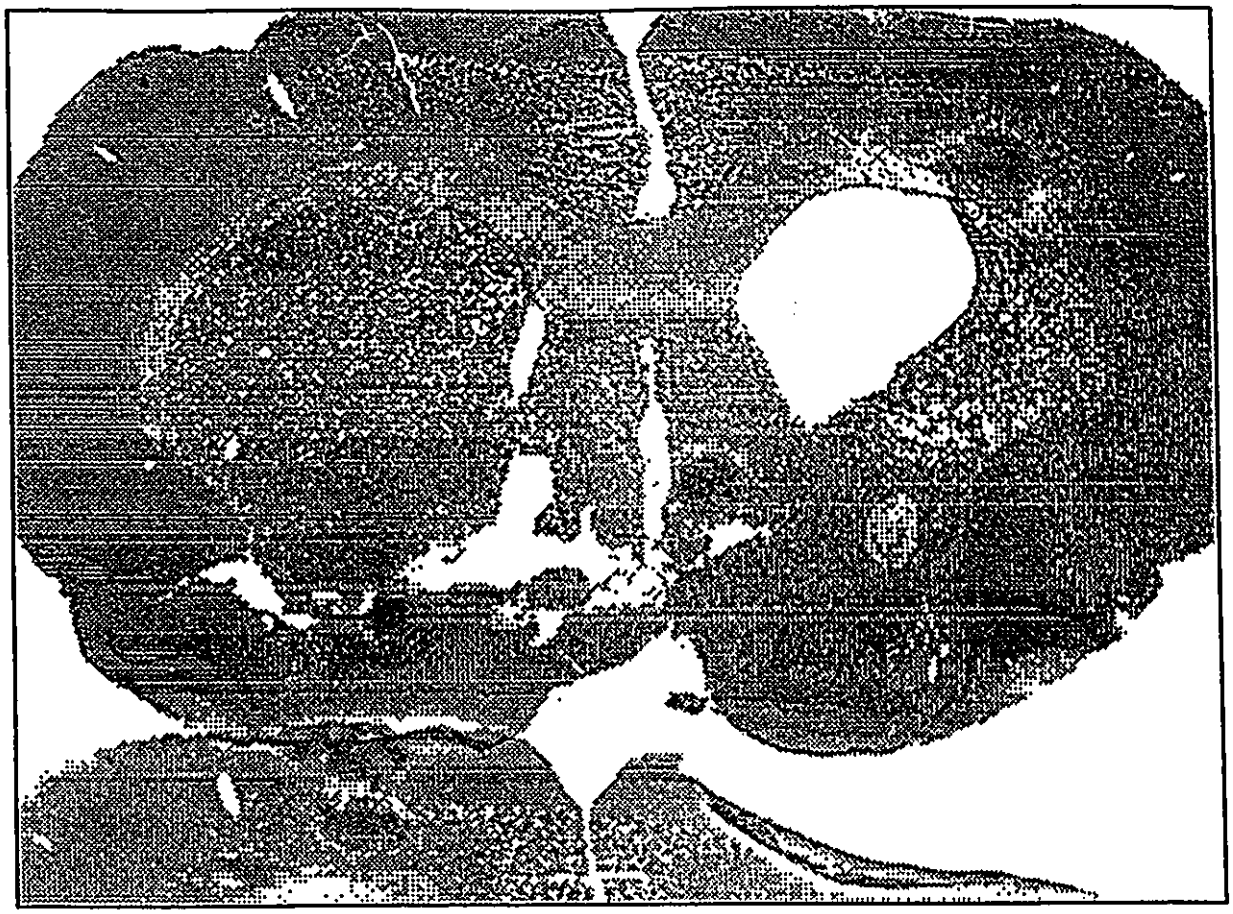
Two different stains were used in order to delineate certain structures. A Nissl stain would produce a clearer delineation of striatum than would AChE. More importantly, the AChE stain was used to differentiate various subnuclei of the thalamus; resolution with a Nissl stain is not nearly as clear. Representative sections appear in figures 6.1-6.3.

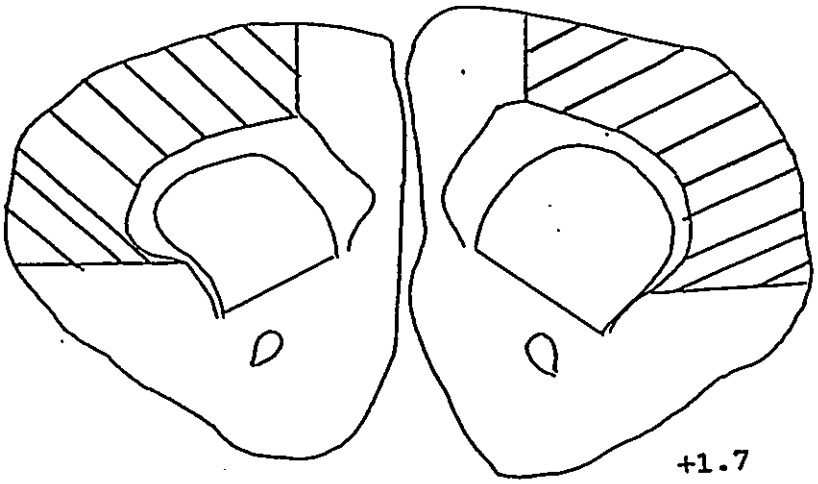
Morphometric Analyses. Areal measurements of various brain structures were taken at various coronal levels, as defined by the atlas of Paxinos and Watson (1982). These coronal planes were (relative to bregma): +1.7, +1.2, -0.3, -0.8, -1.8, -2.3. Sections at these levels for each animal in each group were expanded and traced using a Bausch and Lomb projector. (The boundary delineations appear in figures 6.4-6.7.) Structures of interest included the frontal cortex, striatum,

Figures 6.1-6.7 Morphometry study. Control and unilateral KAIN and IBO lesions; boundary delineations for all structures.









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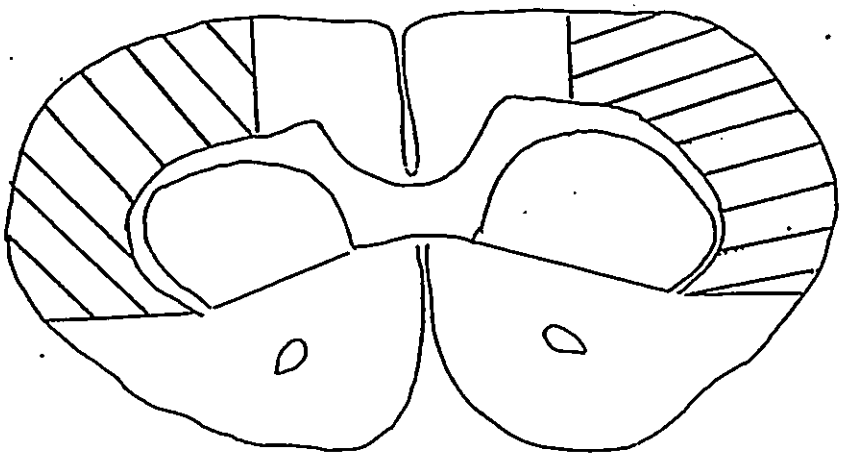

+1.2

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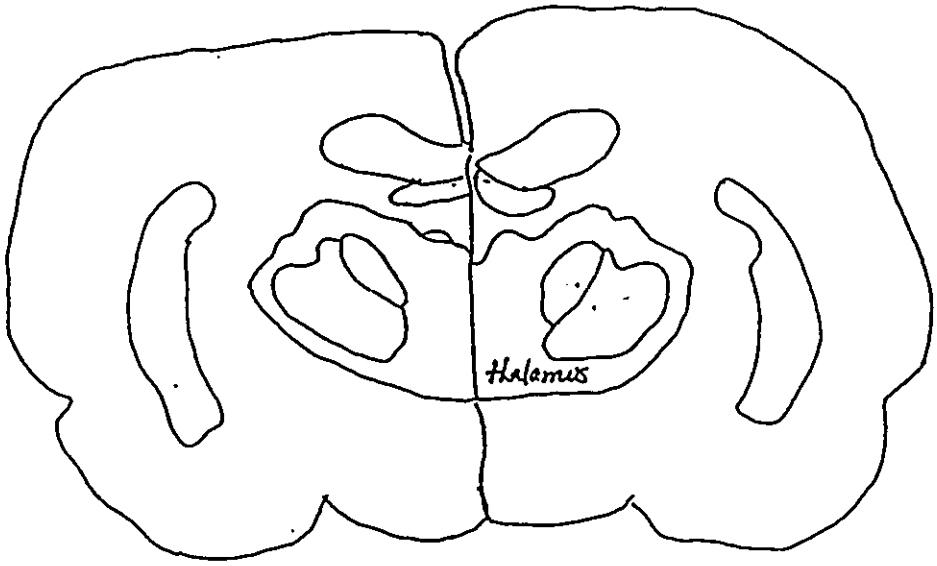
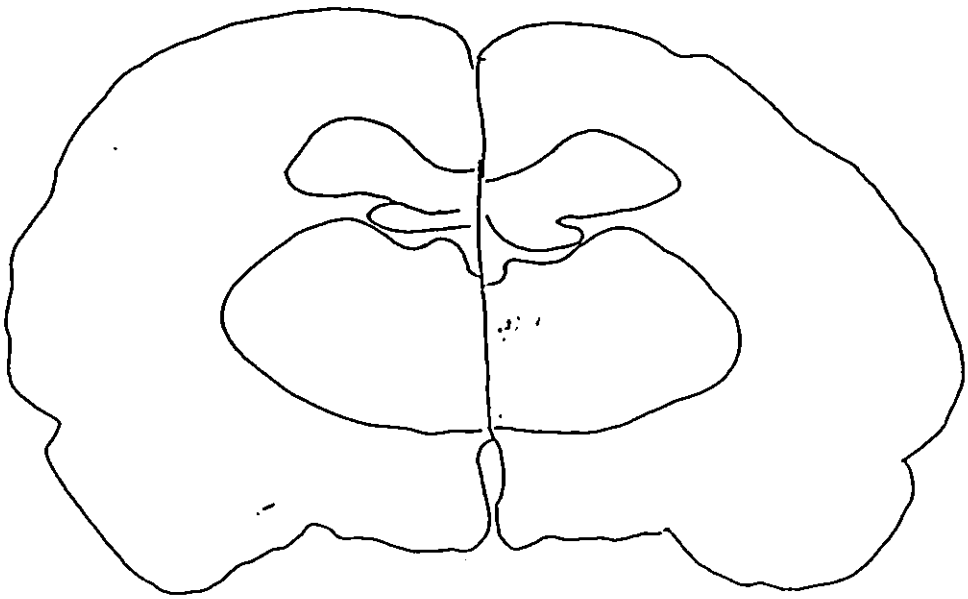


-1.8



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ventricle, ventrolateral (VL) and ventroanterior (VA) nuclei of the thalamus, and the entire thalamus itself at these various planes. Some structures were measured at more than one plane.

The boundaries for striatum and cortex were similar to those used in Chapter 4. Area was assessed using a Bioquant System IV software package (R and M Biometrics, Nashville, Tennessee). The individual tracings were placed on a Hipad Digitizer software tablet (Houston Instruments, Austin, Texas) connected to an IBM Personal Computer. Area was measured using a cursor (Houston Instruments) attached to the software tablet. For each individual structure, two measurements were taken and the mean of the two was calculated.

Results

Mean lengths and areas for all structures and all groups in all planes appear in tables 6.1-6.8 and figures 6.8-6.17.

Striatum. Striatal area was assessed in three coronal planes. The analysis of variance revealed a significant effect for Group ($F(3,110)=8.115$, $p<0.001$), indicating that the overall striatal area was smaller in the three groups lesioned with excitotoxin than the control group. The effect Coronal Plane was also significant ($F(2,110)=5.728$, $p<0.01$), indicating the presence of more striatal area with rostro-caudal progression. The effect of Side was significant ($F(1,110)=174.836$, $p<0.0001$), reflective of the overall effect of the lesion in the right striatum in the three excitotoxin-lesioned groups. The interaction Group x Side was significant

($F(3,110)=25.734$, $p<0.0001$), reflective the striatal degeneration sustained in the right hemisphere by the three excitotoxin-lesioned groups but no degeneration in the controls. The analyses in general describe significant loss of tissue in the right striatum as a consequence of excitotoxin injection.

Ventricle. The ventricle was measured in one coronal plane. All effects were significant. This included a significant effect for Group ($F(3,38)=5.637$, $p<0.01$), Side ($F(1,38)=32.597$, $p<0.0001$) and Group x Side ($F(3,38)=5.635$, $p<0.01$). The statistical analysis was reflective of a significant dilation of the ventricle on the right side of the brain due to significant loss of striatal tissue in the three groups with excitotoxin-induced striatal lesions.

Ventrolateral Nucleus of the Thalamus (VL). The effect of Group was significant ($F(3,27)=3.758$, $p<0.05$), indicating a significantly smaller VL nucleus (at the plane examined) in the all three lesioned groups when compared to the control group.

Ventroanterior Nucleus of the Thalamus (VA). The VA nucleus was measured at one coronal level; the statistical analysis did not reveal any significant effects.

Thalamus. The thalamus as a whole was measured in two planes: more tissue area was present with rostro-caudal progression. This was reflected in a significant effect for Coronal Plane ($F(1,74)=65.494$, $p<0.01$). The effect of Group was significant ($F(3,74)=3.198$, $p<0.05$), indicating significantly less thalamic area in the Kainic acid-lesioned group when compared to the other three groups (control, quinolinic acid, ibotenic acid). The effect of Side was also significant

($F(1,74)=4.611$, $p<0.05$), reflective of the loss of thalamic area in the kainic acid-lesioned group in the right hemisphere when compared to the other three groups.

Cortex. Cortical area of the frontal cortex was measured in three planes. There was more cortical area in all groups with rostro-caudal progression, hence the significant effect of Coronal Plane ($F(2,109)=22.778$, $p<0.0001$). However, none of the other effects were significant.

Discussion

This study has revealed significant striatal degeneration accompanied by ventricular dilation in rats injected unilaterally with one of three excitotoxins that were sacrificed one year post-lesion. This was accompanied by small changes in the thalamus, and no changes between groups in the frontal cortex. The results of this study for the striatum and cortex are similar to those reported previously in the behavioural study of rats with chronic QUIN-induced striatal lesions (refer to Chapter 4), the primary difference being the much longer post-surgery survival time in the present study. Thus, this study has shown that even one year post-surgery, animals with excitotoxin-induced striatal lesions do not demonstrate morphometric changes in the cortex. The same caveats that were mentioned previously must be discussed. First, it is possible that cell counts in the various cortical areas measured would have revealed changes that might precede gross tissue loss. Second, it is possible that even longer post-surgical survival times might produce gross tissue loss in those areas examined in this study. However, it

Tables 6.1-6.8 Controls, QUIN, Morphometry study. Mean area for all structures.

Mean area (mm2) and associated SEs

Group				
CONT	Cortical area Hemisphere		Striatal area Hemisphere	
Plane	left	right	left	right
+1.7	1671.6	1839.4	713.84	891.15
	125.9	93.4	25.9	55.62
+1.2	1884.6	1711.9	1051.3	1083.3
	20	89.8	175.2	21.9
-0.3	2006.8	2084.5	940.3	849.4
	59.9	60.6	103.2	82.4

Mean area (mm2) and associated SEs

Group

QUIN

Plane	Cortical area Hemisphere left	Cortical area Hemisphere right	Striatal area Hemisphere left	Striatal area Hemisphere right
+1.7	1776.8	1830.5	839.9	441.2
	33.7	26.5	81.9	67.01
+1.2	1740.2	1582.1	1067.7	362.22
	60.6	65	90.4	117.4
-0.3	2120.1	1956.3	1125.4	410.9
	96.9	89.9	73.9	70.1

Mean area (mm2) and associated SEs

Group

IBO

Plane	Cortical area Hemisphere left	Cortical area Hemisphere right	Striatal area Hemisphere left	Striatal area Hemisphere right
+1.7	1866.7	1643.3	1000.5	555.71
	47.2	88.1	44.1	92.2
+1 2	1890.4	1754.1	1207.7	516.1
	132.1	125.3	63.4	45.8
-0.3	2013.4	1883.4	1131.1	434.9
	102.1	72.5	68.9	58.5

Mean area (mm2) and associated SEs

Group

KAIN

Plane	Cortical area Hemisphere left	Cortical area Hemisphere right	Striatal area Hemisphere left	Striatal area Hemisphere right
+1.7	1679.9	1508.7	862.01	541.8
	109.1	139.3	52.9	62.1
+1.2	1807.8	1477.6	1149.9	608.7
	49.1	85.8	55.9	119.8
-0.3	1914.8	2089.9	1101.6	543.2
	181.2	107.5	41.6	85.3

Mean area (mm2) and associated SEs

Group

CONT

Plane	Thalamic area		VA area	
	left	right	left	right
-1.8	1225.2	1326.2	152.3	136.2
	69.3	78.8	27.9	6
-2.3	1627.8	1613.3	-	-
	67.9	86.7		

Plane	VL area	
	left	right
-1.8	354.1	366.4
	58.8	65.6
-2.3	-	-

Mean area (mm2) and associated SEs

Group

QUIN

Plane	Thalamic area		VA area	
	left	right	left	right
-1.8	1460.8 92.9	1279.4 153.7	132.3 22.7	96.9 38.4
-2.3	1810.9 65	1515.4 89.7	-	-

Plane	VL area	
	left	right
-1.8	254.3 53.7	172.4 41.3
-2.3	-	-

Mean area (mm2) and associated SEs

Group

IBO

Plane	Thalamic area		VA area	
	left	right	left	right
-1.8	1205.7	1213.2	122.9	102.1
	103.1	63.8	18.6	11.7
-2.3	1720.2	1665.1	-	-
	96.7	57.2		

Plane	VL area	
	left	right
-1.8	230.2	250.7
	44.7	26.3
-2.3	-	-

Mean area (mm2) and associated SEs

Group

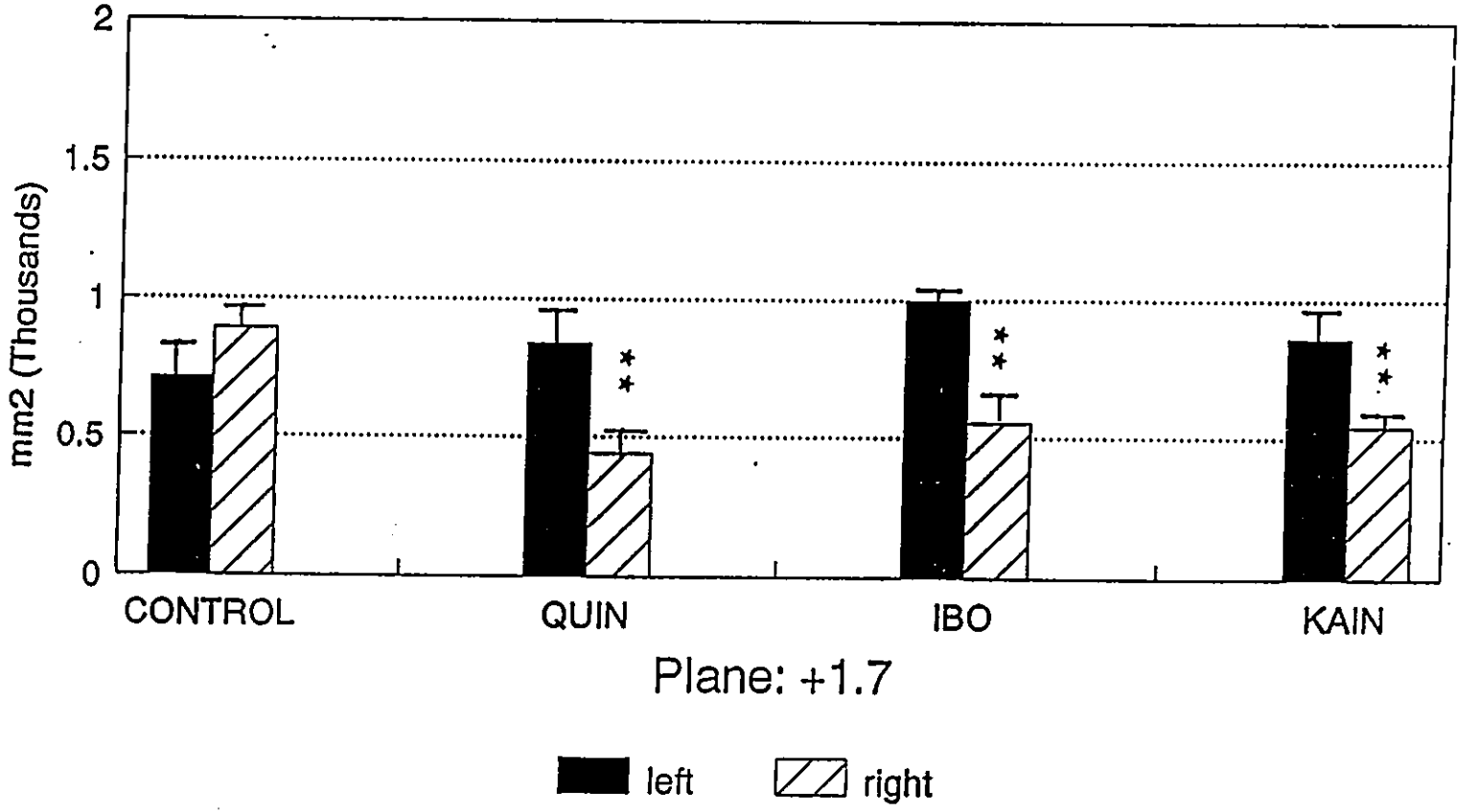
KAIN

Plane	Thalamic area		VA area	
	left	right	left	right
-1.8	1254.4 39.9	1134.1 84.1	144.2 24.9	116.2 22.2
-2.3	1562.6 47.8	1376 81.9	-	-

Plane	VL area	
	left	right
-1.8	259.2 55.2	189.5 52.7
-2.3	-	-

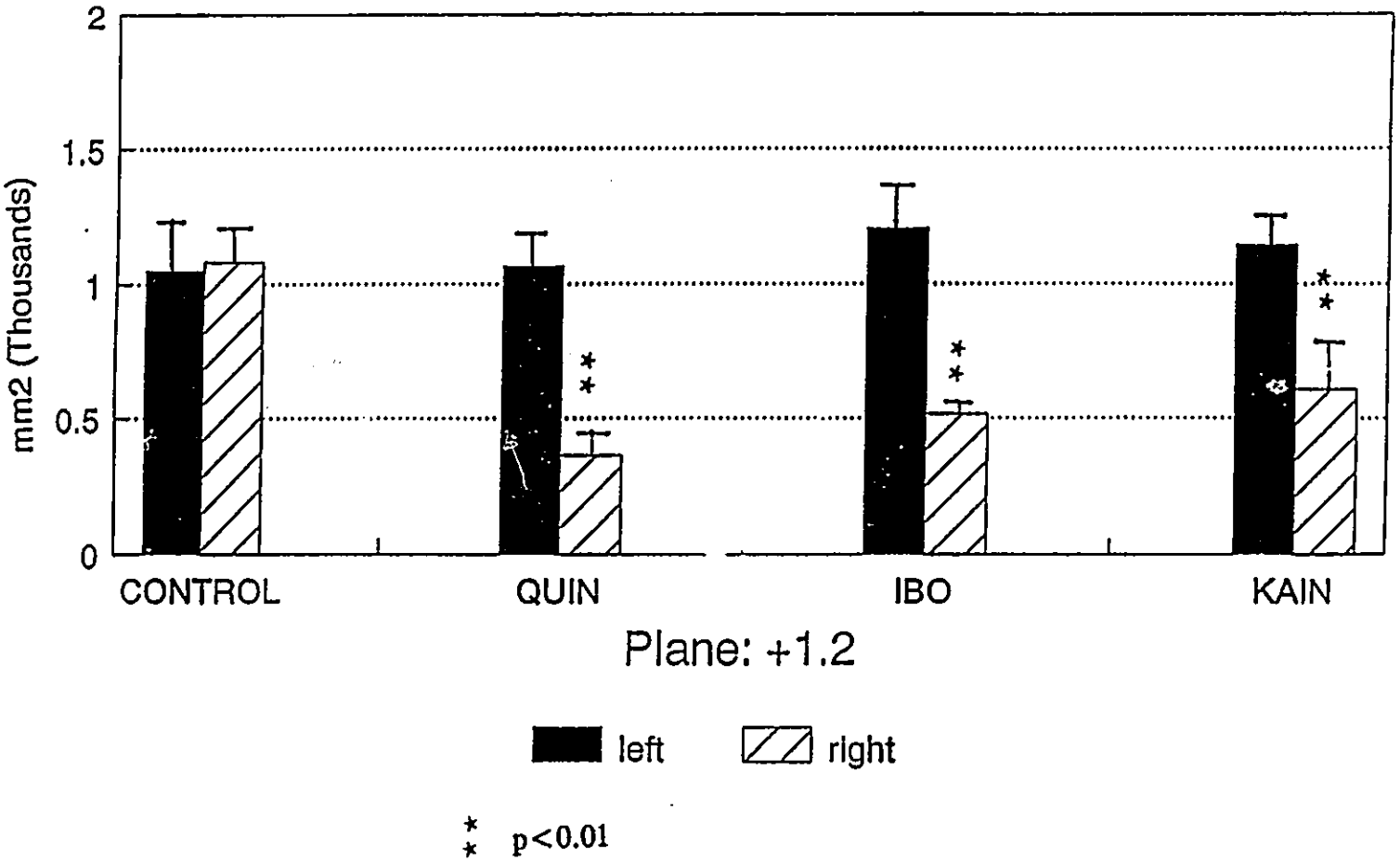
Figures 6.8-6.17 Morphometry study. Graphic description of mean area for all structures.

Morphometry: Striatum

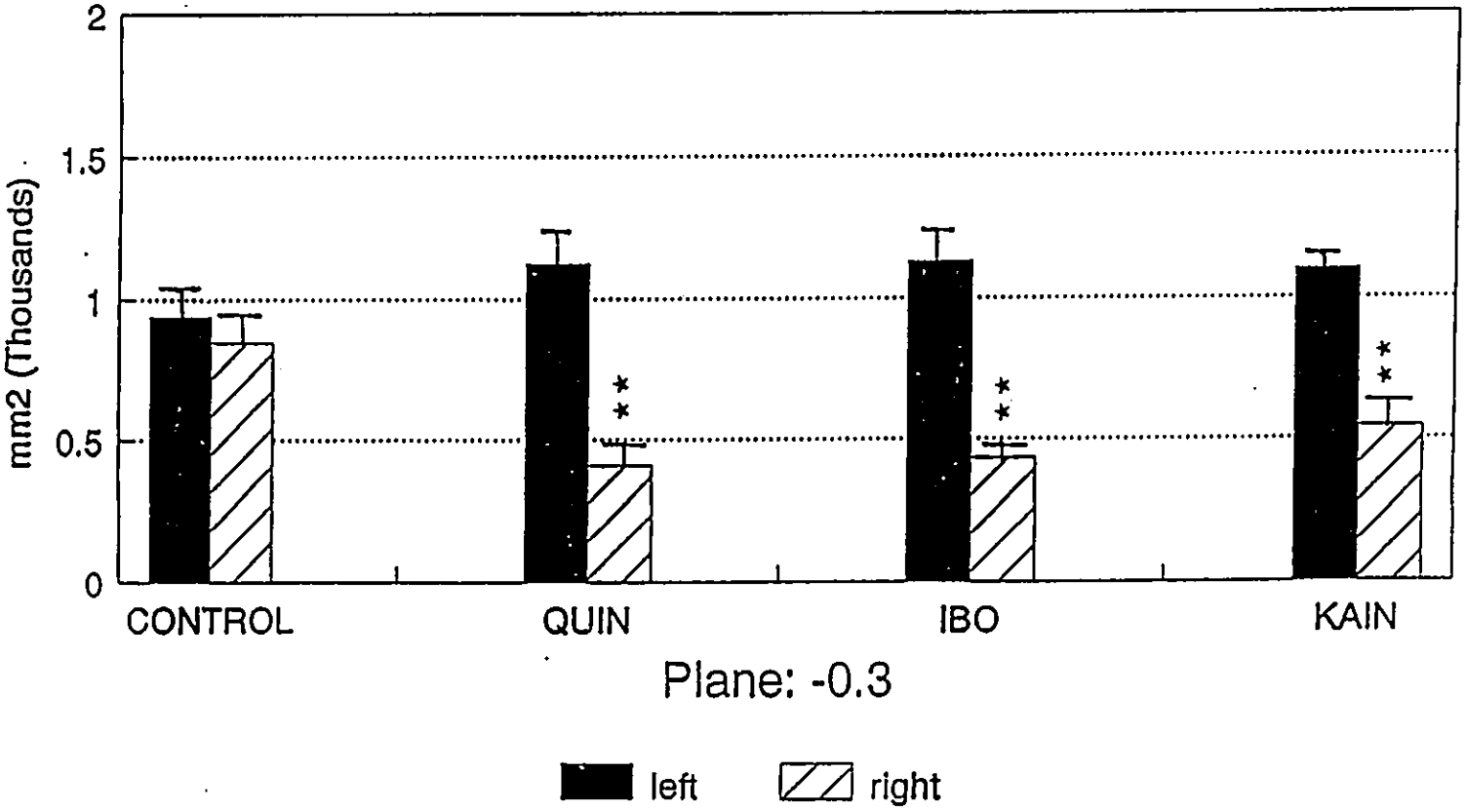


* p<0.01

Morphometry: Striatum

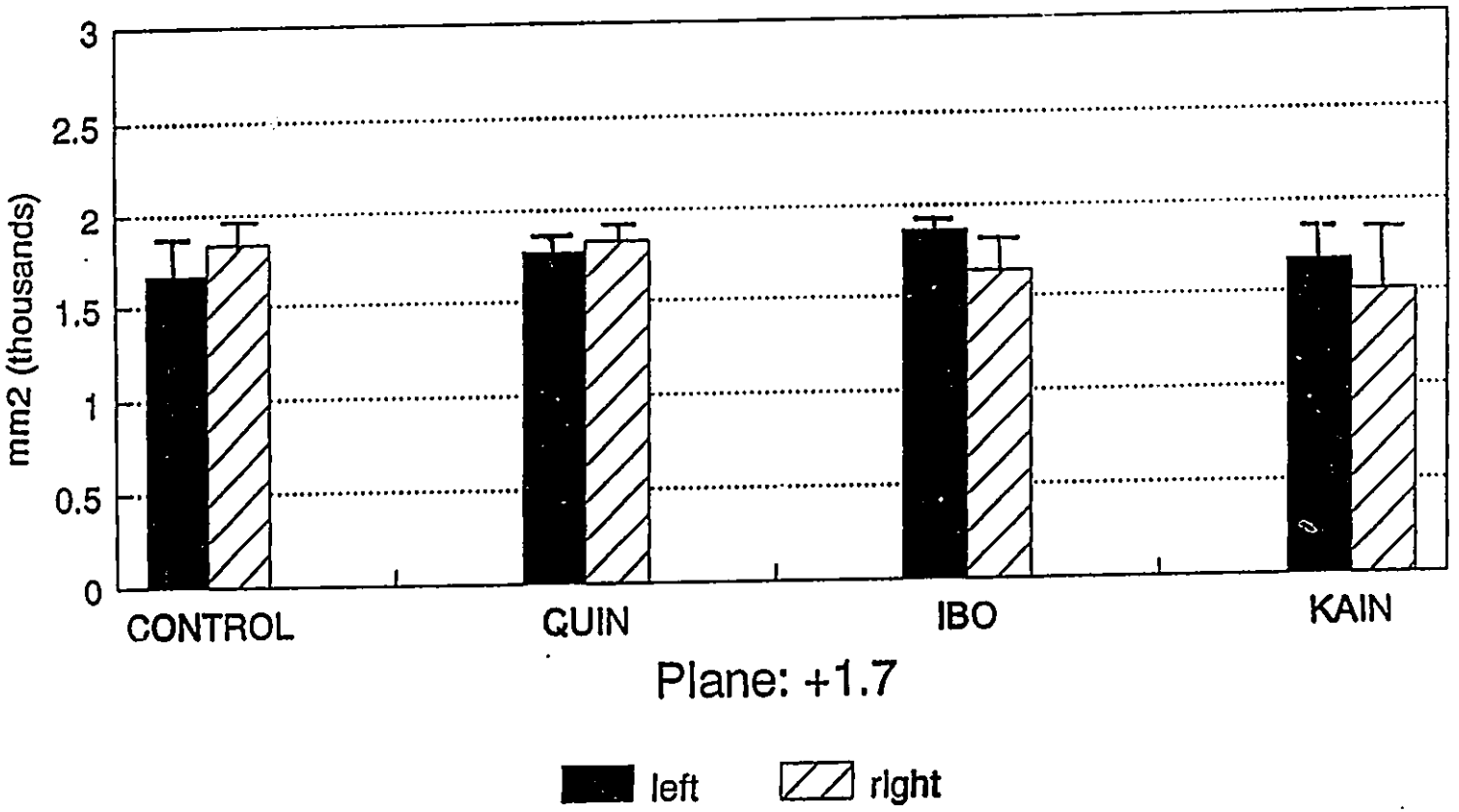


Morphometry: Striatum

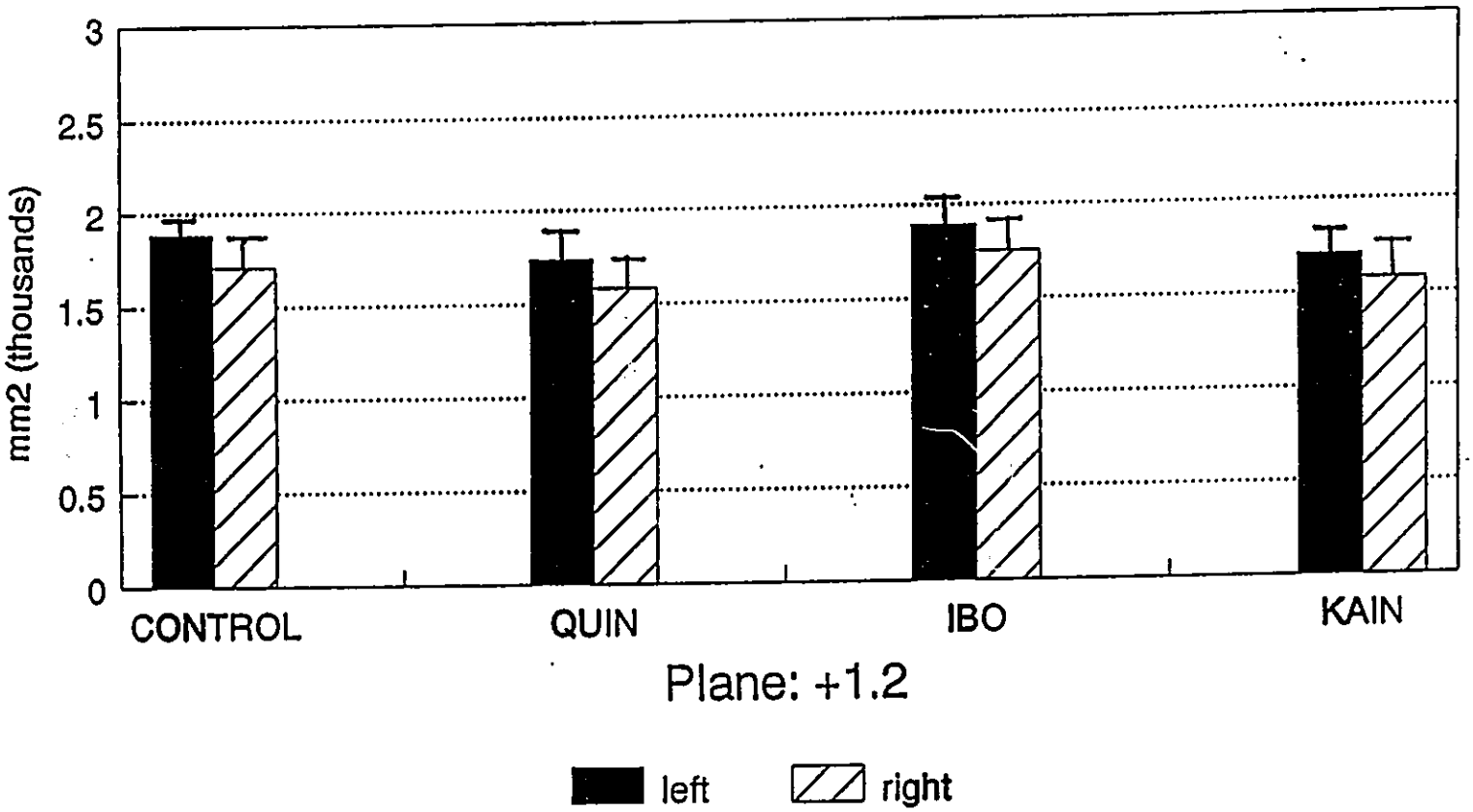


* p<0.01

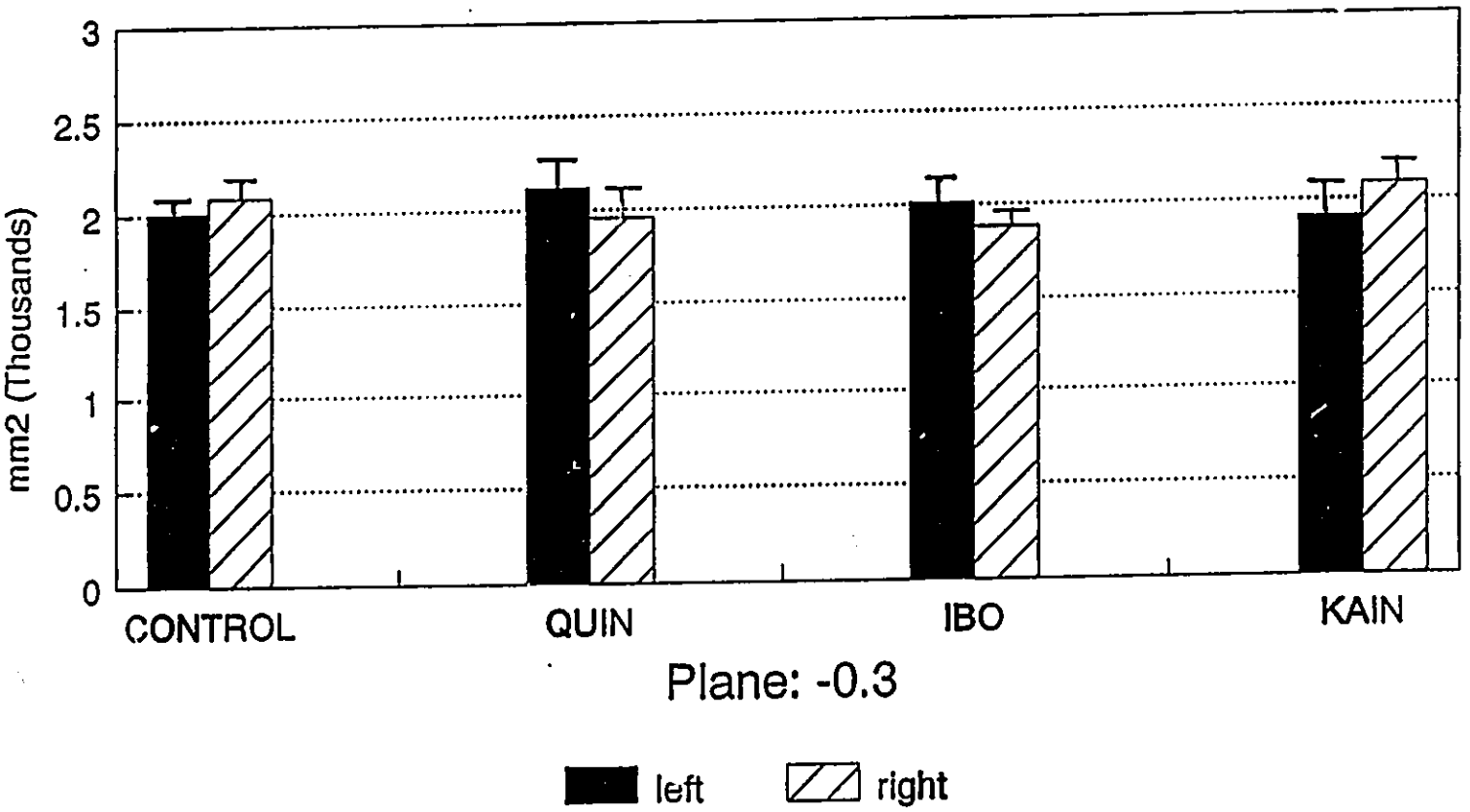
Morphometry:Cortical Area



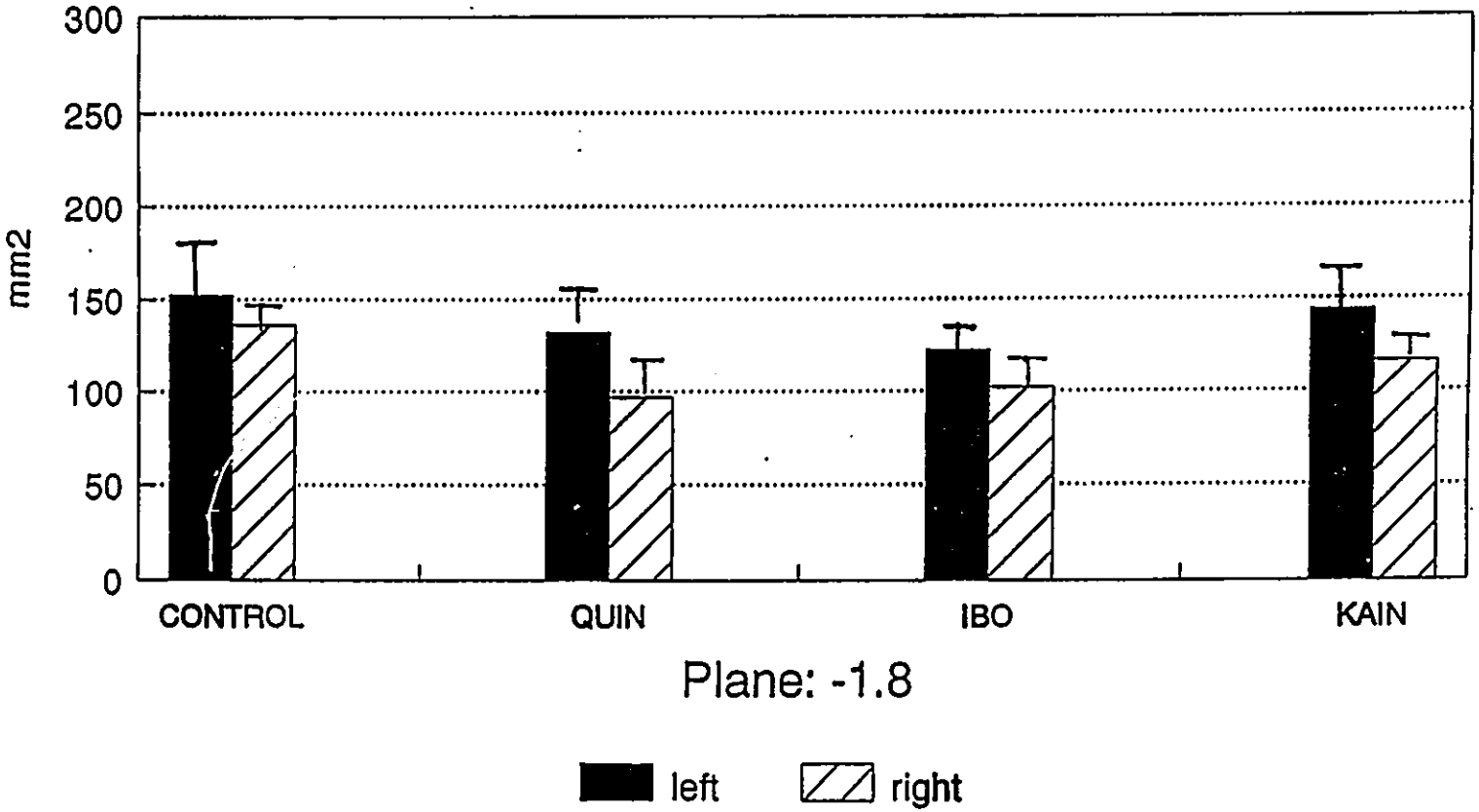
Morphometry: Cortex



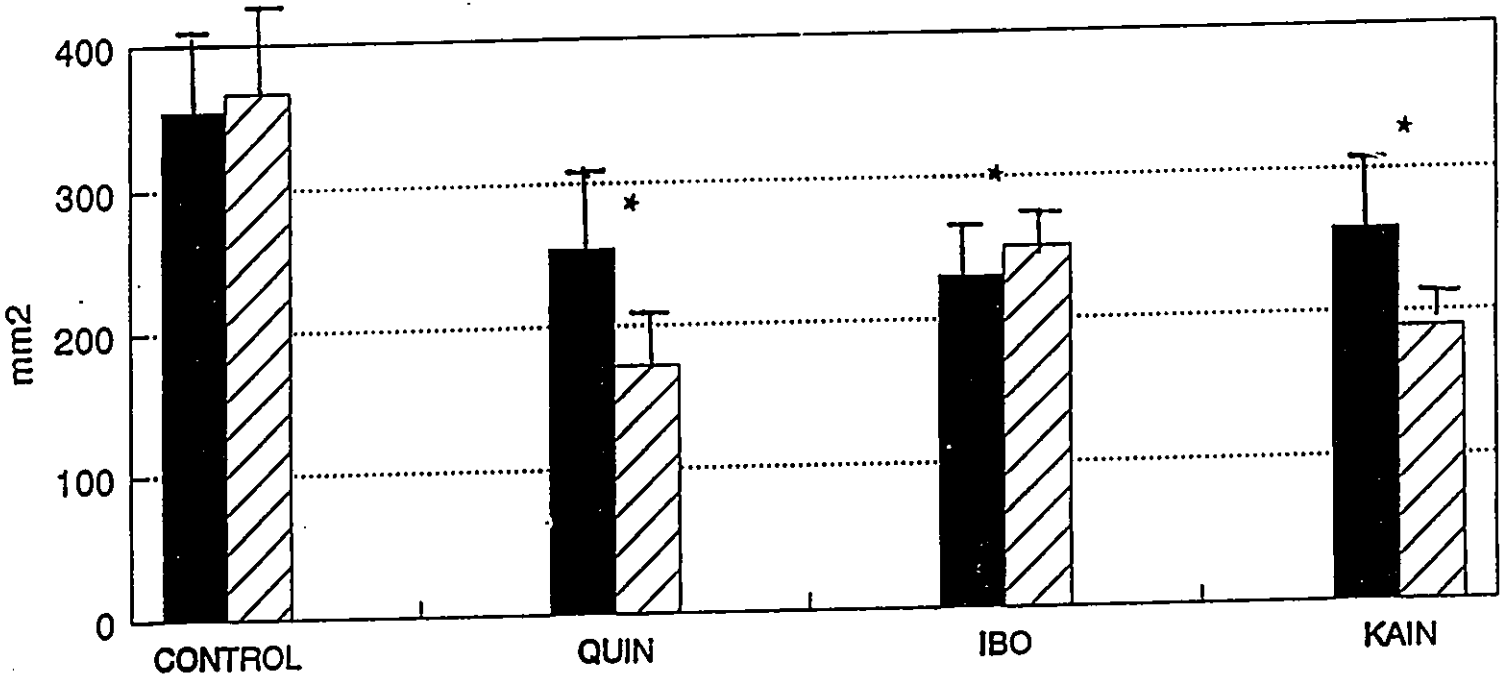
Morphometry: Cortex



Morphometry: VA nucleus

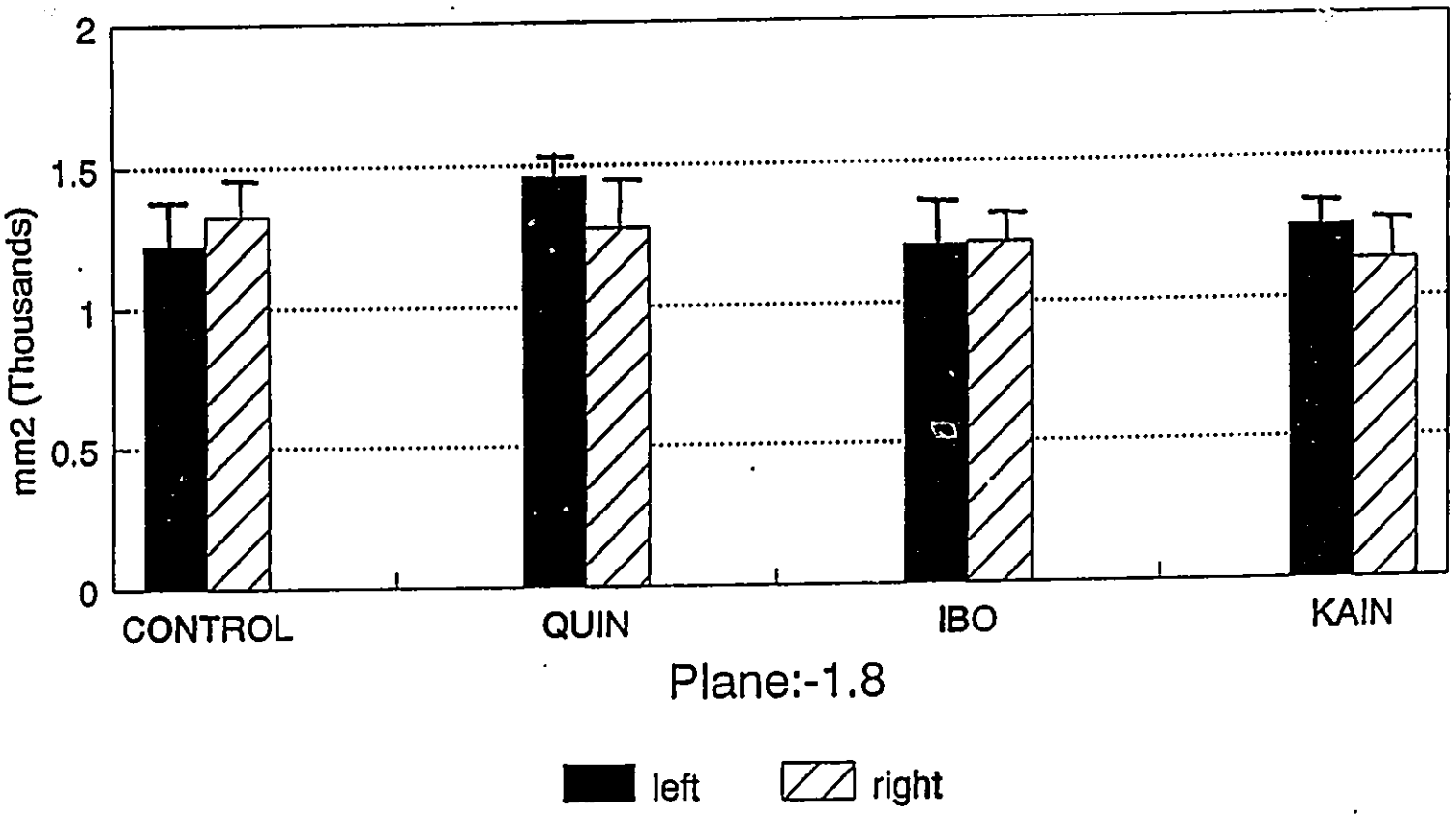


Morphometry: VL nucleus

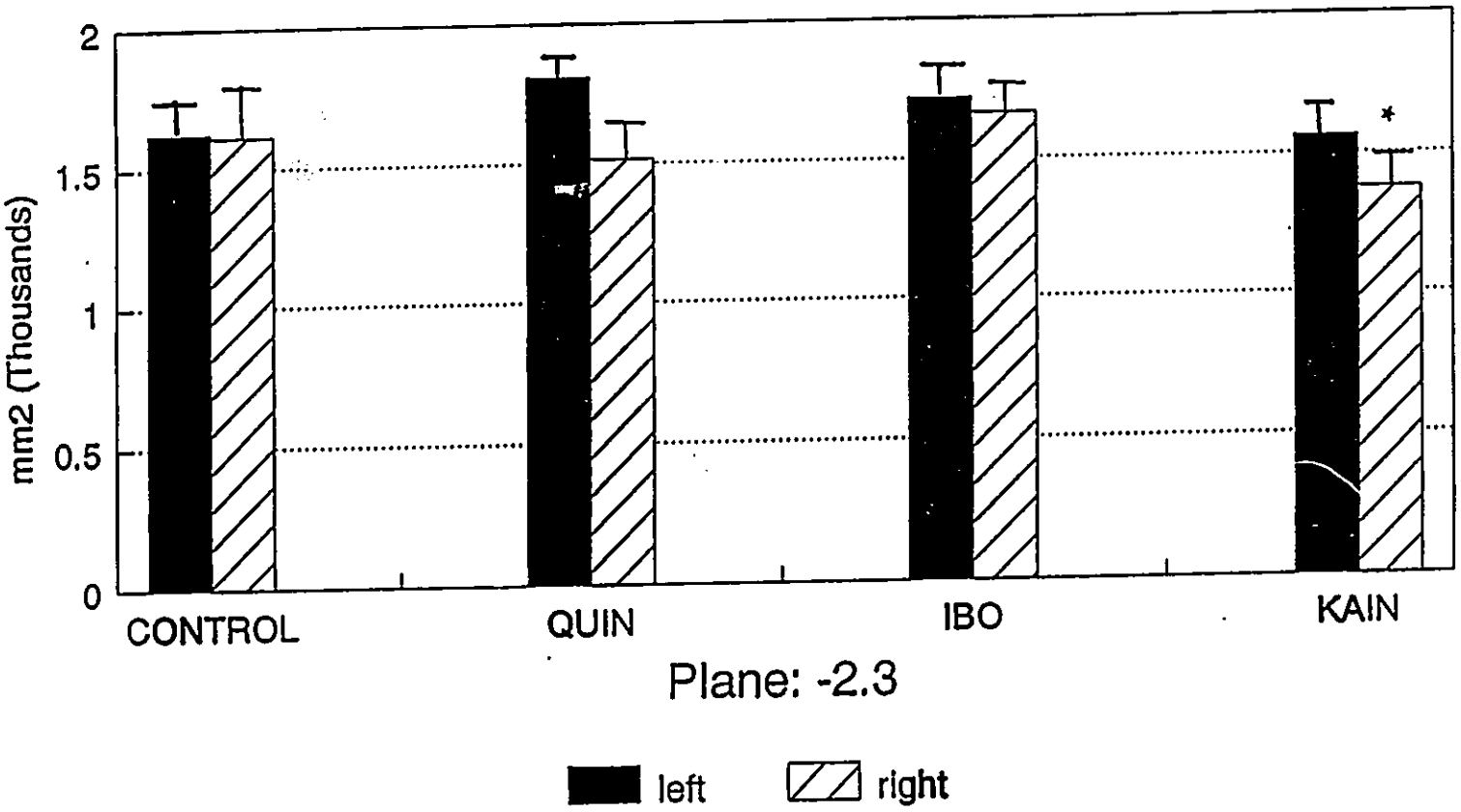


* $p<0.05$

Morphometry: Thalamus



Morphometry: thalamus



* $p<0.05$

may also be the case that the short life-span of the rat precludes much longer survival times; this would not make the rat a good subject in the study of animal models of HD.

The results of this study may be compared with those of Vonsattel, de la Monte, Sotrel and their colleagues who reported gross extrastriatal changes in HD post-mortem tissue (refer to literature review on gross changes in HD brain). Using morphometric techniques similar to those used in this study, these investigators have shown significant loss of tissue area in the thalamus and cortex; largely speaking, no such changes were revealed here.

I. Morphometric Analysis of Striatal and Extrastriatal Structures in Rats with Chronic QUIN-induced Striatal Lesions

B. Immunocytochemistry: tyrosine hydroxylase and substance P

This part of the morophometric study used immunocytochemistry to characterize the substantia nigra in rats with chronic QUIN-induced striatal lesions. Again, the question is whether the extrastriatal pathology observed in HD is secondary to striatal degeneration or the result of an independent pathological process for the various extrastriatal structures. The extrastriatal area in question is the subtantia nigra.

Methods

Surgery. The animals used in this experiment received unilateral striatal lesions created via the injection of quinolinic acid. Male, Sprague-Dawley rats (200-250 g) were anaesthetized and placed into a Kopf small animal stereotaxic apparatus. Four were controls receiving intrastriatal injections of 0.9% saline; six received unilateral injections of one ul of quinolinic acid (240 nm) into the right hemisphere at the following co-ordinates: A/P 0.7; M/L 2.5; D/V 5.5 (from skull). The vehicle and excitotoxin were delivered via a 10 ul Hamilton syringe (Hamilton Company, Reno, NV) fitted with a 30 gauge blunt-tipped needle. After the needle had been lowered to the proper co-ordinates, it remained in place for one minute prior to commencing the injection. Following the protocols of previous researchers, the QUIN was delivered at a rate of one ul per minute. After the injection was complete, the needle remained in place for an additional two minutes prior to

retraction.

Histology. Five months after surgery the CONT and QUIN-lesioned-rats were deeply anaesthetized with sodium pentobarbital, and perfused with 0.9% saline followed by 4% paraformaldehyde. The brains were removed and placed in the same fixative overnight at 4 degrees, Celsius. The following day, the tissue was rinsed with 0.1 M phosphate buffer, and the tissue subsequently stored in a solution of 30% sucrose in 0.1 M phosphate buffer at 4 degrees, C until the tissue sank (approximately 2 days). Care was taken to ensure that the brain tissue from the different groups was treated in the same way.

Each brain was blocked and frozen. The tissue was attached to cryostat chucks using Histoprep (Fisher Scientific), and the brains were subsequently sectioned in the coronal plane at a thickness of 40 um. Striatal sections were collected on chrom-alum subbed slides; sections from the substantia nigra were collected in 0.1 M phosphate-buffered saline (PBS).

The striatal sections were stained with cresyl violet (Nissl stain) while the sections from the substantia nigra were processed for immunocytochemistry. The tissue collected from the substantia nigra was incubated for 48 hours at 4 Celsius, with the following primary antibodies: tyrosine hydroxylase (EugeneTech, 1:1000) and substance P (IncStar, 1:1000). The tissue was then washed in PBS (3 changes, 10 minutes each) and incubated in biotinylated secondary antibody for 90 minutes. After another wash, the tissue was incubated in the avidin-biotin complex (ABC; Vectastain, Dimension Laboratories)) for 60 minutes. Visualization of the end

product occurred through incubation in diaminobenzidine (DAB; Sigma). Further washings were followed by mounting on chrom-alum subbed slides, subsequent dehydration through a a series of alcohols and mounting out of xylene with DPX.

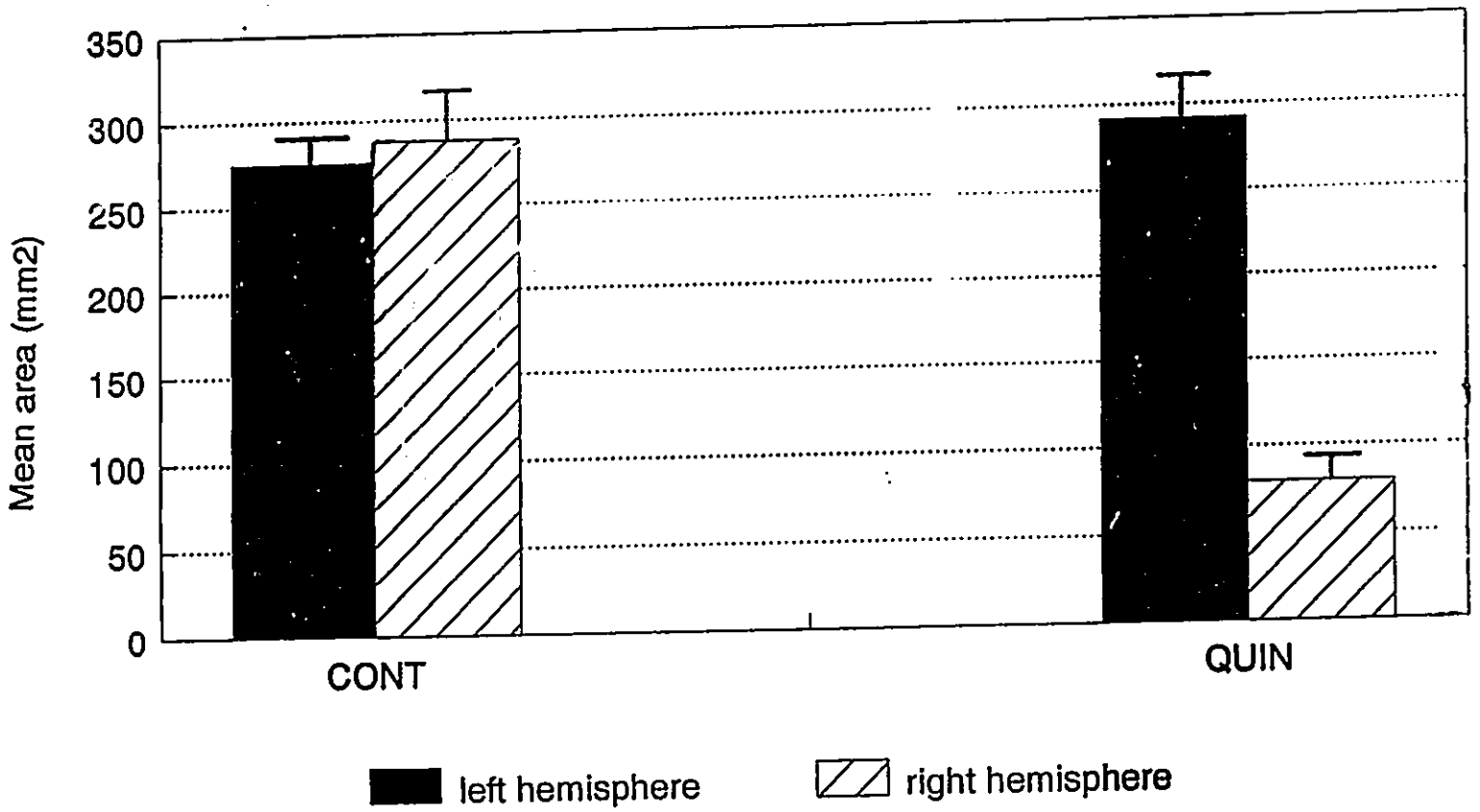
Morphometric Analysis. Areal measurements of the substantia nigra were taken. Sections each animal in each group were expanded and traced using a Bausch and Lomb projector. Area was assessed using a Bioquant System IV software package (R and M Biometrics, Nashville, Tennessee). The individual tracings were placed on a a Hipad Digitizer software tablet (Houston Instruments, Austin, Texas) connected to an IBM Personal Computer. Area was measured using a cursor (Houston Instruments) attached to the software tablet. For each individual structure, two measurements were taken and the mean of the two was calculated.

Results

Nissl staining revealed significant degeneration in the right striatum in the QUIN lesioned animals. The substantia nigra was well delineated by the immunocytochemical processing. The right substantia nigra (lesion side) was markedly and significantly shrunken in relation to the left substantia nigra (refer to figure 6.18 and table 6.9). No differences in area were noted between the left and right substantia nigra in the control animals or between the controls and the unlesioned side in the QUIN animals.

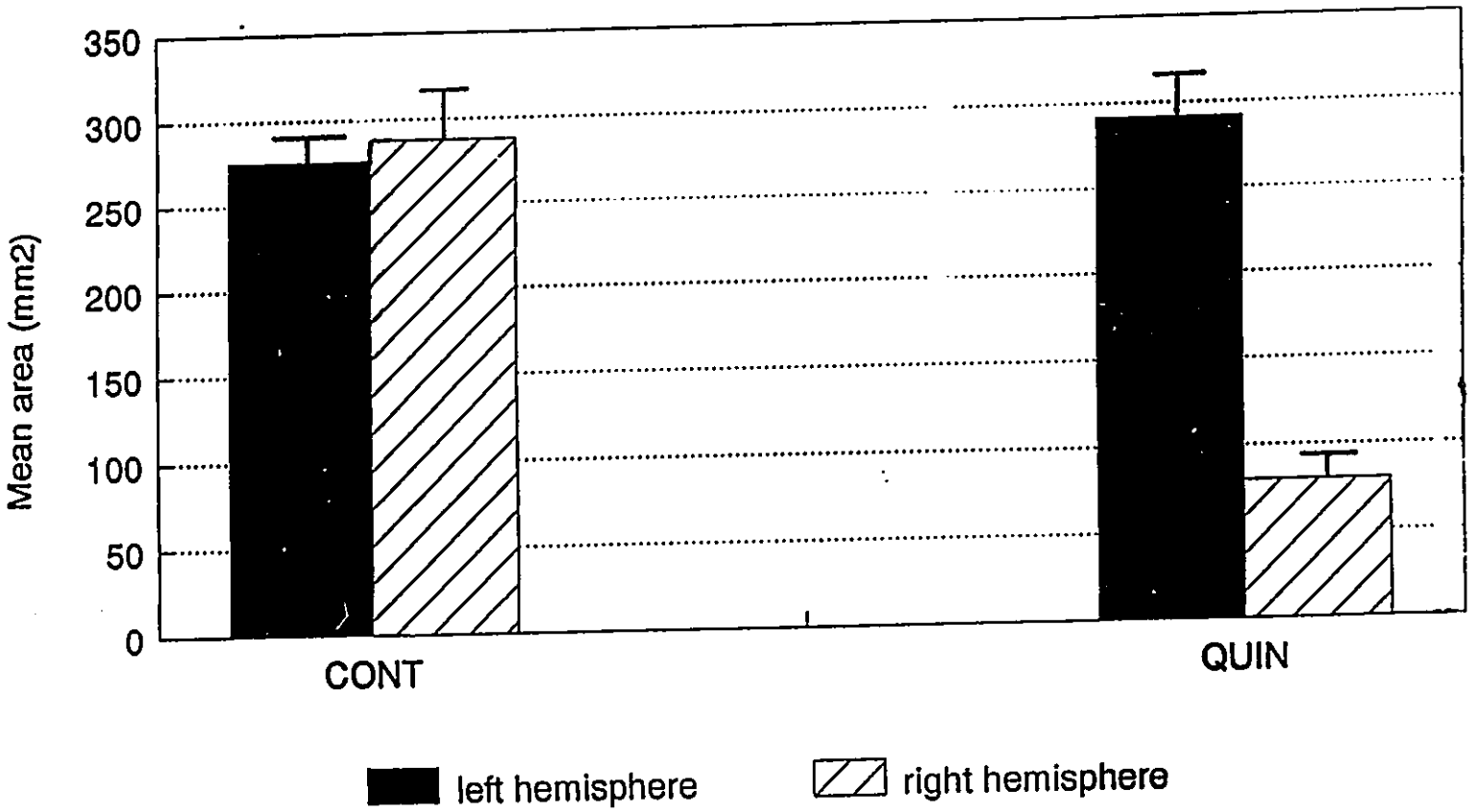
Figure 6.18 Mean area of substantia nigra.

Mean area of substantia nigra



substance P stain

Mean area of substantia nigra



substance P stain

Table 6.9 Mean area of substantia nigra.

Mean area and associated SEs of substantia nigra (mm^2) in control (CONT) and chronic QUIN-lesioned (QUIN) rats

tyrosine hydroxylase stain

	Left	Right (lesioned)
CONT	309.2 (10.4)	325.2 (8.9)
QUIN	315.7 (12.3)	107.2 (8.5)

Substance P stain

	Left	Right (lesioned)
CONT	275.8 (12.2)	288.8 (8.3)
QUIN	291.9 (10.5)	79.9 (7.6)

Discussion

The area of the substantia nigra on the lesioned side was significantly smaller than that of the unlesioned side. This is in agreement with the areal analysis of the substantia nigra in HD (Ferrante et al., 1989; Oyanagi et al., 1989) and is likely reflective of the destruction of the medium spiny neurons of the striatum, and the degeneration of the projection from these cells to the substantia nigra.

II. Neurochemical Analysis of Cortex and Striatum in Rats with Chronic QUIN-induced striatal lesions

The morphometric studies performed revealed no cortical changes in animals with chronic QUIN-induced striatal lesions. However, one potential criticism of this work might be that the negative finding in the cortex was the result of the employment of a technique (i.e., morphometry) that lacked sufficient sensitivity. In this study, the striatum and cortex of rats with chronic (i.e., one year) QUIN-induced striatal lesions were examined neurochemically for a variety of neuropeptides.

Methods.

Surgery Sixteen, Sprague-Dawley rats (200-250 g; Charles-River, Quebec) were anaesthetized and placed into a Kopf small animal stereotaxic apparatus. Eight animals received unilateral injections of one ul of vehicle while the remaining eight received unilateral injections of one ul of quinolinic acid (240nmols) (QUIN; Sigma, St. Louis, MO) into right hemisphere at the following co-ordinates: A/P 0.7; M/L 2.5; D/V 5.5 (from skull). The vehicle and excitotoxins were delivered via a 10 ul Hamilton syringe (Hamilton Company, Reno, NV) fitted with a 30 gauge blunt-tipped needle. The needle remained in place for 1 minute after it was lowered to the proper co-ordinates. The vehicle and QUIN were delivered at a rate of one ul per minute. After the injection was complete, the needle remained in place for an additional two minutes prior to retraction and closing of the wound.

Tissue Preparation and Neurochemical Analysis. Twelve months post-surgery, the eight controls and eight QUIN-lesioned animals were killed by decapitation, the brains removed and put on ice. Using a rat brain matrix (Harvard Apparatus, South Natick, MA), each brain was sectioned into 2 mm coronal sections. A number of cortical areas (frontal pole, dorsolateral prefrontal cortex, anterior motor cortex, anterior somatosensory cortex, posterior motor cortex, posterior somatosensory cortex, entorhinal cortex and striate cortex) were dissected and placed in 1ml 0.1N HCl. The striatum was also dissected for analysis. All samples were boiled for 10 minutes and three 250 ul aliquots were taken and lyophilized for later analysis via radioimmunoassay (RIA).

Lyophilized tissue extracts were reconstituted in one ml of standard assay buffer and samples assayed for the peptides substance P (SP), somatostatin (SS), neuropeptide Y (NPY) and cholecystokinin (CCK) using either a six day equilibrium assay (CCK, SS, SP) or a three day/three day disequilibrium assay (NPY). The primary antisera for all the assays except for SP had been previously raised in New Zealand rabbits; the SP primary antibody was raised in guinea pigs. For specific details on how the primary antibodies were raised refer to Beal et al. (1988a, 1988) and Mazurek et al., 1989). All primary antibodies were previously characterized for RIA. Neuropeptide Y showed a cross-reactivity of 0.28% with peptide YY; substance P showed cross-reactivity of 1.3% with eledoisin-related peptide and 0.9% with neurokinin A; cholecystokinin showed a cross-reactivity of less than 0.001% with a number of peptides (Beal et al., 1988a, 1988b; Mazurek et

al., 1989). The intra-assay variation is 5%; interassay variation is 10%. The final primary antisera dilutions were as follows: CCK, 1:200 K; NPY, 1:100K; SS 1:600K; SP 1:3000K.

The sample, appropriate antisera and radioactive label (New England Nuclear), were incubated at 4 degrees, Celsius for the first period of incubation. Subsequently, the secondary antibody was added (goat anti-rabbit antibody/normal rabbit serum (Linco Research), for CCK, NPY, and SS; goat-anti guinea pig/normal guinea pig serum (Biotek)in the case of SP), and the tubes incubated for a further 24 hours. (In the disequilibrium assay for CCK, the radioactive label was added on the fourth day, instead of the first.) The next day the tubes were centrifuged at 3500 rpm for 30 minues, the supernatant aspirated, and the pellets counted on a gamma counter (Micromedic) to measure radioactivity.

Results

Neurochemistry. All data are expressed as a percent of control values. Substance P showed a mean decrease of 50% in the striatum on the lesioned side ($p<0.01$) in the QUIN group (refer to figure 6.19). There were no significant differences between the two groups for SS, NPY, or CCK in any of the eight cortical areas measured (see figure 6.20).

Figure 6.19 Chronic QUIN-induced striatal lesions. Neurochemical analysis for substance P.

CHRONIC STRIATAL LESION: QUIN STRIATUM

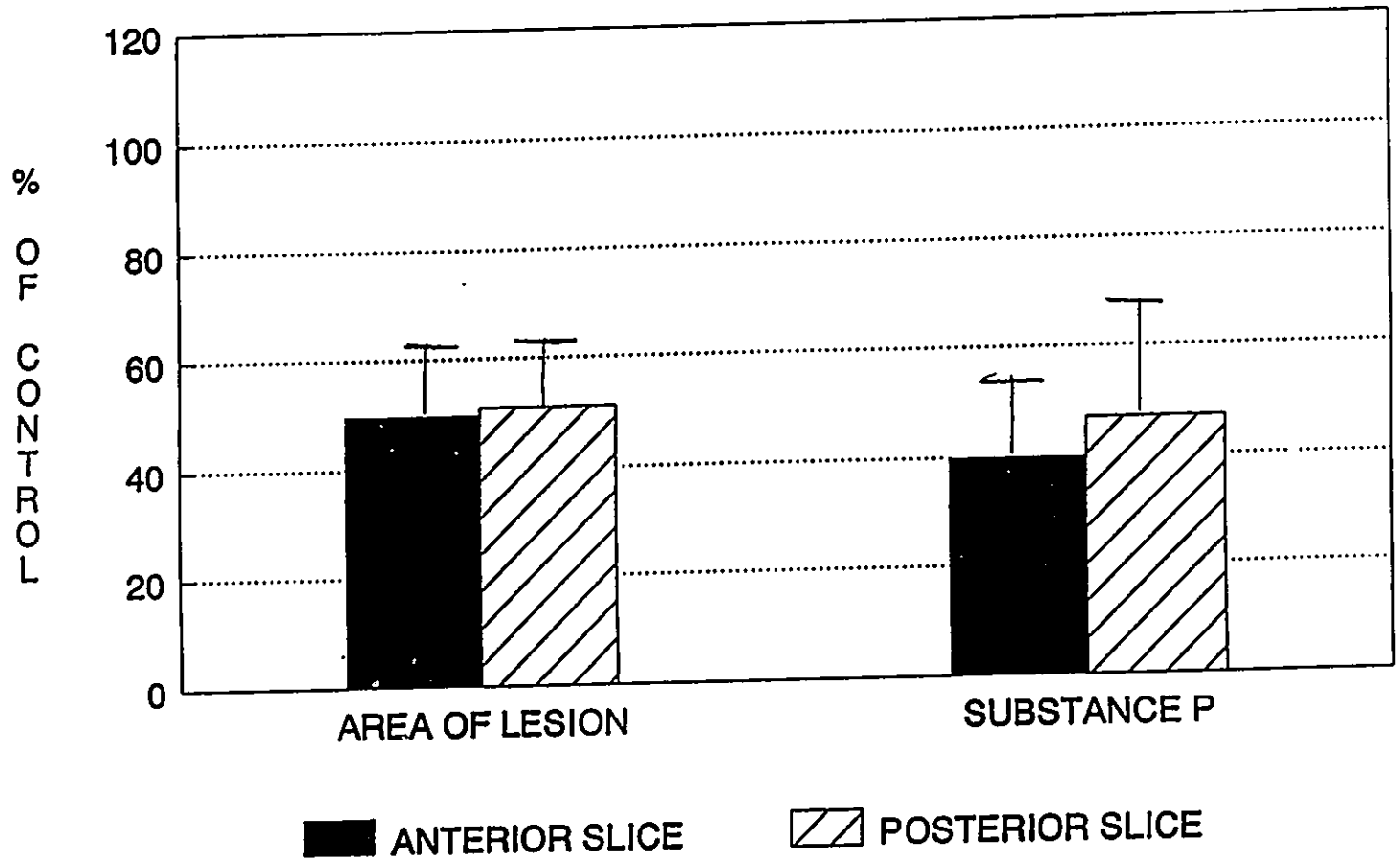
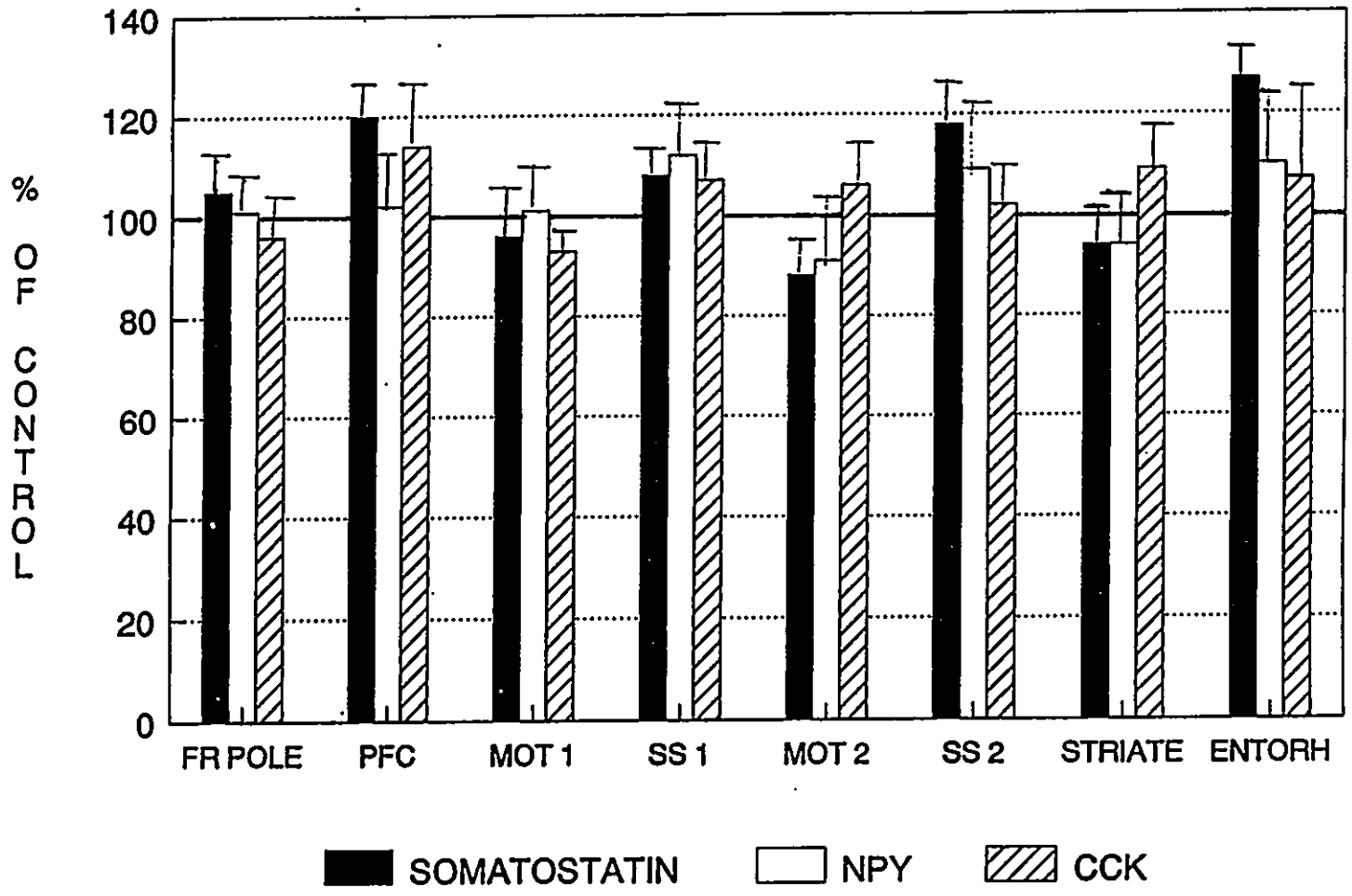


Figure 6.20 Chronic QUIN-induced striatal lesions. Neurochemical analysis for neuropeptides.

CHRONIC STRIATAL LESION: CORTEX



Discussion

Animals with chronic striatal QUIN lesions developed extensive striatal degeneration and ventricular dilation. This is reflected in the 50% depletion of SP-like immunoreactivity in the striatum. However, even one year later, no significant neurochemical changes were found in a variety of cortical areas. My results are supported by a recent study which has examined rats with QUIN-induced striatal lesions sacrificed six months and one year post-surgery and found significant changes in striatal transmitter levels but no changes in transmitter levels in the single area of cortex directly overlying the striatal injection site (Beal et al., 1991b). The present neurochemical study is also in agreement with my examination of cortical morphometry in animals with chronic (i.e., one year) QUIN-induced striatal lesions.

General Discussion

This group of experiments has examined striatal and extrastriatal structures in animals with chronic excitotoxin-induced striatal lesions. In addition to degeneration in the striatum, morphoemtric changes have been found in certain thalamic nuclei and substantia nigra. No changes have been found in the thalamus as a whole (morphometry) or various areas of cortex (morphometry and neurochemistry).

As mentioned previously, one of the reasons for carrying out this study was related to the question of whether the extrastriatal pathology demonstrated in

patients with HD is secondary to the primary problem of striatal degeneration, or whether it arises independent of the striatal problem. The combination of positive and negative findings in the present study leave the question unresolved. The finding of changes in the nigra and certain thalamic nuclei in this study suggests that these changes may be the result of striatal damage, and that, given sufficient time, the thalamus as a whole and perhaps cortical areas, will also show similar secondary changes. It must also be acknowledged that the rodent striato-pallido-thalamo-cortical connections may be sufficiently different from that of the human to account for the failure of this study to show cortical and thalamic changes similar to those reported by Vonsattel and his colleagues (Vonsattel et al., 1985, 1987; de la Monte et al., 1987, 1988; Sotrel and Myers, 1990). Alternatively, the negative changes in cortex found in this study may suggest that the changes in HD cortex are the result of a disease process separate from that in the striatum.

CHAPTER 7

CONCLUSIONS

CONCLUSIONS

The present thesis has been primarily concerned with the examination of the behavioural, neuroanatomical and neurochemical consequences of quinolinic acid-induced lesions of the medial striatum and medial frontal cortex in the rat. The choice of research questions has been directed by what is known about Huntington's Disease, a degenerative disease of the CNS in which the striatum undergoes marked degeneration. A number of questions have been addressed. The first three are primarily concerned with behaviour. The first question is whether nocturnal hyperactivity in rats with excitotoxin-induced damage of the striatum may be considered an adequate marker of striatal dysfunction. The second question is whether quinolinic acid-induced lesions of the medial striatum can produce a behavioural profile analogous to that found in HD. The third question is concerned with exploring the consequences of quinolinic acid-induced damage to the medial frontal cortex. The fourth question addresses the issue of extrastriatal effects following excitotoxin-induced striatal damage (neurochemical and neuroanatomical analyses) and the extent to which this model resembles the pathological profile of HD.

The first question, that of the adequacy of nocturnal hyperactivity as a marker of striatal dysfunction after excitotoxin-induced striatal damage, arose from a combination of the literature on animal models of HD as well and my own initial research with the QUIN model of HD. The literature clearly indicated that kainic acid and ibotenic acid-induced lesions of the striatum would produce a marked, significant spontaneous nocturnal hyperactivity. Yet, my initial experiments with the QUIN model failed to

confirm this finding. Further experimentation confirmed the presence of significant, though not permanent, nocturnal hyperactivity in animals lesioned with kainic and ibotenic acid, but I have never been able to find comparable hyperactivity in animals lesioned with quinolinic acid. Additional findings of this set of experiments have been that the hyperactivity in kainic and ibotenic acid-lesioned animals attenuates with time to the point where the lesioned animals are indistinguishable from controls and that hyperactivity in ibotenic acid-lesioned animals may develop gradually. The neural basis for the hyperactivity and its attenuation remains unclear, although I have proposed a number of possible mechanisms. Although I believe the basic phenomena to be replicable, I also believe that the time course of hyperactivity in kainic acid and ibotenic acid-lesioned animals may be somewhat variable. Regardless, these experiments have important implications for the use of hyperactivity as an output signal to monitor the effectiveness of transplants and other measures aimed at reversing the effects of excitotoxin-induced striatal lesions.

The second question has been concerned with the behavioural analysis of rats with quinolinic acid-induced lesions of the medial striatum. Detailed behavioural study has revealed these animals to be impaired in the performance of the place task version of the Morris Water Maze and delayed alternation task, but unimpaired on a variety of motor tests, suggesting that the deficits observed are attributable to a cognitive impairment, rather than some other interpretation. Extensive water maze testing has revealed that given a new platform location after a delay, controls learn the new platform task much faster than they learned the original platform location, whereas the

lesioned animals do not demonstrate a similar transfer of learning. Examination of the time spent in the old training quadrant after the platform was moved to a new location revealed that the lesioned animals spent a significantly greater percentage of their time in the old training quadrant that controls, seemingly indicative of some sort of perseveration, an impairment also demonstrated in HD patients. These experiments has also suggests a role for the striatum in facilitating the learning of a new task after the learning of a similar task, as well as in the switching of mental sets. To the extent that it is possible to compare animals and humans, the cognitive deficits expressed by these groups of animals may be considered analogous to those exhibited by patients with HD. Histological analysis revealed significant striatal degeneration without cortical atrophy (morphometric analysis) indicating that it seems to be possible to produce cognitive impairments analogous to those demonstrated in HD by lesions restricted to the striatum. This has important implications for the direction of therapeutic strategies for Huntington's Disease.

The knowledge that both the frontal cortex and striatum sustain degeneration in HD has left the neural basis for the neuropsychiatric impairment open to interpretation. The previous experiments have shown that it is possible to produce impairments analogous to those in HD with damage that is seemingly restricted to the striatum. This does not, however, rule out the involvement of the cortex in the neuropsychiatric syndrome. This problem was approached through the behavioural analysis of quinolinic acid-induced lesions of the medial frontal cortex in rats. These animals were impaired in the performance of the Morris Water Maze, but seemingly unimpaired on

a number of motor tests. Histological analysis confirmed the presence of cortical damage, but also suggested the presence of striatal damage, indicating that it may not be possible to restrict quinolinic acid-induced damage to the cortex alone and that the resulting behavioural profile may be an expression of damage to both areas. Future research in for these last two questions could involve the investigation of ways to attenuate the behavioural impairment, as well as an investigation into the behavioural profile of animals with QUIN-induced lesions of the lateral striatum. I have already begun some of these investigations.

The fourth question addressed extrastriatal effects after excitotoxin-induced damage of the medial striatum. Neurochemical and/or neuroanatomical changes were found in the striatum, substantia nigra and thalamus, but no changes were found in the neocortex. Excluding more pedestrian considerations, this suggests that the extrastriatal damage found in HD cortex may represent pathological processes unrelated to those found in the striatum.

The present thesis has been concerned with the behavioural, neuroanatomical and neurochemical evaluation of animal models of HD. The findings of this thesis can certainly be interpreted only from the viewpoint of understanding the neural basis of behaviour, and while this is certainly the higher goal, one of the values of an animal model of a disease is to begin to address questions concerning the disease; I believe this thesis has been successful addressing both these aspects.

REFERENCES

Adams RD and Victor M. 1989. Principles of Neurology, Fourth Edition. New York: McGraw-Hill

Albert MS, Butters N, and Brandt J. 1981. Patterns of remote memory in amnesic and demented patients. Archives of Neurology, 38, 495-500.

Albin RL, Young AB, Penney JB, Handelin B, Balfour R, Anderson KD, Markel DS, Tourtellote WW and Reiner A. 1990. Abnormalities of striatal projection neurons and NMDA receptors in presymptomatic Huntington's Disease. New England Journal of Medicine, 322(18), 1293-1298.

Alexander GE, Crutcher MD and DeLong MR. 1990. Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. In HBM Uylings, CG Van Eden, JPC De Bruin, MA Corner and MGP Feenstra (Eds.) The Prefrontal Cortex. Its Structure, Function and Pathology, Progress in Brain Research, 45, 119-146.

Alexander GE, DeLong MR and Strick PL. 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annual Review of Neuroscience,19, 357-381.

Anderson KD and Reiner A. 1990. Extensive co-occurrence of substance P and dynorphin in striatal projection neurons: an evolutionarily conserved feature of basal ganglia organization. Journal of Comparative Neurology, 295, 339-369.

Aquilonius S-M, Eckernäs S-A and Sundwall A. 1975. Regional distribution of choline acetyltransferase in Huntington's chorea. Journal of Neurology, Neurosurgery and Psychiatry, 38, 669-677.

Arai H, Emson PC and Carrasco LH. 1987. Huntington's Disease: Changes in tachykinin content in postmortem brains. Annals of Neurology, 22, 587-594.

Aronin N, Cooper PE, Lorenz LJ, Bird ED, Sagar SM, Leeman SE and Martin JB. 1983. Somatostatin is increased in the basal ganglia in Huntington's Disease. Annals of Neurology, 13, 519-526.

Beal MF, Ellison DW, Mazurek MF, Swartz KJ, Malloy JR, Bird ED and Martin JB. 1988a. A detailed examination of substance P in pathologically graded cases of Huntington's Disease. Journal of the Neurological Sciences, 84, 51-61.

Beal MF, Mazurek MF, Ellison DW, Swartz KJ, McGarvey U, Bird ED and Martin JB. 1988b. Somatostatin and neuropeptide Y concentrations in pathologically graded cases of Huntington's Disease. Annals of Neurology, 23, 562-569.

Beal MF, Ferrante RJ, Swartz KJ and Kowall NW. 1991a. Chronic quinolinic acid lesions in rats closely resemble Huntington's Disease. Journal of Neuroscience, 11(6), 1649-1659.

Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ and Martin JB. 1986. Replications of the neurochemical characteristics of Huntington's Disease by quinolinic acid. Nature, 321, 168-171.

Beal MF, Kowall NW, Swartz KJ, Ferrante RJ, and Martin JB. 1989. Differential sparing of somatostatin-neuropeptide Y and cholinergic neurons following striatal excitotoxin lesions. Synapse, 3, 38-47.

Beal MF, Marshall PE, Burd GD, Landis DMD and Martin JB. 1985. Excitotoxin lesions do not mimic the alteration of somatostatin in Huntington's Disease. Brain Research, 361, 135-145.

Beal MF, Swartz KJ, Finn SF, Mazurek MF and Kowall NW. 1991b. Neurochemical characterization of excitotoxin lesions in the cerebral cortex. Journal of Neuroscience, 11(1), 147-158.

Beatty WW. 1989. Remote memory for visuospatial information in patients with Huntington's Disease. Psychobiology, 17, 431-434.

Beatty WW, Salmon DP, Butters N, Heindel WC and Granholm EL. 1988. Retrograde amnesia in patients with Alzheimer's or Huntington's Disease. Neurobiology of Aging, 9, 181-186.

Ben-Ari Y, Tremblay E, Ottersen OP, Naquet R. 1979. Evidence suggesting secondary epileptogenic lesions after kainic acid: pretreatment with diazepam reduces distant but not local brain damage. Brain Research, 165, 362-365.

Bernheimer H and Hornykiewicz O. 1973. Brain amines in Huntington's chorea. Advances in Neurology, 1, 525-531.

Bing G, Notter MFD, Hansen JT, Kellogg C, Kordower JH and Gash DM. 1990. Cografts of adrenal medulla in rats with 6-OHDA-induced lesioins. Neuroscience, 34(3), 687-697.

Bird ED. 1980. Chemical pathology of Huntington's Disease. Annual Review of Pharmacology and Toxicology, 20, 533-551.

Bird ED, Caro AJ and Pilling JB. 1974. A sex related factor in the inheritance of Huntington's chorea. Annals of Human Genetics, 37, 255-260.

Bird ED and Iversen LL. 1974. Huntington's chorea. Post-mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. Brain, 97, 457-472.

Bird ED, MacKay AVP, Rayner CN and Iversen LL. 1973. Reduced glutamic acid-decarboxylase activity of post-mortem brain in Huntington's Chorea. Lancet, 2, 1090-1092.

Boegman RJ and Parent A. 1988. Differential sensitivity of neuropeptide Y, somatostatin and NADPH-diaphorase containing neurons in rat cortex and striatum to quinolinic acid. Brain Research, 445, 358-362.

Boegman RJ, Smith Y and Parent A. 1987. Quinolinic acid does not spare striatal neuropeptide Y-immunoreactive neurons. Brain Research, 415, 178-183.

Bohn MC, Cupit L, Marciano F and Gash DM. 1987. Adrenal medulla grafts enhance recovery of striatal dopaminergic fibres. Science, 237, 913-916.

Brandeis R, Brandys Y and Yehuda S. 1989. The use of the Morris water maze in the study of memory and learning. International Journal of Neuroscience, 48, 29-69.

Brandt J and Butters N. 1986. The neuropsychology of Huntington's Disease. TINS, 9, 118-120.

Brooks WJ, Jarvis MF and Wagner JC. 1989. Astrocytes as a locus for the conversion of MPTP into MPP+. Journal of Neural Transmission, 76, 1-12.

Brouwers P, Cox C, Martin A, Chase T and Fedio P. 1984. Differential perceptual-spatial impairment in Huntington's and Alzheimer's dementias. Archives of Neurology, 41, 1073-1076.

Bruyn GW, Bots GThAM, and Dom R. 1979. Huntington's Chorea: Current neuropathological status. In TN Chase, NS Wexler and A Barbeau (eds).

Advances in Neurology, Vol 23, Huntington's Disease, pgs. 83-93. New York: Raven Press.

Bruyn RPM and Stoof JC. 1990. The quinolinic acid hypothesis in Huntington's chorea. Journal of the Neurological Sciences, 95, 29-38.

Butters N, Wolfe J, Martone M, Granholm E and Cermak L. 1985. Memory disorders associated with Huntington's disease: verbal recall, verbal recognition and procedural memory. Neuropsychologia, 23(6), 729-743.

Caine ED and Fisher JH. 1985. Dementia in Huntington's Disease. Handbook of Clinical Neurology, 46, pgs. 305-310. JAM Frederiks, Ed.

Caine E, Hunt RD, Weingartner H and Ebert MH. 1978. Huntington's dementia. Clinical and neuropsychological features. Archives of General Psychiatry, 35, 377-384.

Chesselet MF and Graybiel MF. 1986. Striatal neurons expressing somatostatin-like immunoreactivity: evidence for a peptidergic internueonral system in the cat. Neuroscience, 17(3), 547-571.

Choi DW. 1988. Glutamate neurotoxicity and diseases of the nervous system. Neuron, 1, 623-634

Choi DW, and Rothman SM. 1990. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annual Review of Neuroscience, 13, 171-182.

Clarke DJ, Dunnett SB, Isacson O, Srinathsinghji DJS and Björklund A. 1988. Striatal grafts in rats with unilateral neostriatal lesion-I. Ultrastructural evidence of afferent synaptic inputs from the host nigrostriatal pathway. Neuroscience, 24(3), 791-801.

Cooper JR, Bloom FE and Roth RH. 1991. The Biochemical Basis of Neuropharmacology, New York: Oxford University Press, sixth edition.

Côté L and Crutcher MD. 1985. Chapter 40. Motor functions of the basal ganglia and diseases of transmitter metabolism. In ER Kandel and JH Schwartz (eds). Principles of Neural Science, pgs. 523-535 New York: Elsevier.12

Coyle JT and Schwarcz R. 1976. Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. Nature, 263, 244-246.

Coyle JT, McGeer EG, McGeer PG and Schwarcz R. 1978. Neostriatal injections: a model for Huntington's chorea. In EG McGeer, J Olney and PL McGeer (eds).

Kainic Acid as a Tool in Neurobiology, pgs. 139-159. New York: Raven Press.

Cudkowicz M and Kowall NW. 1990. Degeneration of pyramidal projection neurons in Huntington's Disease cortex. Annals of Neurology, 27, 200-204

Davies K. 1992. Slow search for the Huntington's Disease gene. Nature, 574, 95.

Davies SW and Roberts PJ. 1987. No evidence for preservation of somatostatin containing neurons after intrastriatal injection of quinolinic acid. Nature, 327, 326-329.

Dawbarn D, De Quidt ME and Emson PC. 1985. Survival of basal ganglia neuropeptide Y-somatostatin neurones in Huntington's Disease. Brain Research, 340, 251-260.

Dawbarn D, Zamir N, Waters CM, Hunt SP, Emson PC, and Brownstein MJ. 1986. Peptides derived from prodynorphin are decreased in basal ganglia of Huntington's Disease brains. Brain Research, 372, 155-158.

de la Monte SM, Vonsattel J-P, Hedley-Whyte ET, Ulcickas M, and Richardson EP. 1987. Global loss of cerebral cortex and white matter in Huntington's Disease. Journal of Neuropathology and Experimental Neurology, 46, 342.

de la Monte SM, Vonsattel J-P, and Richardson EP. 1988. Morphometric demonstration of atrophic changes in the cerebral cortex, white matter and neostriatum in Huntington's Disease. Journal of Neuropathology and Experimental Neurology, 47(5), 516-525.

Diamond A and Goldman-Rakic PS. 1987. Comparison of human infants and rhesus monkeys on Piaget's AB task: evidence for dependence on dorsolateral prefrontal cortex. Experimental Brain Research, 74, 24-40.

DiFiglia M, Pasik T and Pasik P. 1980. Ultrastructure of Golgi-impregnated and gold-toned spiny and aspiny neurons in the monkey neostriatum. Journal of Neurocytology, 9, 471-492.

DiFiglia M, Schiff L, and Deckel AW. 1988. Neuronal organization of fetal striatal grafts in kainate- and sham-lesioned rat caudate nucleus: light and electron-microscopic observations. Journal of Neuroscience, 8(4), 1112-1130.

Divac I, Markowitsch HJ and Pritzel M. 1978. Behavioural and anatomical consequence of small intrastriatal injections of kainic acid in the rat. Brain Research, 151, 523-532.

Divac I, Wikmark RGE and Gade E. 1975. Spontaneous alternation in rats with lesions in the frontal lobes: an extension of the frontal lobe syndrome. Physiological Psychology, 3(1), 39-42.

Dunnett SB, Isacson O, Srinathsinghji DJS, Clarke DJ and Björklund A. 1988. Striatal grafts in rats with unilateral neostriatal lesions-III. Recovery from dopamine dependent motor asymmetry and deficits in skilled paw reaching. Neuroscience, 24(3), 813-820.

Dunnett SB, and Iversen SD. 1981. Learning impairments following selective kainic acid-induced lesions within the neostriatum of rats. Behavioural Brain Research, 2, 189-209.

Dunnett SB, Low WC, Iversen SD, Stenevi U, and Björklund A. 1982. Septal transplants restore maze learning in rats with fornix-fimbria lesions. Brain Research, 251, 335-348.

Dunnett SB, Ryan CN, Levin PD, Reynolds M, and Bunch ST. 1987a. Functional consequences of embryonic neocortex transplanted to rats with prefrontal cortex lesions. Behavioural Neuroscience, 101(4), 489-503.

Dunnett SB, Whishaw, IQ, Jones GH and Bunch ST. 1987b. Behavioural, biochemical and histochemical effects of different neurotoxic amino acids injected into the nucleus basalis magnocellularis of rats. Neuroscience, 20(2), 653-669.

Dure LS, Young AB and Penney JB. 1991. Excitatory amino acid binding sites in the caudate nucleus and frontal cortex of Huntington's Disease. Annals of Neurology, 30, 785-793.

Earle KM. 1973. Pathology and experimental models of Huntington's chorea. Advances in Neurology, 1, 341-350.

Ellison DW, Beal MF, Mazurek MF, Malloy JR, Bird ED and Martin JB. 1987. Amino acid neurotransmitter abnormalities in Huntington's Disease and the quinolinic acid animal model of Huntington's Disease. Brain, 110, 1657-1673.

Emson PC, Arregui A, Clement-Jones V, Sandberg BEB, and Rossor M. 1980. Regional distribution of methionin-enkephalin and substance P immunoreactivity in normal human brain and in Huntington's Disease. Brain Research, 199, 147-160.

Fedio P, Cox CS, Neophytides A, Canal-Frederick G and Chase TN. 1979. Neuropsychological profile of Huntington's Disease: patients and those at risk. In TN Chase (ed.) Advances in Neurology, 23, 239-255.

Ferrante RJ, Beal MF, Kowall NW, Richardson EP and Martin JB. 1987a. Sparing of acetylcholinesterase-containing striatal neurons in Huntington's Disease. Brain Research, 411, 162-166.

Ferrante RJ and Kowall NW. 1987b. Tyrosine hydroxylase-like immunoreactivity is distributed in the matrix compartment of normal human and Huntington's Disease striatum. Brain Research, 416, 141-146.

Ferrante RJ, Kowall NW, Martin JB and Richardson EP. 1985a. Characteristics of a selectively spared subset of neurons in Huntington's Disease. Journal of Neuropathology and Experimental Neurology, 44, 1325.

Ferrante RJ, Kowall NW, Beal MF, Richardson EP, Bird ED and Martin JB. 1985b. Selective sparing of a class of striatal neurons in Huntington's Disease. Science, 230, 561-563.

Ferrante RJ, Kowall NW, Richardson EP, Bird ED and Martin JB. 1986. Topography of enkephalin, substance P and acetylcholinesterase in Huntington's Disease striatum. Neuroscience Letters, 71, 283-288.

Ferrante RJ, Kowall NW and Richardson EP. 1989. Neuronal and neuropil loss in the substantia nigra in Huntington's Disease. Journal of Neuropathology and Experimental Neurology, 48, 380.

Fiandaca MS, Kordower JH, Hansen JT, Shou-Shu J, and Gash DM. 1988. Adrenal medullary autografts into the basal ganglia of Cebus monkeys: injury-induced regeneration. Experimental Neurology, 102, 76-91.

Fibiger HC. 1978. Kainic acid lesions of the striatum: a pharmacological and behavioural model of Huntington's Disease. In E McGeer, J Olney and PL McGeer (eds). Kainic Acid as a Tool in Neurobiology, pgs. 161-176. New York: Raven Press.

Folstein SE. 1989. Huntington's Disease: A Disorder of Families, Baltimore: Johns Hopkins University Press.

Folstein SE, Abbott MH, Chase GA, Jensen BA and Folstein MF. 1983a. The association of affective disorder with Huntington's Disease in a case series and in families. Psychological Medicine, 13, 537-542.

Folstein SE, Franz ML, Jensen BA, Chase GA and Folstein MF. 1983b. Conduct disorder and affective disorder amoung the offspring of patients with Huntington's Disease. Psychological Medicine, 13, 45-52.

Folstein SE, Leigh J, Parhad IM and Folstein MF. 1986. The diagnosis of Huntington's Disease. Neurology, 36, 1279-1283.

Freedman M, and Oscar-Berman M. 1986. Bilateral frontal lobe disease and selective delayed response deficits in humans. Behavioural Neuroscience, 100 (3), 337-342.

Furtado JCS and Mazurek MF. 1992. A role for the lateral striatum in visuospatial cognition. Society for Neuroscience Abstracts.

Gale JS, Bird ED, Spokes EG, Iversen LL and Jessell T. 1978. Human brain substance P: distribution in controls and Huntington's chorea. Journal of Neurochemistry, 30, 633-634.

Garnett ES, Firnau G, Nahmias C, Carbotte R, and Bartolucci G. 1984. Reduced striatal glucose consumption and prolonged reaction time and early features in Huntington's Disease. Journal of the Neurological Sciences, 65, 231-237.

Gerfen C. 1992a. The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annual Review of Neuroscience, 15, 285-320.

Gerfen C. 1992b. The neostriatal mosaic: multiple levels of compartmental organization. TINS, 15(4), 133-139.

Giordano M, Hagenmeyer-Houser SH and Sanberg PR. 1988. Intraparenchymal fetal striatal transplants and recovery in kainic acid lesioned rats. Brain Research, 446, 183-188.

Goldman PS and Nauta WJH. 1977. An intricately patterned prefronto-caudate projection in the rhesus monkey. Journal of Comparative Neurology, 171, 369-386.

Goldman-Rakic PS. 1987. Development of cortical circuitry and cognitive function. Child Development, 58, 601-622.

Gordon WP and Illes J. 1987. Neurolinguistic characteristics of language production in Huntington's Disease: A preliminary report. Brain and Language, 31, 1-10.

Gouras P. 1985. The Oculomotor System. In ER Kandel and JH Schwartz (eds). Principles of Neural Science, pgs. 573-590, New York: Elsevier.

Grafe MR, Forno LS, and Eng LF. 1985. Immunocytochemical studies of substance P and met-enkephalin in the basal ganglia and substantia nigra in Huntington's,

Parkinson's and Alzheimer's Diseases. Journal of Neuropathology and Experimental Neurology, 44(1), 47-59.

Graybiel AM, Hirsch EC and Agid YA. 1987. Differences in tyrosine hydroxylase-like immunoreactivity characterize the mesostriatal innervation of striosomes and extrastriosomal matrix. PNAS, 84, 303-307.

Graybiel AM and Ragsdale CW. 1978. Histochemically distinct compartments in thee striatum of human, monkey and cat demonstrated by acetylthiocholinesterase staining. PNAS, 75(11), 5723-5726.

Graybiel AM and Ragsdale CW. 1983. Biochemical anatomy of the striatum. In PC Emson (ed) Chemical Neuroanatomy, New York: Raven Press.

Graveland GA, Williams RS, and DiFiglia M. 1985. Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's Disease. Science, 227, 770-773.

Greenamyre JT, Penney JB, Young AB, D'Amato CJ, Hicks SP and Shoulson I. 1985. Alterations in L-glutamate binding in Alzheimer's and Huntington's Diseases. Science, 227, 1496-1498.

Guldin WO and Markowitsch HJ. 1981. No detectable remote lesions following massive intrastriatal injections of ibotenic acid. Brain Research, 225, 446-451.

Guldin WO and Markowitsch HJ. 1982. Epidural kainate, but not ibotenate produces lesions in local and distant regions of the brain. A comparison of the intracerebral actions of kainic acid and ibotenic acid. Journal of Neuroscience Methods, 5, 83-93.

Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY, Young, AB, Shoulson I, Bonilla E, and Martin JB. 1983. A polymorphic DNA marker genetically linked to Huntington's Disease. Nature, 306, 234-238.

Haber S and Elde R. 1981. Correlation between met-enkephalin and substance P immunoreactivity in the primate globus pallidus. Neuroscience, 6(7), 1291-1297.

Hammond P, and Brimijoin S. 1988. Acetylcholinesterase in Huntington's and Alzheimer's Diseases: simultaneous enzyme assay and immunoassay of multiple brain regioins. Journal of Neurochemistry, 50, 1111-1116.

Hattie WH. 1909. Huntington's Chorea. American Journal of Insanity (Proceedings of

the American Medico-Psychological Association, 16, 171-176.

Hayden MR. 1981. Huntington's Chorea. Berlin: Springer-Verlag.

Hayden MR, Hewitt J, Stoessl AJ, Clark C, Ammann W and Martin WRW. 1985. The combined use of positron emission tomography and DNA polymorphisms for preclinical detection of Huntington's Disease. Neurology, 37, 1441-1447.

Hayden MR, Hewitt J, Wasmuth JJ, Kastelein JJ, Langlois S, Conneally M, Haines J, Smith B, Hilbert C and Allard D. 1988. A polymorphic DNA marker that represents a conserved expressed sequence in the region of the Huntington Disease Gene. American Journal of Human Genetics, 42, 125-131.

Hayden MR, Kastelein JJ, Wilson RD, Hilbert C, Hewitt J, Langlois S, Fox S and Bloch M. 1987. First trimester prenatal diagnosis for Huntington's Disease with DNA probes. Lancet, 1284-1285.

Hayden MR, Martin WRW, Stoessl AJ, Clark C, Hollenberg S, Adam MJ, Ammann W, Harrop R, Rogers G, Ruth T, Sayre C, and Pate BD. 1986. Positron emission tomography in the early diagnosis of Huntington's Disease. Neurology, 36, 888-894.

Hefter H, Homberg V, Lange HW, and Freund H-J. 1987. Impairment of rapid movement in Huntington's Disease. Brain, 110, 585-612.

Heindel WC, Salmon DP, Shults CW, Walicke PA and Butters N. 1989. Neuropsychological evidence for multiple implicit memory systems: a comparison of Alzheimer's, Huntington's and Parkinson's Disease patients. Journal of Neuroscience, 9(2), 582-587.

Heindel WC, Salmon DP and Butters N. 1990. Pictorial priming and cued recall in Alzheimer's and Huntington's Disease. Brain and Cognition, 13, 282-295.

Hiley CR and Bird ED. 1974. Decreased muscarinic receptor concentration in post-mortem brain in Huntington's chorea. Brain Research, 80, 355-358.

Hodges JR, Salmon DP and Butters N. 1990. Differential impairment of semantic and episodic memory in Alzheimer's and Huntington's diseases: a controlled prospective study. Journal of Neurology, Neurosurgery and Psychiatry, 53, 1089-1095.

Huntington G. 1872. On Chorea. Medical and Surgical Reporter, 26, 317-321.

Huntington G. 1910. Recollections of Huntington's Chorea as I saw it at East

Hampton, Long Island, during my boyhood. Journal of Nervous and Mental Disease, 37, 255-257.

Illes J. 1989. Neurolinguistic features of spontaneous language production dissociate three forms of neurodegenerative diseases: Alzheimer's, Huntington's and Parkinson's. Brain and Language, 37, 628-642.

Isacson O, Brundin P, Kelly PAT, Gage F, and Björklund A. 1984. Functional and neuronal degeneration of striatal neurones in the ibotenic acid-lesioned rat striatum. Nature, 311, 458-460.

Isacson O, Brundin P, Gage FH and Björklund A. 1985. Neural grafting in a rat model of Huntington's Disease: progressive neurochemical changes after neostriatal ibotenate lesions and striatal tissue grafting. Neuroscience, 16(4), 799-817.

Isacson O, Dunnett SB, and Björklund A. 1986. Graft-induced behavioural recovery in an animal model of Huntington's disease. Proceedings of the National Academy of Sciences, USA, 83, 2728-2732.

Isacson O, Dawbarn O, Brundin P, Gage FH, Emson PC and Björklund A. 1987a. Neural grafting in a rat model of Huntington's Disease: Striosomal-like organization of striatal grafts as revealed by acetylcholinesterase histochemistry, immunocytochemistry and receptor autoradiography. Neuroscience, 22(2), 481-497.

Isacson O, Fischer W, Wictorin K, Dawbarn D and Björklund A. 1987b. Astroglial response in the excitotoxically lesioned neostriatum. Neuroscience, 20(3), 1043-1056.

Jason GW, Pajurkova EM, Suchowersky O, Hewitt J, Hilbert C, Reed J and Hayden MR. 1988. Presymptomatic neuropsychological impairment in Huntington's Disease. Archives of Neurology, 45, 769-773.

Johnston JB, Gerfen CR, Haber SN and van der Kooy D. 1990. Mechanisms of striatal pattern formation: conservation of mammalian compartmentalization. Journal of Comparative Neurology, 57, 93-102.

Josiassen RC, Curry LM and Mancall EL. 1983. Development of neuropsychological deficits in Huntington's Disease. Archives of Neurology, 40, 791-796.

Kanazawa I, Bird ED, Gale JS, Iversen LL, Jessell TM, Muramoto O, Spokes EG and Sutoo D. 1985. Substance P: Decrease in substantia nigra and globus pallidus in Huntington's Disease. In TN Chase, NS Wexler and A Barbeau (eds). Advances in Neurology, 23, Huntington's Disease, pgs. 495-504.

Kanazawa I, Bird ED, O'Connell R and Powell D. 1977. Evidence for a decrease in substance P in Huntington's chorea. Brain Research, 120, 387-392.

Kemp JM and Powell TPS. 1970. The corticostriate projection in the monkey. Brain, 93, 525-546.

Kesslak JP, Brown L, Steichen C and Cotman CW. 1986. Adult and embryonic frontal cortex transplants after frontal cortex ablation enhance recovery on a reinforced alternation task. Experimental Neurology, 94, 615-626.

Kish SJ, Shannak K, and Hornykiewicz O. 1987. Elevated serotonin and reduced dopamine in subregionally divided Huntington's Disease striatum. Annals of Neurology, 22, 386-389.

Knopman D and Nissen MJ. 1991. Procedural learning is impaired in Huntington's Disease: Evidence from the serial reaction time test. Neuropsychologia, 29(3), 245-254.

Koh J-Y and Choi D. 1988. Cultured striatal neurons containing NADPH-diaphorase or acetylcholinesterase are selectively resistant to injury by NMDA receptor agonists. Brain Research, 446, 374-378.

Koh J-Y, Peters S and Choi D. 1986. Neurons containing NADPH-diaphorase are selectively resistant to quinolinate toxicity. Science, 234, 73-76.

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

Kolb B. 1974. Dissociation of the effects of lesions of the orbital or medial aspect of the prefrontal cortex of the rat with respect to activity. Behavioural Biology, 10, 329-343.

Kolb B. 1987. Recovery from early cortical damage in rats. I. Differential behavioural and anatomical effects of frontal lesions at different ages of neural maturation. Behavioural Brain Research, 25, 205-220.

Kolb B. 1990. Prefrontal cortex. In B Kolb and RC Tees (eds). The Cerebral Cortex of the Rat, pgs. 437-458. Cambridge, MA: MIT Press.

Kolb B and Holmes C. 1983. Neonatal motor cortex lesions in the rat: absence of sparing of motor behaviours and impaired spatial learning concurrent with abnormal cerebral morphogenesis. Behavioural Neuroscience, 97(5), 697-709.

Kolb B and Milner B. 1981a. Performance of complex arm and facial movements after

focal brain lesions. Neuropsychologia, 19(4), 491-503.

Kolb B and Milner B. 1981b. Observations on spontaneous facial expression after focal cerebral excisions and after intracarotid injection of sodium amytal. Neuropsychologia, 19(4), 505-514.

Kolb B, Pittman K, Sutherland RJ and Whishaw IQ. 1982. Dissociation of the contributions of the prefrontal cortex and dorsomedial thalamic nucleus to spatially guided behaviour in the rat. Behavioural Brain Research, 6, 365-378.

Kolb B, Reynolds B and Fantie B. 1988. Frontal cortex grafts have opposite effects at different postoperative recovery times. Behavioural and Neural Biology, 50, 195-200.

Kolb B, Sutherland RJ and Whishaw IQ. 1983. A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats. Behavioural Neuroscience, 97(1), 13-27.

Kolb B and Tees RC. 1990. The rat as a model of cortical function. In B Kolb and RC Tees (eds). The Cerebral Cortex of the Rat, pgs. 3-18. Cambridge, MA: MIT Press

Kolb B and Whishaw IQ. 1983a. Problems and principles underlying interspecies comparisons. In TE Robinson (ed). Behavioural approaches to brain research, pgs. 237-263. New York:Oxford University Press.

Kolb B and Whishaw IQ. 1983b. Dissociation of the contributions of the prefrontal, motor, and parietal cortex to the control of movement in the rat: an experimental review. Canadian Journal of Psychology, 37(2), 211-232.

Kolb B and Whishaw IQ. 1990. Fundamentals of Human Neuropsychology, Third Edition. New York: WH Freeman.

Koller WC and Trimble J. 1985. The gait abnormality of Huntington's Disease. Neurology, 35, 1450-1454.

Kuhl DE, Metter EJ, Riege WH and Markham CH. 1984. Patterns of cerebral glucose utilization in Parkinson's Disease and Huntington's Disease. Annals of Neurology, 15(suppl), s119-s125.

Kuhl DE, Phelps ME, Markham CH, Metter J, Riege WH and Winter J. 1982. Cerebral metabolism and atrophy in Huntington's Disease determined by ^{18}FDG and computed tomographic scan. Annals of Neurology, 12, 425-434.

Kunzle H. 1975. Bilateral projections form precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in Macaca fascicularis, Brain Research, 88, 195-209.

Kunzle H. 1977. Projections from the primary somatosensory cortex to basal ganglia and thalamus in the monkey. Experimental Brain Research, 36, 481-492.

Kuwert T, Lange HW, Langen K-J, Herzog H, Aulich A and Feinendegen LE. 1990. Cortical and subcortical glucose consumption measured by PET in patients with Huntington's Disease. Brain, 113, 1405-1423.

Labbe R, Firl A, Mufson EJ and Stein DG. 1983. Fetal brain transplants: reduction of cognitive deficits in rats with frontal cortex lesions. Science, 221, 470-472.

Lange H, Thorner G, Hopf A and Schroder KF. 1976. Morphometric studies of the neuropathological changes in choreatic disease. Journal of the Neurological Sciences, 28, 401-425.

Laplane D, Levasseur M, Pillon B, Dubois M, Mazoyer B, Tran Dinh S, Sette G, Danze F and Baron J-C. 1989. Obsessive-compulsive and other behavioural changes with bilateral basal ganglia lesions. Brain, 119, 699-725.

Larsen JK and Divac I. 1978. Selective ablations within the prefrontal cortex of the rat and performance of delayed alternation. Physiological Psychology, 6(1), 15-17.

Lasker AG, Zee DS, Hain TC, Folstein SE and Singer HS. 1987. Saccades in Huntington's Disease: initiation defects and distractability. Neurology, 37, 364-370.

Lavoie B and Parent A. 1991. Serotoninergic innervation of the thalamus in the primate: an immunohistochemical study. Journal of Comparative Neurology, 312, 1-18

Levivier M, Pearlman SH, Collier TJ, Sladek JR and Gash DM. 1989. Fetal striatal transplants protect against quinolinic acid lesions in the striatum: a behavioral analysis. Society for Neuroscience Abstracts, 533.9.

Lindsay RM. 1979. Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurones. Nature, 282, 80-82.

Ludlow CL, Connor NP, and Bassich CJ. 1987. Speech timing in Parkinson's and Huntington's Disease. Brain and Language, 32, 195-214.

Lyle OE and Gottesman II. 1977. Premorbid psychometric indicators of the gene for

Huntington's Disease. Journal of Consulting and Clinical Psychology, 45(6), 1011-1022.

Lynch GS. 1970. Separable forebrain systems controlling different manifestations of spontaneous activity. Journal of Comparative and Physiological Psychology, 70(1), 48-59.

Marshall PE, Landis DMD, Zalneraitis EL. 1983. Studies of substance P and leucine-enkephalin in Huntington's Disease. Brain Research, 289, 11-26.

Martin LJ, Spicer DM, Lewis MH, Gluck JP and Cork LC. 1991. Social deprivation of infant rhesus monkeys alters the chemoarchitecture of the brain. I. Subcortical regions. Journal of Neuroscience, 11(11), 3344-3358.

Martin WRW, Clark C, Ammann W, Stoessl AJ, Shtybel W and Hayden MR. 1992. Cortical glucose metabolism in Huntington's Disease. Neurology, 42, 223-229.

Martone M, Butters N, Payne M, Becker JT and Sax DS. 1984. Dissociations between skill learning and verbal recognition in amnesia and dementia. Archives of Neurology, 41, 965-970.

Mason ST and Fibiger HC. 1979a. Kainic acid lesions of the striatum in rats mimic the spontaneous motor abnormalities of Huntington's Disease. Neuropharmacology, 18, 403-407.

Mason ST and Fibiger HC. 1979b. On the specificity of kainic acid. Science, 204, 1339-1341.

Massman PJ, Delis DC, Butters N, Levin BE and Salmon DP. 1990. Are all subcortical dementias alike? Verbal learning and memory in Parkinson's and Huntington's Disease patients. Journal of Clinical and Experimental Neuropsychology, 12(5), 729-744.

Mazurek MF, Beal MF, Ellison DW, Knowlton SF, Swartz KW and Martin JB. 1989. Cholecystokinin in Huntington's Disease: Widespread increases in postmortem cerebral cortex. In J Hughes, G Dockray and G Woodruff (eds). The Neuropeptide Cholecystokinin, pgs 28-32, New York: John-Wiley and Sons.

McCaughey WTE. 1961. Pathologic spectrum of Huntington's chorea. Journal of Nervous and Mental Disease, 133(2), 91-103.

McDonald JW and Johnston MV. 1990. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. Brain Research Reviews, 15, 41-70.

McGeer PL, McGeer EG, and Fibiger HC. 1973a. Choline acetylase and glutamic decarboxylase in Huntington's chorea. Neurology, 23, 912-917.

McGeer PL, McGeer EG and Fibiger HC. 1973b. Glutamic-acid decarboxylase and choline acetylase in Huntington's chorea and Parkinson's Disease. Lancet, 623-624.

McGeer EL and McGeer PG. 1976. Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. Nature, 263, 517-519.

McGeorge AJ and Faull RLM. 1989. The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience, 29(3), 503-507.

Melamed E, Hefti F and Bird ED. 1982. Huntington's chorea is not associated with hyperaactivity of nigrostriatal dopaminergic neurons: studies in postmortem tissues and rats with kainic acid lesions. Neurology, 32, 640-644.

Morris R. 1984. Developments of a water maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods, 11, 47-60.

Morris RGM, Garrud P, Rawlins JNP and O'Keefe J. 1982. Place navigation impaired in rats with hippocampal lesions. Nature, 297, 681-683.

Moses JA, Golden CJ, Berger PA and Wisniewski AM. 1981. Early, middle and late stage Huntington's Disease as measured by the Luria-Nebraska neuropsychological battery. International Journal of Neuroscience, 14, 95-100.

Myers RH, Goldman D, Bird ED, Sax DS, Merril CR, Schoenfeld M and Wolf PA. 1983. Maternal transmission in Huntington's Disease. Lancet, 2(1), 208-210.

Nemeroff CB, Youngblood WW, Manberg PJ, Prange AJ and Kizer JS. 1983. Regional brain concentrations of neuropeptides in Huntington's chorea and schizophrenia. Science, 221, 972-975.

Newcombe RG, Walker DA and Harper PS. 1981. Factors influencing age at onset and duration of survival in Huntington's chorea. Annals of Human Genetics, 45, 387-396.

Nieuwenhuys R 1985. Chemoarchitecture of the Brain, Berlin: Springer-Verlag.

Nieuwenhuys R, Voogd J, and van Huijzen C. 1988. The Human Central Nervous System, Berlin: Springer-Verlag.

Öberg RGE and Divac I. 1979. "Cognitive" functions of the neostriatum. In I Divac and RGE Öberg (eds). The Neostriatum, pgs. 291-313. Oxford: Pergamon Press.

Osler W. 1893. Remarks on the varieties of chronic chorea and a report upon two families of the hereditary form, with one autopsy. Journal of Nervous and Mental Disease, 18, 97-111.

Oyanagi K, Takeda S, Takahashi H, Ohama E and Ikuta I. 1989. The quantitative investigation of the substantia nigra in Huntington's Disease Annals of Neurology, 26, 13-19.

Packard M and White N. 1990. Lesions of the caudate nucleus selectively impair "reference memory" acquisition in the radial maze. Behavioural and Neural Biology, 53, 39-50.

Pasinetti GM, Morgan DG and Finch CE. 1991. Disappearance of GAD-mRNA and tyrosine hydroxylase in substantia nigra following striatal ibotenic acid lesions: evidence for transneuronal regression. Experimental Neurology, 112, 131-139.

Paxinos G, and Watson C. 1982. The Rat Brain in Stereotaxic Co-ordinates. New York: Academic Press.

Pearlman SH, Levivier M, and Gash DM. 1990. Striatal implants provide long term protection against behavioral, regulatory, and morphological changes induced by quinolinic acid. Society for Neuroscience Abstracts, 203.3.

Pearson SJ, Heathfield KWG and Reynolds GP. 1990. Pallidal GABA and chorea in Huntington's disease. Journal of Neural Transmission, 81, 241-246.

Pearson SJ and Reynolds GP. 1989. Tryptophan metabolism and quinolinic acid in the brain in Huntington's Disease. In AR Crossman and MA Sambrook (eds). Neural Mechanisms in Disorders of Movement, pgs. 343-346. London: John Libbey.

Penney JB, Young AB, Shoulson I, Starosta-Rubinstein S, Snodgrass SR, Sanchez-Ramos J, Ramos-Arroyo M, Gomez F, Penchaszadeh G, Alyir J, Esteves J, DeQuiroz I, Marsol N, Moreno H, Conneally PM, Bonilla E and Wexler NS. 1990. Huntington's Disease in Venezuela: Seven years of follow-up on symptomatic and asymptomatic individuals. Movement Disorders, 5(2), 93-99.

Perry TL, Hansen S, and Kloster M. 1973. Huntington's chorea. Deficiency of gamma-amino butyric acid in brain. New England Journal of Medicine, 288(7)), 377-

342.

Pisa M. 1988a. Motor somatotopy in the striatum of the rat: manipulation, biting and gait. Behavioural Brain Research, 27, 21-35.

Pisa M. 1988b. Motor functions of the striatum in the rat: critical role of the lateral region in tongue and forelimb reaching. Neuroscience, 24(2), 453-463.

Pisa M, and Cyr J. 1990. Regionally selective roles ofthe rat's striatum in modality-specific discrimination learning and forelimb reaching. Behavioural Brain Research, 37, 281-292.

Pisa M, Sanberg PR, and Fibiger HC. 1981. Striatal injections of kainic acid selectively impair serial memory performance in the rat. Experimental Neurology, 74, 633-653.

Potegal M. 1971. A note on spatial-motor deficits in patients with Huntington's Disease: a test of a hypothesis. Neuropsychologia, 9, 233-235.

Pritzel M, Isacson O, Brundin P, Wilklund L, and Björklund A. 1986. Afferent and efferent connections of striatal grafts implanted into the ibotenic acid-lesioned neostriatum in adult rats. Experimental Brain Research, 65, 112-126.

Reynolds GP and Pearson SJ. 1989. Increased brain 3-hydroxykynurenine in Huntington's Disease. Lancet, 2, 979-980.

Reynolds GP, Pearson SJ, Halket J, and Sandler M. 1988. Brain quinolinic acid in Huntington's Disease. Journal of Neurochemistry, 50(6), 1959-1960.

Robbins TW, Everitt BJ, Marston HM, Willkinson J, Jones GH and Page KJ. 1989a. Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. Behavioural Brain Research, 35, 221-240.

Robbins TW, Everitt BJ, Ryan CN, Marston HM, Jones GH and Page KJ. 1989b. Comparative effects of quisqualic and ibotenic acid-induced lesions of the substantia innominata and globus pallidus on the acquisition of a conditional visual discrimination: differential effects on cholinergic mechanisms. Neuroscience, 28(20), 337-352.

Roberts RC and DiFiglia M. 1988. Localization of immunoreactive GABA and Enkephalin and NADPH-diaphorase-positive neurons in fetal striatal grafts in the quinolinic-acid lesioned rat neostriatum. Journal of Comparative Neurology,

274, 406-421.

Roberts RC and DiFiglia M. 1990. Evidence for synaptic proliferation, reorganization and growth in the excitotoxic adult rat caudate nucleus. Experimental Neurology, 107(1), 1-10.

Rosvold HE and Swarczbart MK. 1964. Neural structures involved in delayed-response performance. In JM Warren and K Akert (eds). The Frontal Granular Cortex and Behaviour, pgs. 1-15. New York: McGraw-Hill.

Saint-Cyr JA, Taylor AE and Lang AE. 1988. Procedural learning and neostriatal dysfunction Brain, 111, 941-979.

Saji M and Reis DJ. 1987. Delayed transneuronal death of substantia nigra neurons prevented by gamma-aminobutyric acid agonist. Science, 235, 66-69.

Sanberg PR, Henault MA, and Deckel WA. 1986. Locomotor hyperactivity: effects of multiple striatal transplants in an animal model of Huntington's Disease. Pharmacology, Biochemistry and Behaviour, 25, 297-300.

Sanberg PR, Calderon SF, Giordano M, Tew JM, and Norman AB. 1989. The quinolinic acid model of Huntington's Disease: locomotor abnormalities, Experimental Neurology, 105, 45-53.

Sanberg PR and Coyle JT. 1984. Scientific approaches to Huntington's Disease. CRC Reviews in Clinical Neurobiology, 1-44.

Sarter M and Dudchenko P. 1991. Dissociative effects of ibotenic and quisqualic acid-induced basal forebrain lesions on cortical acetylcholinesterase-positive fibre density and cytochrome oxidase. Neuroscience. 41(2/3), 729-738.

Sax DS, O'Donnell B, Menzer L, Montgomery K, and Kayne HL. 1983. Computed tomographic, neurologic, and neuropsychological correlates of Huntington's Disease. International Journal of Neuroscience, 18, 21-36.

Schoenfeld M, Myers RH, Cupples LA, Markman B, Sax DS and Clark E. 1984. Increased rate of suicide among patients with Huntington's Disease. Journal of Neurology, Neurosurgery and Psychiatry, 47, 1283-1287.

Schwarcz R, and Coyle JT. 1977. Striatal lesions with kainic acid: neurochemical characteristics. Brain Research, 127, 235-249

Schwarcz R, Hökfelt T, Fuxe K, Jonsson G, Goldstein M and Terenius L. 1979. Ibotenic acid- induced neuronal degeneration: a morphological and

neurochemical study. Experimental Brain Research, 37, 199-216.

Schwarcz R, Okuno E, White RJ, Bird ED and Whetsell WO. 1988. 3-hydroxyanthranilate oxygenase activity is increased in the brains of Huntington's Disease victims. PNAS, 85, 4079-4081.

Schwarcz R, Whetsell WO and Mangano RM. 1983. Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. Science, 219, 316-318.

Schwob JE, Fuller T, Price JL and Olney JW. 1980. Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid: a histological study. Neuroscience, 5, 991-1014.

Seizinger BR, Liebisch DC, Kish SJ, Arendt RM, Hornykiewicz O and Herz A. 1986. Opiod peptides in Huntington's Disease: alterations in prodynorphin and proenkephalin system. Brain Research, 378, 405-408.

Shelton PA and Knopman DS. 1991. Ideomotor apraxia in Huntington's Disease. Archives of Neurology, 48, 35-41.

Shoulson I. 1982. Huntington's Disease: anti-neurotoxic therapeutic strategies. In K Fuxe, P Roberts and R Schwarcz (Eds). Excitotoxins, pgs 343-353. New York: Plenum Press.

Singh VK, and Van Alstyne D. 1978 Glial cells from normal adult rat brain established in continuous culture. Brain Research, 155, 418-421.

Sofroniew MV, Isacson O and Björklund A. 1986. Cortical grafts prevent atrophy of cholinergic basal nucleus neurons induced by excitotoxic cortical damage. Brain Research , 378, 409-415.

Sofroniew MV and Pearson RCA. 1985. Degeneration of cholinergic neurons in the basal nucleus following kainic or N-methyl-D-aspartic acid application to the cerebral cortex. Brain Research, 339, 186-190.

Sotrel A and Myers RH. 1991. Morphometric analysis of the prefrontal cortex in Huntington's Disease. Annals of Neurology, 41(6), 1117-1125.

Speedie LJ, Brake N, Folstein SE, Bowers D, and Heilman KM. 1990. Comprehension of prosody in Huntington's Disease. Journal of Neurology, Neurosurgery and Psychiatry, 53, 607-610.

Spokes EGS. 1980. Neurochemical alterations in Huntington's chorea. A study of post-

mortem brain tissue. Brain, 103, 179-210.

Srinathsinghji DJS, Dunnett SB, Isacson O, Clarke DJ, Kendrick K and Björklund A. 1988. Striatal grafts in rats with unilateral neostriatal lesions. II. In vivo monitoring of GABA release in globus pallidus and substantia nigra. Neuroscience, 24(3), 803-811.

Stahl WL and Swanson PD. 1974. Biochemical abnormalities in Huntington's Chorea brains. Neurology, 24, 813-819.

Stewart J and Kolb B. 1988. The effects of neonatal gonadectomy and prenatal stress on cortical thickness and asymmetry in rats. Behavioral and Neural Biology, 49, 344-360.

Sutherland RJ, Kolb B, and Whishaw IQ. 1987. Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in rat. Neuroscience Letters, 31, 271-276.

Takemoto T. 1978. Isolation and structural identification of naturally occurring excitatory amino acids. In E McGeer, J Olney and PL McGeer (eds). Kainic Acid as a Tool in Neurobiology, pgs. 1-15, New York: Raven Press.

Thompson PD, Berardelli A, Rothwell JC, Day BL, Dick JPR, Benecke R and Marsden CD. 1988. The coexistence of bradykinesia and chorea in Huntington's Disease and its implications for theories of basal ganglia control of movement. Brain, 111, 223-244.

Tian JR, Zee DS, Lasker AG and Folstein SE. 1991. Saccades in Huntington's Disease. Predictive tracking and interaction between release of fixation and initiation of saccades. Neurology, 41, 875-881.

van der Loos JI. 1987. The structure of spontaneous language production in Alzheimer's, Huntington's and Parkinson's Disease. PhD Thesis, Stanford University.

van Eden CG, Uylings HBM and Van Pelt J 1984. Sex difference and left-right asymmetries in the prefrontal cortex during postnatal development in the rat. Developmental Brain Research, 12, 146-153.

Vincent SR, Johansson O, Hökfelt T, Skirboll L, Elde RP, Terenius L, Kimmel J and Goldstein M. 1983. NADPH-diaphorase: a selective histochemical marker for striatal neurons containing both somatostatin and avian pancreatic polypeptide (APP)-like immunoreactivities. Journal of Comparative Neurology, 217 252-263.

Vincent SR and Kimura H. 1992. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience, 46(4), 755-784.

Volpe BT and Baker H. 1989. Delayed degeneration in septal cholinergic neurons after primary ischemic or ibotenic acid hippocampal injury. Society for Neuroscience Abstracts, 177.2

Vonsattel J-P, Myers RH, Stevens TJ, Ferrante RJ, Bird ED and Richardson EP. 1985. Neuropathological classification of Huntington's Disease. Journal of Neuropathology and Experimental Neurology, 44(6), 559-577.

Vonsattel J-P, Myers RH, Stevens TJ, Ferrante RJ, Paskevich PA, Richardson EP and Bird ED. 1987. Huntington's Disease: Neuropathological Grading. In MB Carpenter and A Jayaraman (eds). The Basal Ganglia II: Structure and Function-Current Concepts, pgs. 515-531. New York: Plenum Press.

Warren M. 1964. The behaviour of carnivores and primates with lesions in the prefrontal cortex. In JM Warren and K Akert (eds). The Frontal Granular Cortex and Behaviour, pgs. 168-186. New York: McGraw-Hill.

Wastek GJ, Stem LZ, Johnson PC and Yamamura HI. 1976. Huntington's Disease: Regional alternation in muscarinic cholinergic receptor binding in Human Brain. Life Sciences, 19, 1033-1040.

Watkins JC and Olverman HJ. 1987. Agonists and antagonists for excitatory amino acid receptors. TINS, 10(7).

Weingartner H, Caine ED and Ebert MH. 1979. Imagery, encoding and retrieval of information from memory: some specific encoding-retrieval changes in Huntington's Disease. Journal of Abnormal Psychology, 88(1), 52-58.

Whetsell WO and Schwarcz R. 1989. Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. Neuroscience Letters, 97, 271-275.

Whishaw IQ, Kolb B, and Sutherland RJ. 1983. The analysis of behaviour in the laboratory rat. In TE Robinson (ed.) Behavioural Approaches to Brain Research, pgs. 141-211. New York, Oxford: Oxford University Press.

Whishaw IQ, Mittleman G, Bunch ST, and Dunnett SB. 1987. Impairments in the acquisition, retention and selection of spatial navigation after medial caudate-putamen lesions in rats. Behavioural Brain Research, 24, 125-138.

Wictorin K and Björklund A. 1989. Connectivity of striatal grafts implanted into the

ibotenic acid-lesioned striatum. Neuroscience, 30(2), 297-311.

Wictorin K, Isacson O, Fischer W, Nothias F, Peschanski M and Björklund A. 1988. Connectivity of striatal grafts implanted in the ibotenic acid-lesioned striatum. I. Subcortical afferents. Neuroscience, 27(2), 547-562.

Wictorin K, Clarke DJ, Bolam JP and Björklund A. 1989. Host corticostriatal fibres establish synaptic connections with grafted striatal neurons in the ibotenic acid-lesioned striatum. Neuroscience, 31(3), 189-195.

Wictorin K, Clarke DJ, Bolam JP and Björklund A. 1990. Fetal striatal neurons grafted into the ibotenate lesioned adult striatum: efferent projections and synaptic contacts in the host globus pallidus. Neuroscience, 37(2), 301-315.

Wolfensberger M, Arnster U, Cuénod M, Foster AC, Whetsell WO and Schwarcz R. 1983. Identification of quinolinic acid in rat and human brain tissue. Neuroscience Letters, 41, 247-252.

Young AB, Greenamyre JT, Hollingsworth L, Albin R, D'Amato C, Shoulson I, and Penney JB. 1988. NMDA receptor losses in putamen from patients with Huntington's Disease. Science, 241, 981-983.

Young AB, Penney JB, Starosta-Rubinstein S, Markel DS, Berent S, Giordani B, Ehrenkaufer R, Jewett D and Hichwa R. 1986. PET Scan investigations of Huntington's Disease: cerebral metabolic correlates of neurological features and functional decline. Annals of Neurology, 20, 296-303.

Zaczek R, and Coyle JT. 1982. Excitatory amino acid analogues: neurotoxicity and seizures. Neuropharmacology, 21, 15-26.

Zaczek R, Simonton S, and Coyle JT. 1980. Local and distant neuronal degeneration following intrastriatal injection of kainic acid. Journal of Neuropathology and Experimental Neurology, 39, 245-264.

Zweig RM, Ross CA, Hedreen JC, Peyser C, Cardillo JE, Folstein SE and Price DL. 1992. Locus coeruleus involvement in Huntington's Disease. Archives of Neurology, 49, 152-156.