

**ABNORMAL MOTOR BEHAVIOUR AND
THE BASAL GANGLIA**

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B.Sc., MANCHESTER 1982

A thesis submitted to the University of Manchester for
the degree of Ph.D in the Faculty of Medicine

1985

Department of Physiology

I, Gary Noy, declare that

No portion of the work referred to in this thesis has been
submitted in support of an application for another degree
or qualification of this or any other university or
institution of learning.

G. Noy

October 1985

ACKNOWLEDGEMENTS

I would like to thank Professors Maynard Case and Roger Green for permitting me to conduct this research in the Department of Physiology. I wish also to express my deep gratitude to Drs. Ann Latham and Paul Slater, my supervisors, for their invaluable guidance and criticism during these studies and in the preparation of this manuscript.

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ABSTRACT

The motor abnormalities occurring in Parkinson's disease are tremor, rigidity and akinesia. Similar motor effects can be produced in rats by administration of centrally acting cholinomimetics such as tremorine. Although the major lesion in Parkinson's disease is in the basal ganglia, the evidence concerning the involvement of the basal ganglia in the action of tremorine is controversial. In this study the role of the basal ganglia in tremorine-induced hindlimb rigidity has been evaluated. An apparatus was constructed which could reliably and consistently quantify the hindlimb tone of conscious rats using a mechanographical method. The effects of lesions and pharmacological manipulations of neurotransmitter function of various basal ganglia nuclei have been investigated.

Tremorine (20mg/Kg i.p.) was found to produce muscle rigidity which lasted for approximately 1h. Upon the first trial, the rigidity produced by tremorine was less than that measured on subsequent trials but these subsequent administrations produced a consistent and reproducible degree of rigidity. The mechanism of this change in effectiveness is unknown but was circumvented in this study by disregarding the results of the first administration.

The present results indicate that the basal ganglia play only a minor role in the production of rigidity by tremorine. Whereas the local application of oxotremorine (the active metabolite of tremorine) to the striatum and substantia nigra pars reticulata (SNR) mimicked the effects of systemically administered tremorine, the doses required were higher than the total amount of oxotremorine in brain following tremorine

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administration as estimated by bioassay on guinea pig ileum. However the local administration of atropine to these areas did not reduce tremorine-induced rigidity, lesions of the striatum and its dopamine-containing afferent pathway increased the rigidity induced by tremorine and intrastriatal neostigmine reduced limb tone.

Single unit extracellular electrophysiological recordings showed that the systemic administration of tremorine produced an atropine-sensitive increase in spontaneous activity of SNR and globus pallidus neurones. Intrastriatal infusions of oxotremorine mimicked this effect on SNR but not on pallidal units. Intranigral and intrapallidal baclofen reduced the magnitude of tremorine-induced rigidity, indicating that the basal ganglia has some influence over the rigidity induced by tremorine.

Lesions of the inferior olive and other hindbrain nuclei by systemically administered 3-acetylpyridine (3-AP) produced an increase in resting hindlimb tone, selectively antagonised tremorine-induced rigidity (having no effect on that produced by morphine) and exacerbated the tremor produced by tremorine. All of the motor effects produced by 3-AP can be explained in terms of the lesion involving the cerebellum suggesting that tremorine may produce rigidity via the cerebellum although other hindbrain structures may be involved.

Thus, it appears that the basal ganglia are not a major site of action for tremorine-induced hindlimb rigidity in the conscious rat, whereas the involvement of the cerebellum and lower motor centres is more likely. Further, more selective studies on the cerebellum and its associated hindbrain nuclei are required to confirm this possibility.

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SECTION ONE : INTRODUCTION

This study concerns the involvement of various brain structures in the mediation of the hypokinetic-rigid syndrome induced by tremorine in the rat. Although the centrally mediated motor abnormalities induced by tremorine (rigidity, tremor and akinesia) have been well described in a variety of animals including rat, dog and monkey (Everett, Blockus and Shepperd, 1956; Jenden, 1968; Ringdahl and Jenden, 1983) the central mechanisms involved remain ill-understood. This may, in part, be due to the highly complex interaction of the various central structures associated with the control of posture and movement. As the output of these structures is ultimately expressed as a change in the excitability and discharge rate of the α motoneurones (often referred to as the final common path) it is important to understand how the supraspinal motor centres interact with each other and with the α motoneurones before undertaking such a study.

1.1 THE ROLE OF MUSCLE RECEPTORS IN MOTOR CONTROL

Skeletal muscle contains a variety of sensory receptors of which the Golgi tendon organ and the intra-fusal muscle spindle are important in muscle control. The tendon organs, first described by Camillo Golgi (1903), are located in the musculotendinous region of the muscle. These may be up to 1mm long in man (Ralston, Millar and Kasahara, 1960) but are approximately 0.5mm by 0.1mm in the cat (Bridgman, 1968). The receptors are encapsulated and are located in series with the extrafusal muscle fibres in the tendinous fascicles

such that the number of muscle fibres acting upon an individual tendon organ is limited; it has been estimated that only 10 muscle fibres act upon a single tendon organ in the cat (Barker, 1967). The afferent fibres of the cat tendon organ have a conduction velocity of 70-120m/s (Hunt, 1954) and therefore have diameters within the group I classification of Lloyd (1943a). The afferent nerve of the tendon organ has become known as Ib after Bradley and Eccles (1953).

The tendon organ is a mechanoreceptor and responds to tension within the muscle-tendon unit. The older view that the tendon organ had a high mechanical threshold and thereby served as an afferent limb of a safety reflex mechanism now has little support. The evidence for a high mechanical threshold was based upon the tendon organ responses to passive muscle stretch but it has been demonstrated that a passive stretch of 422g of the cat soleus muscle produces no more than 59mg force in a single tendon organ fascicle (Binder, Kronin, Moore and Stuart, 1977). It has also been shown that a tendon organ can respond to the contraction of only one of its connecting muscle fibres (Houk and Henneman, 1967; Stuart, Mosher, Gerlach and Reinking, 1972); Jami and Petit, 1976). The current view is that the tendon organs signal the tension within the muscle over the whole physiological range.

The most studied and the most complicated of all the muscle and joint receptors is the muscle spindle. The description of the muscle spindle provided by

Sherrington (1894) and Ruffini (1898) was accepted without modification for more than 50 years. The classical picture of the muscle spindle describes a thin parallel group of 2-12 intrafusal muscle fibres with the central third of the fibres surrounded by fluid contained within a connective tissue capsule. The intrafusal fibres have well defined striations in the polar regions but the most central region (approximately 400 μ m long in the cat) contains poor striations and a conglomeration of nuclei. This central region has a dense innervation and Ruffini (1898) described three types of what he thought were afferent nerve terminals; primary, secondary and plate endings. It is now known that the plate endings are the terminations of the fusimotor, efferent γ -motor neurones. Due to the morphology of the primary and secondary endings, Ruffini (1898) also referred to them as annulospiral and flower spray endings respectively. However, it is now known that both the primary and secondary afferent neurones may have spiral or flower spray endings (Barker, 1948; Cooper and Daniel, 1963). The primary afferent fibres have a diameter within the range of the group I classification of Lloyd (1943a) and were termed Ia by Bradley and Eccles (1953) whereas the secondary afferents belong to group II.

The muscle spindle is also a mechanoreceptor, responding to muscle stretch. It has been found that the two afferents from the muscle spindle have differing sensitivities, with the primary afferents having a

lower threshold (Lundberg and Winsbury, 1960) and a greater sensitivity to dynamic stimuli than the secondary afferents (Cooper, 1959; 1961). Matthews (1962) described two types of fusimotor fibres which could alter the responsiveness of the sensory afferents. The dynamic fusimotor fibres increased the dynamic response of the primary afferents while the static fusimotor fibres caused a decrease of the dynamic response but an increase in the tonic discharge of both afferents. With the subdivision of the intrafusal fibres into nuclear chain and nuclear bag fibres (Boyd, 1962; Cooper and Daniel, 1963) and the subsequent division of the nuclear bag fibres into bag₁ and bag₂ (Ovalle and Smith, 1972; Gladden, 1976; Banks, Harker and Stacey, 1977) the possibility arose that the static and dynamic sensations could be produced by different intrafusal fibres. Indeed, it is known that the speed of contraction and the degree of equatorial stretch differs in all three intrafusal muscle types (Bessou and Pagès, 1975; Boyd, 1976; Boyd, Gladden, McWilliam and Ward, 1977). It is currently believed that the dynamic fusimotor effects are produced via bag₁ fibres while the static fusimotor effects are produced by bag₂ and/or chain fibres. Therefore, the muscle spindles signal their rate and degree of stretch, which because they are attached to and in parallel with the extrafusal muscle fibres, is equivalent to the changes in the length of the muscle.

The group I afferents of both the Golgi tendon organ and the muscle spindle are known to influence

motor activity at both spinal and supraspinal levels. The stretch reflex (the myotactic reflex of Liddell and Sherrington, 1924) was demonstrated by Lloyd (1943a and b) to involve a monosynaptic pathway and both Granit (1950) and Hunt (1952) suggested that the Ia afferents were involved. This was subsequently confirmed by studies involving intracellular recordings from α -motoneurones (Kuno, 1964; Mendell and Henneman, 1971). The Ia afferents have also been shown to inhibit antagonist muscles via polysynaptic pathways (Eccles and Lundberg, 1958). The only spinal reflex action of Ib afferents which is not disputed is the inhibition of homonymous motoneurones following muscle stretch (Sherrington, 1909; Hunt, 1952). This has been supported by observations from intracellular recordings of α -motoneurones (Eccles, Eccles and Lundberg, 1957). Whilst other reflex effects have been associated with tendon organ activation (e.g. activation of antagonist muscles, Laporte and Lloyd, 1952) recent studies have only been able to demonstrate them if the red nucleus is simultaneously stimulated (Hongo, Jankowska and Lundberg, 1969).

The most important functions of the muscle receptors are to provide information about muscle tension, muscle length and rate of change of muscle length to the higher motor control centres. Afferents from both tendon organs and muscle spindles have been demonstrated to project to the sensorimotor cortex and the cerebellum. The projection from the hindlimbs to

the cortex is via the spinomedullothalamic pathway in the cat (Willis and Coggeshall, 1978), whereas the projection from the forelimbs is via the dorsal column pathway (Rosén, 1969; Willis and Coggeshall, 1978). The projection from the hindlimbs passes in part through the dorsal spinocerebellar tract to nucleus 2 (Johansson and Silfvenius, 1977), a cell group once considered to be part of the vestibular complex (Brodal and Pompeiano, 1957), and thence via the ventral posterior lateral thalamic nucleus to the cortex (Grant, Boivie and Silfvenius, 1973). Although the group I afferents have a cortical projection it is believed that their activation is not brought to "consciousness" as no change in behaviour (Pompeiano and Swet, 1962) or production of conditioned behaviour (Swet and Bourassa, 1967) could be produced using stimuli believed to selectively activate group I afferent neurones. Even though these sensations may not be brought to consciousness themselves, they are believed to be involved in what might be termed "limb position sense". In support of this concept it has been found that during vibratory stimulation of biceps or triceps tendons (a stimulus known to selectively stimulate Ia afferents) errors were made by subjects estimating the degree of angulation of the elbow joint (Goodwin, McClosky and Matthews, 1972).

The muscle receptor projections to the cerebellum are more clearly defined. There are two main direct ascending pathways from the spinal cord to the

cerebellum, the dorsal spino-cerebellar tract and the ventral spino-cerebellar tract. The dorsal spino-cerebellar tract takes its origin in Clarke's column and group I fibres have long been known to make powerful excitatory connections with these cells (Grundfest and Campbell, 1942; Lloyd and McIntyre, 1950). The group Ia linkages here have a high safety factor for transmission as attested by the ability of this synapse to faithfully transmit high frequency presynaptic firing (Oscarsson, 1965). Group II spindle afferents are also known to make excitatory synaptic connections with neurones of Clarke's column (Eccles, Oscarsson and Willis, 1961). The ventral spino-cerebellar tract is not so well understood as the dorsal spino-cerebellar tract, partly due to the uncertainty over where this tract originates. Early investigators believed that the major input to this pathway was excitatory from Ib afferents with widespread convergence from synergistic muscles (Oscarsson, 1957). However, more recently, both excitatory and inhibitory inputs from Ia afferents have been described (Lundberg and Weight, 1971). The reason that these inputs were not described earlier was probably due to the use of electrical threshold differences to distinguish Ia from Ib afferents in the earlier studies. The muscle receptors also project to the cerebellum via the spino-olivary pathways since group I and group II strength volleys in muscle nerves cause changes in the activity in inferior olivary neurones (Armstrong, Eccles, Harvey and Matthews, 1968). However, group III strength volleys

are best at stimulating olivary neurones (Grant, Oscarsson and Rosén, 1966).

Only the muscle spindles receive an efferent projection from the central nervous system. The γ -motorneurone projection terminating in plate endings has been described above. A more diffuse motor termination was described by Hess (1961) which terminates via trail endings (Barker and Ip, 1965). It has also been demonstrated that some motorneurones innervate both extrafusal and intrafusal muscle fibres (Adal and Barker, 1965; Bessou, Emonet-Dénand and Laporte, 1965). These have been termed β motorneurones and have been shown to be associated with a different type of plate ending than the classical γ -motorneurones (Barker, 1966, 1967). The role of these efferents to the muscle spindles is unclear, however several hypotheses concerning the function of the muscle spindles in the control of movement have been proposed. Merton (1953) suggested that the fusimotor system was involved in a follow-up servo system whereby movements are initiated by motor commands exclusively to the γ -motorneurone system. This hypothesis meant that the motor commands go first to the periphery to contract the polar regions of the intrafusal muscle fibres with the resulting increased spindle afferent discharge being conducted back to the spinal cord to excite the α -motorneurones and thereby cause the muscle to contract. However, experiments failed to demonstrate an increase in Ia afferent discharge rate immediately before a movement

and it was also found that a wide range of movements (such as chewing) could still be produced in deafferentated muscles (Goodwin and Luschei, 1974). A second, modified hypothesis of servo-assistance was advanced by Matthews (1964) and subsequently illustrated by Phillips (1969). Matthews postulated that both α - and γ -motorneurons are activated together. The γ -motorneurons set the muscle spindles as a reference for the desired movement and if the movement achieved by the extrafusal muscle fibres does not match them they give servo-assistance via Ia afferent discharge. Therefore, this hypothesis suggests that the muscle spindles measure how well a particular movement is performed. This information is sent to the spinal motor apparatus and also to the supraspinal motor centres where additional modification of the descending motor commands can be made. This model explains why muscles concerned with fine accurate movements contain a relatively high density of muscle spindles and also explains why some movements are not adversely affected by dorsal rhizotomy procedures. Indirect evidence that this hypothesis may be true has come from studies which have demonstrated that γ - and α -motorneurons from the same muscle have similar cortical representations in the cat (Mortimer and Akert, 1961; Fidone and Preston, 1969) and also in the baboon (Grigg and Preston, 1971). This model also suggests a mechanism whereby the muscle rigidity of diseases such as Parkinson's disease may occur. Muscle tone depends upon a number of factors including the

number of motor units activated and the firing frequency in the individual motor units. Abnormal tone could therefore arise by an increase in α -motorneurone excitability caused directly via a descending pathway or indirectly via an increase in γ -motorneurone activity.

The descending pathways which modulate the activity of the motorneurones were originally classified into pyramidal and extrapyramidal systems by Wilson (1914). This was based on purely anatomical grounds, the subdivision being dependent on whether or not the descending tracts passed through the medullary pyramids. The pyramidal tract arises from the pyramidal cells of the motor cortex and passes through the medullary pyramids to influence the activity of all motorneurone groups. This pathway has different terminations in different animals but the effect of a bilateral pyramidotomy in the rhesus monkey is predominantly on steering and extremity movements (Lawrence and Kuypers, 1968a). Immediately post operatively, the monkey can sit up, walk, run and climb but is unable to pick up food with its hands. Some of this distal control is regained after a period of recovery but individual finger movements do not return even after 3-4 years (Kuypers, 1981).

The pyramidal / extrapyramidal classification is still in use but is of limited help when describing the function of various extrapyramidal descending pathways. For instance, both the vestibulo-spinal and rubro-spinal tracts are part of the extrapyramidal motor system

but they have quite different functions: the vestibulo-spinal tract appears to be primarily involved in mediating coarse antigravity reflexes whilst the rubro-spinal tract is concerned with relatively fine control of distal limb musculature. A more satisfactory division of descending motor pathways has been proposed by Kuypers (1981) based upon the terminal distribution of these pathways within the spinal cord. Such a classification provides information on the function of the various pathways due to the detailed spatial organisation of the motoneurones within the spinal cord (Romanes, 1951). The α - and γ -motoneurones innervating a particular muscle are represented in longitudinal columns within the spinal cord (Romanes, 1951; Bryan, Trevino and Willis, 1972). There are two major columns in the ventral horn, one located medially and one laterally. The motoneurones of the medial cell group innervate axial muscles whereas those of the lateral cell group innervate the limb muscles (Sprague, 1948; Sterlin and Kuypers, 1967; Brink, Morrell and Pfaff, 1979). The classification described by Kuypers (1981) divides the descending brainstem pathways into two groups, A and B. Group A pathways terminate in the ventromedial part of the intermediate zone of the spinal cord. This area is known to contain long and intermediate proprio-spinal neurones. The pathways include the tecto-spinal tract, the lateral and medial vestibulo-spinal tracts and the reticulo-spinal tracts from the pontine and medullary medial tegmental field. The pathways pass

through the medial and ventromedial parts of the medullary cross section and continue into the ventral and ventrolateral funiculi of the cervical spinal cord. Therefore, group A pathways are best at influencing large numbers of motor units and muscles simultaneously. Such outputs control the relatively gross movements involved in postural adjustments and since such large groups of motor units are influenced there is only a limited amount of motor fractionation.

Group B pathways comprise the contralaterally descending paths which terminate in the lateral parts of the spinal intermediate zone. This area is known to contain short propriobulbar and propriospinal neurones. Group B pathways include the rubro-spinal tract and the ponto-spinal tract from the ventrolateral pontine tegmentum. The pathways pass through the ventrolateral part of the medullary cross section and continue into the spinal dorsolateral funiculus. The pathways in this group therefore activate smaller muscle units than group A pathways and thus there is a relatively greater capability for motor fractionation. There is evidence that the cortical influences on these two groups of descending pathways are different in the rhesus monkey with group B pathways being modulated by the precentral gyrus cortical area and the group A pathways being influenced by cortical areas rostral to the precentral gyrus (Kuypers, 1981). The differing effects of group A and B brainstem pathways have been demonstrated in the monkey (Lawrence and

Kuypers, 1968b). A bilateral pyramidotomy in conjunction with a bilateral lesion of the group A descending pathways produced postural changes in the trunk and limbs. The monkey had a prolonged inability to right itself and had a severe deficit in steering axial and proximal limb movements. In contrast, the distal limb movements were considerably less impaired. A bilateral pyramidotomy together with a lesion of the group B descending pathways produced quite different motor impairments. There were no severe postural changes and very little effect on the ability of the monkey to sit up and climb about. The use of the distal limb and especially the forelimb was impaired with the wrist and fingers being limp. The forelimb was much less affected during walking and climbing behaviours. Other evidence for the differing actions of group A and group B pathways have been obtained in the cat. When the superior colliculus (group A) is unilaterally stimulated (Roucoux and Crommelinck, 1976) or lesioned (Sprague and Meikle, 1965) turning behaviour is observed. However, if similar procedures are carried out on the red nucleus (group B) much more localised effects are observed. Stimulating the red nucleus produces restricted movements of the contralateral extremities (Ghez, 1975) whereas a lesion of the red nucleus produces a temporary impairment of the manipulatory capacity of the contralateral limb (Gorska and Sybirska, 1978).

The group A and B pathways constitute the routes

whereby the extrapyramidal structures, the cerebellum and the basal ganglia, exert their influence over motor behaviour.

1.2 THE ROLE OF THE CEREBELLUM IN MOTOR CONTROL

The basic concept of the cerebellum as a major supraspinal structure involved in motor control arose from studies made in the 19th century (e.g. Flourens, 1824; Luciani, 1891; reviewed by Millar, 1926). The cerebellum is a large structure being approximately one tenth of the mass of the brain in higher mammals. It is located on the dorsal surface of the hindbrain, being connected to the brainstem by three pairs of peduncles; the superior, the middle, and the inferior cerebellar peduncles. The cerebellum consists of a superficial, highly convoluted layer of grey matter, the cerebellar cortex, overlying a mass of white matter and the cerebellar nuclei. The cortex is divisible into three distinct layers, the outermost molecular layer, the ganglionic or Purkinje cell layer and the granular layer. There are no regional histological differences sufficient to distinguish one part of the cortex from another. The exposed position of the cerebellum, together with its histological uniformity, have been prime reasons why the cerebellum has been so intensively investigated and resulted in its neuronal circuitry being more well defined than other regions of the brain.

The cerebellar cortex is composed of functional connections between five different types of neurone,

the Purkinje cell, the Golgi cell, the basket cell, the stellate cell and the granule cell. The molecular layer has a low density of neuronal cell bodies (only stellate and basket cell bodies are found in this layer); it consists mainly of the dendritic processes of the Purkinje cells and the densely packed granule cell axons. These axons run parallel to each other and to the longitudinal axis of the cerebellar folia, and are therefore termed the parallel fibres. The Purkinje cell layer consists of a single sheet of pear-shaped Purkinje cells which, in the cat, have vertical and transverse diameters of 50-70 μ m and 30-35 μ m respectively. These are the largest cells of the cerebellar cortex and are the only projection neurones; their axons terminate within the deep cerebellar nuclei and in the lateral vestibular nuclei (Jansen and Brodal, 1940). The Golgi neurones are found in close association with the Purkinje cells but they are usually a little deeper in the cortex, within the granular layer. The granular layer is densely packed with small granule neurones ($2.4 \times 10^6 \text{ mm}^{-3}$ in the monkey), their axons ascend to the molecular layer where they bifurcate to form the parallel fibres (Fox and Barnard, 1957; Eccles, Ito and Szentágothai, 1967).

Of the five types of neurone within the cerebellar cortex, only the granule cells are excitatory. The stellate and basket cells are responsible for lateral inhibition of Purkinje cells across the folia (Eccles et al, 1967; Eccles, 1973). The basket cells are the

most effective as they synapse onto the primary dendrites of the Purkinje cells rather than onto the more distal dendrites as do the stellate cells. The Golgi cells are involved in the modulation of inputs through the mossy fibre pathways.

There are two major types of afferents to the cerebellar cortex, the mossy fibres and the climbing fibres. The mossy fibres terminate within the cerebellar glomeruli, which are formed by the dendrites of granule cells, Golgi axons and the mossy fibre terminals (Eccles et al, 1967). The major synaptic contacts of the mossy fibres are with granule cells. In contrast, the climbing fibres form very close and powerful synaptic contacts with Purkinje cells. The mossy fibres produce a diffuse effect within the cerebellar cortex due to the highly divergent nature of their connections. This divergence has been investigated in the cat and it has been estimated that each mossy fibre makes contact with 3-5 granule cells (Eccles, 1973), each of which may be in contact with as many as 300 Purkinje cells (Eccles et al, 1967). In the monkey, a single Purkinje cell can be in synaptic contact with 200,000 parallel fibres (Fox and Barnard, 1957). In contrast, only one climbing fibre is associated with each Purkinje cell (and makes many synaptic contacts), and as climbing fibres have very few collaterals they have a very localised and powerful influence on Purkinje cells. The differing effects these two afferent systems have on Purkinje cell

activity has been demonstrated electrophysiologically. The climbing fibre produces a short burst of action potentials (Ito and Simpson, 1971) with a frequency as high as 500s^{-1} (Eccles, Llinás and Sasaki, 1966a). The parallel fibres produce a smaller increase in discharge rate which may be followed by a disynaptic inhibition mediated by parallel fibre input to stellate and/or basket cells (Eccles, Llinás and Sasaki, 1966b).

The mossy fibre inputs can be subdivided into two groups based on the afferent pathways, direct inputs from the spinal cord and pontine nuclei, and indirect inputs via the precerebellar reticular nuclei (Bloedel and Courville, 1981). The direct mossy fibre inputs are from the dorsal and ventral spino-cerebellar tracts, the dorsal column nuclei, the vestibular organs, and the pontine nuclei. The dorsal spino-cerebellar tract arises in Clarke's column and ascends through the inferior cerebellar peduncle to terminate within the anterior vermis and paravermis and also within the paramedian lobule and dorsal paraflocculus (Grant, 1962). This pathway has been shown to contain five classes of proprioceptive (e.g. muscle receptors) and exteroceptive (e.g. cutaneous receptors) sensory information (Lundberg and Oscarsson, 1960; Oscarsson, 1965). The ventral spino-cerebellar tract enters the cerebellum via the superior cerebellar peduncle and terminates contralaterally to the spinal pathway in the cerebellar vermis of principally the anterior lobe (Vachananda, 1959). The cuneocerebellar system is a direct spino-cerebellar

tract for afferents from the forelimbs (Cooke, Larson, Oscarsson and Sjölund, 1971). This can be subdivided into proprioceptive and exteroceptive subdivisions in a similar way to the dorsal spino-cerebellar tract (Holmqvist, Oscarsson and Rosén, 1963). The cuneo-cerebellar system enters the cerebellum via the inferior peduncle. A projection from the gracile nucleus, which receives primarily cutaneous inputs, to the cerebellum has also been demonstrated (Gordon and Horrobin, 1967; Rinvik and Walberg, 1975), as has a trigemino-cerebellar mossy fibre projection (Carpenter and Hanna, 1961; Cupédo, 1965). The vestibulo-cerebellar projection includes both primary (from vestibular organs) and secondary (from vestibular nuclei) mossy fibre afferents to the vestibulo-cerebellum, uvula and paraflocculus and are undoubtedly concerned with the regulation of posture (Carpenter, Stein and Peter, 1972; Mehler, 1977). The ponto-cerebellar projection is the major pathway by which cortical regions influence the cerebellar cortex. Many studies have demonstrated a topographical organisation of the ponto-cerebellar projection (e.g. Brodal, 1979) and also the somatotopic projection of both the primary motor cortex and the primary somatosensory cortex upon the pontine nuclei (Brodal, 1968).

The indirect mossy fibre afferents arise from three principle precerebellar reticular nuclei, the paramedian reticular nucleus, the lateral reticular nucleus, and the nucleus reticularis tegmenti pontis

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(Bloedel and Courville,1981). These nuclei receive afferents from both ascending and descending pathways. However, only the nucleus reticularis tegmenti pontis receives inputs exclusively from descending pathways and, unlike the other two nuclei, projects only to the cerebellum (Bloedel and Courville,1981). Many of the inputs acting through the direct pathways also activate the indirect nuclei. A further source of mossy fibres has been demonstrated to be the deep cerebellar nuclei (Gould and Graybiel,1976; Tolbert, Bantli, and Bloedel,1976). This suggests a direct route whereby the output of the cerebellum can modulate ongoing activity within the cerebellar cortex.

In contrast to the widespread source of mossy fibre afferents, the climbing fibres arise from a single source, the inferior olive. The inferior olive had been established as a cerebellar afferent relay nucleus (Brodal,1940) many years before it was discovered that it was the source of the cerebellar climbing fibres (Szentágothai and Rajkovits,1959). Although evidence has been presented claiming extra-olivary sources of climbing fibres (Sasaki, Kawaguchi, Shimono and Yoneda,1969; Courville,1975) it is now generally accepted that the inferior olive is the major, if not the only, source of climbing fibres (Courville and Faraco-Cantin,1978). Using neuroanatomical tracing techniques it has been shown that the olivo-cerebellar fibres from each nucleus cross the midline and traverse, as well as surround, the contralateral olive before

entering the cerebellum via the inferior cerebellar peduncle (Courville, 1975; Groenewegen and Voogd, 1977). Although the majority of the fibres cross within the brainstem there is a small ipsilateral component which may cross within the cerebellum since all the labelled terminals were contralateral to the injection sites (Courville, 1975). Other studies using retrograde axonal transport of horseradish peroxidase have demonstrated a very complex but highly organised topographical olivo-cerebellar projection (Groenewegen and Voogd, 1977; Brodal and Walberg, 1977; Groenewegen, Voogd and Freedman, 1979).

The inferior olive receives inputs from many ascending and descending pathways. An important and common feature of these afferents is that they all have a topographic organisation. There are at least two projections from the spinal cord. The spino-olivary pathway was initially studied by Brodal, Walberg and Blackstad (1950) who demonstrated that these fibres ascend in the ventral funiculus. Most cross in the spinal cord and ascend ipsilateral to their termination. All spinal segments contribute to the pathway but most of the afferents arise from the lumbar and sacral levels rather than the thoracic and cervical. Subsequent studies have agreed with these findings (Berkley and Worden, 1978). A second crossed pathway from the dorsal column nuclei has been described (Hand and Liu, 1966; Ebbesson, 1968). It was later shown that the projections to the olive from the dorsal column nuclei and the

spino-olivary tract converge (Boesten and Voogd, 1975). This offers the possibility of a specific stimulation of part of the body affecting a particular group of olivary neurones by two different pathways. Oscarsson (1973) has demonstrated that many pathways in all of the spinal funiculi can activate the olivo-cerebellar system. However, the anatomical substrates involved can only be speculated upon, apart from the established spino-olivary and dorsal column pathways. The older neuroanatomical techniques of the lesion-degeneration principle and the more recent radioactive tracer-axonal transport techniques are only useful for direct pathways; they shed no light upon disynaptic and polysynaptic pathways. However, it is clear that cutaneous and deep receptors have projections to the inferior olive and thereby to the cerebellum. There are also projections to the inferior olive from other caudal brainstem nuclei, including the spinal trigeminal nucleus (Berkley and Hand, 1976), the vestibular nuclei (Saint-Cyr and Courville, 1979), and the lateral reticular nucleus in the rat (Brown, Chan-Palay and Palay, 1977) but not in the cat (Künzle, 1975a).

One of the major sources of afferents to the inferior olive arises in the deep cerebellar nuclei, with the fastigial and interpositus nuclei having the most extensive projection. Anterograde degeneration (Dom, King and Martin, 1973) and autoradiographic tracing studies (Graybiel, Nauta, Lasak and Nauta, 1973) in the cat have demonstrated a bilateral (mostly ipsilateral)

projection from the fastigial nucleus to the caudal region of the olive, along with a crossed projection from the interpositus and dentate nuclei to the rostral portions of the olive. A more well defined topographic projection has been described in the opossum (Martin, Culberson, Laxson, Linauts, Penneton and Tschismadia, 1980). Thus, it appears that the output of the cerebellum can influence the climbing fibre input as well as the mossy fibre input.

A contralateral projection from the superior colliculus to the inferior olive has been described (Nyberg-Hansen, 1964) and has been found to arise from the stratum griseum profundum and project to the dorso-medial portion of the caudal medial accessory olive in the cat (Graham, 1977). In studies involving this same projection in monkeys, it has been stressed that the superior colliculus afferents project to areas of the inferior olive which in turn connect with the vermal visual areas of the cerebellum (Weber, Partlow and Harting, 1978). Pretectal afferents to the inferior olive, which can be activated by visual stimuli, have also been described in the cat (Hoffman, Behrend and Schoppman, 1976).

The largest projection to the inferior olive is from the mesencephalic nuclei. Saint-Cyr and Courville (1980) have demonstrated an olivary projecting from a continuous column of nuclei lying adjacent to the central grey in the cat. These nuclei include the Darkschewitsch nucleus, the interstitial nucleus of

Cajal and the parvocellular red nucleus. These are purely ipsilateral projections to most of the olivary complex, excluding the rostral dorsal accessory olive and the mediolateral portion of the medial accessory olive. There is also a suggestion that in the rat the Edinger-Westphal nucleus may also project to the olive (Brown, Chan-Palay and Palay, 1977). There is little information concerning these mesencephalic nuclei except that some receive afferents from vestibular and oculomotor nuclei and have been implicated in eye movements (Carpenter, Harbison and Peter, 1970).

A sparse direct cortico-olivary projection has been demonstrated in both the monkey (Mettler, 1935) and the cat (Walberg, 1956). A study employing more refined histological techniques has shown that these projections are mainly crossed, are distributed topographically to the principle and accessory olives and arise from the anterior sigmoid gyrus and the rostral part of the coronal gyrus (Sousa-Pinto and Brodal, 1969). However, some electrophysiological evidence is in direct contradiction to the known neuroanatomy (Armstrong, 1974), as cerebrocortical evoked potentials have been recorded in the contralateral parts of the cerebellum as opposed to the expected ipsilateral cerebellum. Using similar electrophysiological techniques, a descending projection to the inferior olive from the caudate nucleus and globus pallidus has been suggested (Walberg, 1956; Sedgwick and Williams, 1967) but such pathways have not been detected using horseradish peroxidase neuro-

anatomical tracing techniques (Saint-Cyr and Courville, 1980).

The efferent projections from the Purkinje cells of the cerebellar cortex have a clear sagittal projection, from the vermis to the fastigial nucleus, from the intermediate cortex to the interpositus nucleus, and from the lateral cortex to the dentate or lateral nucleus (Jansen and Brodal, 1940). The flocculonodular lobe projects directly to the vestibular nuclei. These different parts of the cerebellar cortex and their associated deep nuclei are functionally different. An early study by Holmes (1939) demonstrated that the lateral cerebellar cortex received a large input from the cerebral association cortex via the pontine nuclei and that most of the output from the dentate nucleus was to the motor cortex via the ventrolateral thalamic nucleus. He suggested that this region of the cerebellum might be involved in the initiation of intentional movements as he had observed defects in the rate, timing, and force of muscle contraction in subjects with cerebellar hemisphere injuries. Such defects have also been observed following reversible cooling lesions of the dentate nucleus in the monkey (Brooks, Kozlovskaya, Atkin, Horvath and Uno, 1973). It is now known that the output of the dentate nucleus passes via the superior cerebellar peduncle to both ventrolateral and ventro-anterior thalamic nuclei (Dom, 1973) and it is currently believed that the lateral cerebellum preprograms some aspects of the motor command signals from the motor cortex

(Allen and Tsukahara, 1974).

The intermediate cerebellar cortex receives its afferents from both the motor cortex and the spinal cord. The major efferents of the interpositus nucleus are to the motor cortex, via the ventrolateral thalamic nucleus, and to the spinal cord via the red nucleus. Some of the efferent fibres are known to project to both the thalamus and the red nucleus (King, Dom, Conner and Martin, 1973). The projection from the interpositus nucleus to the red nucleus is somatotopically arranged (Eccles, Scheid and Taborikova, 1973) and as the rubro-spinal tract also displays a somatotopical organisation, the influence of the cerebellum mediated by this pathway is very precise. It is therefore believed that the intermediate cerebellar cortex-interpositus nucleus circuits provide a corrective function over the motor commands signalled by the motor cortex via the rubro- and cortico-spinal tracts. It is clear that the lateral cerebellar cortex-dentate nucleus and the intermediate cerebellar cortex-interpositus nucleus modulate motor activity via the pyramidal and group B brainstem pathways of Kuypers (1981).

The cerebellar vermis receives inputs from the spinal cord via the ventral spino-cerebellar, and to a lesser extent, the dorsal spino-cerebellar tracts as well as visual, auditory and vestibular afferents (Armstrong, 1978). The lateral part of the anterior lobe vermis and the posterior lobe vermis project directly to the vestibular nuclei (especially to the dorsal half of

Deiters' nucleus), whereas the rest of the vermis projects to fastigial nucleus. However, the main projection of fastigial nucleus is to the whole of Deiters' nucleus and to the pontomedullary reticular formation (Armstrong, 1978), although there is a sparse projection to ventrolateral thalamus (Massion and Rispal-Adel, 1972) and cervical spinal cord (Wilson, Uchino, Susswein and Fukushima, 1977). Deiters' nucleus, together with the other nuclei of the vestibular complex, gives rise to the vestibulo-spinal tracts and the pontomedullary reticular formation contributes a large part of the reticulo-spinal tract. These two descending pathways form a major part of the group A brainstem descending fibres of Kuypers (1981). As midline cerebellar injuries produce major deficits in postural control it is currently believed that the vermis of the cerebellum is involved in the coordination of axial muscle groups.

The flocculonodular lobe of the cerebellum also projects in a topographic fashion to the vestibular complex, although mostly to areas other than Deiters' nucleus (Brodal, 1974). Some of these target cells project to oculomotor nuclei (Ito, Nisimaru and Yamamoto, 1973) and it appears that this area of the cerebellum may be primarily involved in regulating vestibulo-ocular reflexes.

Pathological lesions of the cerebellum produce a wide range of motor deficits which include; disturbance of gait, ataxia, hypotonia, dysmetria and tremor. The range of disturbances exhibited depend upon the region

of the cerebellum which is damaged. In general, three types of cerebellar lesion are recognised. These are, archiocerebellar syndrome involving lesions of the flocculonodular lobe, paleocerebellar syndrome involving lesions of the medial parts of the cerebellar cortex, and neocerebellar syndrome involving the lateral parts of the cerebellar cortex (Dow and Moruzzi, 1958). All three syndromes produce a disturbance in gait whereas only lesions involving the lateral parts of the cerebellar cortex produce symptoms such as hypotonia and tremor. The disturbances in gait are not the same in all three syndromes, as would be predicted from the anatomical connections described above. Lesions involving the flocculonodular lobe affect predominantly balance and produce these effects by group A pathways of Kuypers (1981). Unilateral lesions of the fastigial nucleus in the cat and rat support this view, producing powerful extensor hypertonus of the contralateral limbs and extensor atonia of the ipsilateral limbs (Batini and Pompeiano, 1957; Imperato, Nicoletti, Diana, Scapagnini and Di Chiara, 1984). Imperato et al (1984) have also shown that these postural changes can also be produced by the local administration of muscimol (a gamma-aminobutyric acid (GABA)-mimetic agent) to the fastigial nucleus. Disturbances of gait produced by lesions of the lateral parts of the cerebellar cortex are a result of poor muscle control of the limbs rather than a deficit in balance or posture. Similarly, other motor deficits produced by such lesions are a result of

failing to coordinate muscle activity to start and stop at the correct time, which might be expected as this region of the cerebellum is intimately associated with the motor cortex and receives many peripheral afferents from cutaneous and muscle receptors.

Whilst the motor deficits which result from cerebellar dysfunction have long been recognised, more recently it has been shown that similar effects can be produced by lesions of the inferior olive (Soechting, Ranish, Palmineri and Terzuolo, 1976). Due to this, and the very intimate association of the climbing fibres with Purkinje cells, the inferior olive has been the subject of intense investigation. Many studies have used nicotinamide antagonists to produce lesions within the inferior olive, the most commonly used agent has been 3-acetylpyridine (Hicks, 1955; Desclin, 1974; Batini and Billard, 1985), although 6-aminonicotinamide produces similar effects (Sternberg and Philips, 1958). These agents have been reported to produce widespread damage to both the central and peripheral nervous systems (Hicks, 1955; Sternberg and Philips, 1958) although Desclin and Escubi (1974) claim that the central lesions are located mainly in the medulla oblongata. The use of harmaline and nicotinamide together with 3-acetylpyridine has been claimed to confine the central lesion to the inferior olive (Llinás, Walton, Hillman and Sotelo, 1975) although this has been disputed by Balaban (1985). Ultrastructural changes within the Purkinje cells have been described following the chemical destruction of the

inferior olive (Desclin and Colin, 1980). However, what is not disputed by any of these studies is that the inferior olive is particularly sensitive to the toxic effect of nicotinamide antagonists and therefore many studies have used these agents to destroy the olivo-cerebellar projection. Such studies have led to the proposal that the climbing fibre projection to the cerebellar cortex is in some way involved in learning motor tasks (Marr, 1969). In support of this hypothesis evidence has been presented which shows that the olivo-cerebellar neurones exert a trophic effect upon the Purkinje cells (Ito, Nisimaru and Shibuki, 1979) such that following the destruction of the inferior olive, the Purkinje cells lose their inhibitory action on Deiters' nucleus (Ito, Orlov and Shimoyama, 1978). This may be the result of the loss of climbing fibres or may be a direct result of the 3-acetylpyridine upon the Purkinje cells.

Whatever the true function of the inferior olive might be, it is clear that it is important for the correct functioning of the cerebellum and that the nicotinamide antagonists provide a convenient method to study the olivo-cerebellar projection.

1.3 THE ROLE OF THE BASAL GANGLIA IN MOTOR CONTROL

The basal ganglia are large subcortical nuclei within the telencephalon and can be divided into two major subdivisions, the corpus striatum and the amygdaloid nuclear complex. The latter is regarded as being a component of the limbic system. Evidence that

the corpus striatum is involved in motor behaviour dates back to the 19th century when Magendie (1841) reported that the injection of chromic acid into the corpus striatum of the rabbit caused the animal to keep running forwards, not stopping even when it met an obstruction. The corpus striatum can be further subdivided into the neostriatum (or more usually, the striatum), consisting of the caudate nucleus and putamen, and the paleostriatum, consisting of the medial and lateral globus pallidus segments (the entopeduncular nucleus and globus pallidus, respectively, in sub-primates). The striatum and pallidum are closely associated with two brainstem nuclei, the substantia nigra and the subthalamic nucleus. The striatum has massive reciprocal connections with the substantia nigra (Szabo, 1962; Nauta and Mehler, 1966) whereas the two pallidal segments have reciprocal connections with the subthalamic nucleus (Nauta and Mehler, 1966; Carpenter, Fraser and Shriver, 1968; Nauta and Cole, 1978). The striatum is regarded as the receptive component whereas the medial pallidal segment and the substantia nigra pars reticulata are regarded as the output components of the motor basal ganglia.

As the caudate nucleus and the putamen have a similar histological appearance and a similar embryological origin they are considered as being a single functional entity (Carpenter, 1981). The neurones of the striatum are densely packed and are considered to be of two types, medium achromatic and large meta-chromatic neurones, both of which may or may not have

spiny dendrites (Fox, Andrade, Hillman and Schwyn, 1971a; Fox, Andrade, Schwyn and Rafols, 1971b; Di Figlia, Pasik and Pasik, 1976). Traditionally, the large cells were believed to be the source of the striatal efferent fibres (Fox et al, 1971a) but more recent studies with retrograde transport of horseradish peroxidase have challenged this concept. Such studies in the cat (Grofová, 1975), rat (Bunny and Aghajanian, 1976) and monkey (Szabo, 1977) show labelling of mainly medium sized neurones within the striatum. The reason that the efferent nature of these cells was not discovered in earlier studies using Golgi staining may be that the fine calibre of the striatofugal axons prevented full impregnation of the Golgi stain.

Many neuroactive compounds have been found in the striatum, including monoamines such as dopamine and noradrenaline and peptides such as met-enkephalin and substance P (see Graybiel and Ragsdale, 1983 for review). The striatum contains one of the highest concentrations of acetylcholine in the brain and there is abundant evidence that cholinergic neurotransmission is fundamental to normal striatal function since the levels of acetylcholine are changed in diseased states associated with basal ganglia dysfunction such as Huntington's chorea and Parkinson's disease and also Alzheimer's disease (McGeer and McGeer, 1975; Spokes, 1980). The current view is that a population of both medium and large striatal neurones contain acetylcholine and that most are interneurons, although a few may project to

the cortex (Butcher and Butcher, 1974). Another major and important neurotransmitter in the striatum is GABA. There is direct evidence that this is located in medium-sized neurones (Ribak, Vaughn and Roberts, 1979; Bolam, Freund, Hammond, Smith and Somogyi, 1982), and indirect evidence that many of these neurones are projection neurones to the substantia nigra and to the globus pallidus (Fonnum, Gottesfeld and Grofova, 1978; Nagy, Carter and Fibiger, 1978). Apart from substance P, cholecystokinin, dynorphin, and the enkephalins, which are implicated in striatofugal projections (see below), the functions of the many other neuroactive compounds within the striatum are unknown. Unlike the cerebellum, very little is known about the neuronal circuitry within the striatum but much more is known about the inter-connecting pathways between the various nuclei of the basal ganglia.

The striatum receives three major afferent connections which are from the cerebral cortex, the intralaminar nuclei of the thalamus and the substantia nigra pars compacta. The projection from the cortex is the largest and retrograde horseradish peroxidase transport studies have demonstrated that these are direct cortico-striatal projections rather than collaterals of fibres in corticofugal pathways to other motor areas of the brain (Jones, Coulter, Burton and Porter, 1977; Jones and Wise, 1977). The neurotransmitter in this pathway is believed to be glutamic acid (Spencer, 1976; Fonnum and Storm-Mathisen, 1977). Virtually all regions of the

cortex project to the striatum and all regions of the striatum receive fibres from the cortex (Webster, 1961; Kemp and Powell, 1970). Kemp and Powell (1970) have shown that the cortico-striatal projection is topographically organised with the medial surface of the frontal lobe projecting to the lateral parts of the striatum and the orbital surface projecting to the medial parts of the striatum. The cortical input to the striatum includes bilateral projections (Carman, Cowan, Powell and Webster, 1965; Künzle, 1975b). Different regions of the cortex project to different volumes of striatum, so that the projection from the sensorimotor cortex goes to the greatest volume whereas the visual cortex projects only to a small posterior part of the striatum (Webster 1961). Although the striatum has been traditionally regarded as a single functional entity it is now known that the caudate nucleus and putamen receive their cortical afferents from different areas of the cerebral cortex, at least in primates. It has been shown that the caudate nucleus receives its cortical input from association areas, particularly the premotor regions of the frontal lobe and the posterior parietal areas, whereas the putamen receives most of its cortical inputs from sensorimotor cortex (Künzle, 1975b, 1978). This anatomical organisation, together with observations from behavioural studies has led to the suggestion that the caudate nucleus is more involved in cognitive activity than in motor activity, whereas the reverse is true of the putamen (Öberg and Divac, 1979). Whether

such an organisation and functional division is true for the rat is unclear. However, a laterally organised functional division has been reported, with the dorso-lateral striatum receiving afferents from the motor cortex and the ventromedial striatum receiving its cortical input from the hippocampus (Kelley, Domesick and Nauta, 1982).

Early evidence of a thalamostriate projection from the intralaminar (non-specific) thalamic nuclei was provided by retrograde and anterograde degeneration studies (Powell and Cowan, 1967). Using radiolabelled tracers it has been shown that the greater part of this projection arises in the parafascicular nucleus and centrum medianum (the CM-PF complex) although other thalamic nuclei are also involved (Herkenham, 1978; Royce, 1978). These studies provide evidence of a topographically organised efferent projection from the intralaminar nuclei to the striatum with the more rostral intralaminar nuclei projecting to the more rostral parts of the striatum. These fibres terminate within the striatum in a characteristic mosaic fashion which is similar to that of the cortico-striatal projection (Herkenham, 1978; Carpenter, 1981). Collaterals of the thalamostriatal fibres have been shown to project to widespread areas of the cerebral cortex (Jones and Leavitt, 1974; Carpenter, 1981). In spite of the large size of the thalamostriatal projection, the neurotransmitter involved is unknown. Contrary to some reports, it appears unlikely that glutamic acid,

aspartic acid or acetylcholine are the major transmitters (McGeer, Staines and McGeer, 1984).

The nigro-striatal projection was the first of the striatal afferent systems to be identified. As early as 1895 von Monakow demonstrated that cerebral ablations which included part of the striatum produced extensive degeneration within the substantia nigra pars compacta. Subsequent studies revealed that cortical ablations per se did not cause the nigral degeneration and that the degeneration observed in von Monakow's experiments was directly related to the degree of striatal damage (Mettler, 1943). Fluorescent histochemical techniques have demonstrated an extremely rich innervation of the striatum by dopamine-containing neurones of the substantia nigra pars compacta (Falk, Hillarp, Thieme and Torp, 1962; Bédard, Larochelle, Parent and Poirier, 1969). Such studies have shown that the dopaminergic terminals form a distinct matrix of fine varicose fibres containing small granular vesicles (approximately 50µm in diameter) around both small and large striatal neurones. Anterograde degeneration studies in the monkey have suggested that both the striato-nigral and the nigro-striatal fibres are topographically and reciprocally organised (Szabo, 1962, 1967) and may therefore form a closed feedback loop (Carpenter and Peter, 1972). Such a loop arrangement has been demonstrated by axonal transport studies using horseradish peroxidase in the rat (Nauta, Kaiserman-Abramof and Lasek, 1975). The topography of the nigro-striatal projection appears

to be that the more lateral regions of the nigra project to dorsal portions of the putamen while medial regions project to ventral parts of the putamen. Rostral parts of the nigra project to both the head of the caudate nucleus and rostral putamen (Carpenter and Peter, 1972). The termination of the nigro-striatal fibres are evenly distributed over broad regions of the striatum without the characteristic mosaic patterns of the cortico- and thalamo-striate projections. Although dopamine-containing neurones account for 85-95% of the nigro-striatal tract, there has been the suggestion that at least one other neurotransmitter is involved (Hedreen, 1978; van der Kooy, Coscina and Hattori, 1981). Subsequently, it has been suggested that neurotensin is a possible neurotransmitter here (McGeer et al, 1984) as it has been found in both the nigra and striatum and is known to facilitate dopamine release from striatal slices (de Quidt and Emson, 1983). The possibility of a second neurotransmitter in this pathway may account for the conflicting reports of both excitatory and inhibitory effects on striatal neurones following stimulation of the nigro-striatal pathway or iontophoretic application of dopamine (Hull, Bernardi and Buchwald, 1970; Spehlmann, 1975). However, most workers agree that dopamine has an inhibitory action on striatal neurones.

A projection from the dorsal raphe nucleus to the striatum has been demonstrated (Nauta, Pritz and Lasek, 1974) with the highest concentration of 5-hydroxytryptamine and most dense projection being to the ventrocaudal regions of the putamen (Ternaux, Héry, Bourgoin, Adrien,

Glowinski and Hamon, 1977). The action of 5-hydroxytryptamine is believed to be inhibitory on striatal neurones (Olpe and Koella, 1977) although there may be a second neurotransmitter in the raphe-striatal pathway (Steinbusch, van der Kooy, Verhofstad and Pelligrino, 1980). Several minor pathways to the striatum have been identified. These include a topographically organised pallido-striatal projection (Jayaraman, 1983), a sparse projection from the subthalamic nucleus which may arise from displaced pallidal cells (Ricardo, 1980), and a pedunclopontine nucleus-striatal projection (Saper and Loewy, 1982). An amygdalo-striatal pathway involving neurones which may contain cholecystokinin and somatostatin has been described (Kelly et al, 1982; Meyer, Beinfeld, Oertel and Brownstein, 1982). In addition, there may be a histaminergic pathway from the mammillary body and reticular formation (Barbin, Garbarg, Lorens-Cortes, Palcios, Pollard and Schwartz, 1977).

The major route by which the striatum exerts its influence on cortical activity involves relays, not only in the thalamus, but also in the globus pallidus. As described previously, the globus pallidus consists of two parts, the lateral and medial pallidal segments. These two segments have similar histological appearances, with two types of neurone having been described in primates; large polygonal neurones with long thick dendrites and small neurones with very few dendrites (Fox, Andrade, Lu Qui and Rafols, 1974). The largest afferent projection to the pallidum is from the striatum, which is

topographically organised in rostrocaudal and medio-lateral dimensions to both pallidal segments. The nucleus accumbens and limbic striatum projects to the subcommisural part of the external pallidal segment in the rat (Nauta and Domesick, 1978). This region has been termed the ventral pallidum by Heimer and Wilson (1975). The remainder of the striatum projects to the supracommisural pallidum in such a way that neurones from the head of the caudate nucleus project to dorsal and rostral parts of the pallidum whilst the putamen has efferent projections to ventral and caudal parts of the pallidum (Szabo, 1962; Cowan and Powell, 1966; Szabo, 1967). The efferents from the striatum are very thin myelinated fibres which form slender fascicules (sometimes referred to as Wilson's pencils) that converge upon the globus pallidus like spokes of a wheel (Wilson, 1914).

It is difficult to determine if the same striatal fibres innervate both segments of the pallidum. Recent immunohistochemical studies in the rat have found that the external pallidal segment contains numerous enkephalin-positive fibres but (with the exception of the ventral pallidum) only a few substance P-positive fibres, whereas the reverse is true in the internal segment and the substantia nigra (Haber and Nauta, 1983). This suggests that the two pallidal segments are, at least in part, innervated by separate striatal fibres. Two neurotransmitters are known to be associated with the striato-pallidal projection, these are GABA (Fonnum

et al, 1978; Nagy et al, 1978) and enkephalin (Del Fiacco, Paxinos and Cuello, 1982). It has also been suggested that there are some striato-pallidal substance P-containing neurones (Staines, Nagy, Vincent and Fibiger, 1980) and it is possible that some fibres of the striato-nigral dynorphin-containing projection may give off collaterals as they pass through the pallidum.

The only other major afferent pathway to the pallidum is from the subthalamic nucleus. Many of the early anatomical studies suggested that the subthalamo-pallidal projection was mainly, if not exclusively, to the medial pallidal segment (Whittier and Mettler, 1949; Carpenter and Strominger, 1967). Reports which did suggest that there was a projection to both segments (e.g. Glees and Wall, 1946) were criticised on the grounds that the lesions of the subthalamic nucleus involved in these studies were not sufficiently circumscribed. However, using radiolabelled neuronal tracing and autoradiographic techniques in the monkey and cat, Nauta and Cole (1978) have demonstrated that the subthalamic nucleus does indeed project to both segments of the pallidum. These workers have shown that the subthalamic nucleus projected almost equally to both pallidal segments and that the pathway was topographically organised with the fibres terminating in dense bands parallel to the medullary lamina. Since the subthalamic nucleus also projects to the substantia nigra pars reticulata (Whittier and Mettler, 1949; Nauta and Cole, 1978), it occupies a central position in the circuitry of the basal

ganglia. Indeed, single subthalamic neurones have been shown to project to both the pallidum and the substantia nigra in the rat (Deniau, Hammond, Chevalier and Féger, 1978) and it has been estimated that 94% of the subthalamic neurones project to both these nuclei (van der Kooy and Hattori, 1980).

The neurotransmitters in the subthalamofugal pathways are unknown. Stimulation of the subthalamic nucleus evokes, via monosynaptic pathways, both excitatory (Hammond, Deniau, Rizk and Féger, 1978) and inhibitory responses (Rouzaire-Dubois, Scarnati, Hammond, Crossman and Shibazaki, 1983) in the substantia nigra and entopeduncular nucleus respectively. As the vast majority of subthalamic nuclear cells project to both of these nuclei it suggests that the neurotransmitter has both excitatory and inhibitory actions. As it has been shown that GABA has both excitatory and inhibitory effects on hippocampal pyramidal neurones, depending on whether the amino acid was applied to the soma or dendrites of the cell (Andersen, Dingledine, Gjerstad, Langmoen, and Mosfeld-Laursen, 1980), the suggestion by Rouzaire-Dubois et al (1978) that GABA is the neurotransmitter in the subthalamic nucleus efferent pathways may be correct. Clearly, further experimental evidence is required to determine the true identity of the subthalamo-pallidal neurotransmitter.

Two other very small afferent projections to the pallidum have been described. There is some evidence that the globus pallidus may receive afferents from

the pedunculopontine nucleus, or from the adjacent cholinergic cell group, the nucleus parabrachialis (Saper and Loewy, 1982). In addition a dopaminergic projection from the substantia nigra has been identified (Lindvall and Bjorklund, 1979).

Although the histological morphology of the two pallidal segments is very similar (Fox et al, 1974), they have distinctive projections. The lateral pallidal segment projects predominantly to the subthalamic nucleus (Nauta and Mehler, 1966) in a topographical manner such that rostral parts of the pallidal segment project to the medial half of the subthalamic nucleus and central parts of the segment project to the lateral half of the nucleus (Carpenter et al, 1968; Carpenter, Batton, Carleton and Keller, 1981). It remains uncertain whether the medial pallidal segment contributes to the pallido-subthalamic projection although a recent autoradiographic study suggests that the medial pallidal segment is not involved (Kim, Nakano, Jayaraman and Carpenter, 1976). The neurotransmitter in the pallido-subthalamic pathway is unknown but lesion and biochemical studies have suggested that neither GABA nor acetylcholine are involved (van der Kooy, Hattori, Shannak and Hornykiewicz, 1981).

Several groups of workers have described a pallido-nigral pathway from the lateral pallidal segment (Grofova, 1975; Kanazawa, Marshall and Kelly, 1976; Kim et al, 1976), which preferentially terminates upon dopamine-containing neurones of the pars compacta (Hattori,

Fibiger and McGeer, 1975). Although lesion and biochemical studies suggest that the neurotransmitter in this pathway may be GABA (Hattori, McGeer, Fibiger and McGeer, 1973; Nagy et al, 1978), it is possible that the observed effects may have been due to the interruption of the striato-nigral pathway which is known to pass through the globus pallidus (Fonnum, Grofová, Rinvik, Storm-Mathisen and Walberg, 1974; Kuppersmith and Lieberman, 1980; Nagy and Fibiger, 1980). Similarly, a substance P-containing pallido-nigral projection has been suggested (Mroz, Brownstein and Leeman, 1977; Jessell, Emson, Paxinos and Cuello, 1978), but this again may be associated with interruption of the striato-nigral pathway (Kanazawa, Emson and Cuello, 1977).

The largest pallidofugal projection arises in the medial pallidal segment and projects to the thalamus, habenula and tectum. The lateral pallidal segment is known to contribute to the pallido-thalamic projection, predominantly innervating the non-specific thalamic reticular nucleus (Nauta, 1979) which in turn projects to many of the other thalamic nuclei (Scheibel and Scheibel, 1966). In contrast, the more extensive thalamic projection from the medial pallidal segment is distributed over the thalamic fasciculus to three thalamic areas, the nucleus ventralis lateralis and ventralis anterior (VA-VL complex), the centrum medianum and the lateral habenula nucleus (Nauta and Domesick, 1984). The topography of the pallido-thalamic projection in the monkey is well defined (Kuo and

Carpenter, 1973). The outer portion of the medial pallidal segment gives rise to the ansa lenticularis, which projects mainly to the ventral anterior pars principalis nucleus of the thalamus, and the inner portion of the medial pallidal segment gives rise to the lenticular fasciculus, which projects primarily to the ventral lateral pars oralis of the thalamus. This latter nucleus is thought to project topographically upon the motor cortex (Walker, 1966). Kuo and Carpenter (1973) also found that all parts of the medial pallidal segment project to the rostral parts of the centromedian nucleus.

Studies of the efferent projections of the entopeduncular nucleus in the rat have indicated a similar thalamic distribution, with the major projection terminating within the ventromedian nucleus, the latter being the homologue of the primate VA-VL complex (Carter and Fibiger, 1978). One group of workers have suggested that the globus pallidus of the rat has a wider thalamic distribution than that associated with the lateral pallidal segment in the monkey, projecting to ventroanterior, ventromedial, ventrodorsal and ventroexternal thalamic nuclei (Severin, Young and Massopust, 1976). However, this is not supported by other studies (Carter and Fibiger, 1978; Herkenham, 1979).

The neurotransmitter in the pallido-thalamic pathway is unknown. Following medial pallidal segment stimulation, monosynaptic inhibitory postsynaptic potentials have been recorded in neurones of the VA-VL

complex (Uno and Yoshida,1975). However, excitatory effects have also been reported (Desiraju and Purpura,1969). There is a strong possibility that the neurotransmitter in this pathway is GABA as the pallidum is rich in gamma-aminobutyric acid transaminase (Nagai, McGeer and McGeer,1983). Furthermore, lesions of the entopeduncular nucleus in the rat results in a 50% loss of GABA, glutamic acid dehydrogenase and high affinity GABA uptake in the ipsilateral ventral anterior-lateral thalamus (Penny and Young,1981).

The pallido-habenula projection forms a distinctive bundle in primates and man which is formed as the fibres leave the ansa lenticularis (Wilson,1914; Nauta and Mehler,1966; Carpenter et al,1968). The fibres have a complex course through the thalamus in both primates and cats (Nauta and Mehler,1966; Larsen and McBride,1979) and this projection in the cat is probably the most extensive of any of its pallidofugal pathways (Larsen and McBride,1979). There is also a large pallido-habenula projection in the rat which arises from virtually every neurone in the entopeduncular nucleus (Herkenham and Nauta,1977). This suggests that the projection consists of collaterals of other pallido-thalamic fibres. The habenular complex is an epithalamic structure which is functionally linked with various nuclei of the limbic system (Herkenham and Nauta,1977, 1979; Parent, Gravel and Boucher,1981). On anatomical grounds it would appear that limbic and pallidal inputs are segregated in the habenula since the afferents from

limbic sources terminate in the medial habenula whereas the afferents from the medial pallidal segment terminate in the lateral habenula (Herkenham and Nauta, 1979; McBride, 1981). However, electrophysiological studies suggest that neurones in both medial and lateral parts of the habenula are inhibited by entopeduncular stimulation (McBride, 1980), thus indicating that the habenula may be an important nucleus for the convergence of limbic and corpus striatal influences. Lesion and biochemical studies in the rat have suggested that the neurotransmitter in the pallido-habenula pathway is GABA (Nagy, Carter, Lehman and Fibiger, 1978; Gottesfeld, Brandon and Wu, 1981).

Although early studies suggested that pallidofugal pathways to the brainstem existed (Wilson, 1914; Ranson, Ranson and Ranson, 1941), more recent experiments using silver impregnation techniques have shown that efferent pallidal fibres can only be traced as far as the mesencephalon where they terminate in the compact portion of the pedunculopontine nucleus (Nauta and Mehler, 1966; Kuo and Carpenter, 1973). Autoradiographic and horseradish peroxidase studies have confirmed these results in the monkey (Kim et al, 1976), the cat (Larsen and McBride, 1979) and the rat (Jackson and Crossman, 1981). The pathway has a sparse contralateral component (Nauta, 1979) and double-labelling studies in the rat and monkey have shown that the pedunculopontine projection are collaterals of pallido-thalamic and pallido-habenula fibres (van der Kooy and Carter, 1981; Parent

and Bellefeuille, 1982). A study with horseradish peroxidase has indicated that some neurones of the pedunculopontine nucleus may project to the spinal cord and this has led to the suggestion that this nucleus may represent a link between the corpus striatum and the spinal cord (Ross, Ruggiero and Reis, 1979). However, spinal cord projecting fibres from the pedunculopontine nucleus were not found by Moon-Edley and Graybiel (1983). The nature of the pallido-pedunculopontine neurotransmitter has not been investigated, but since this pathway arises from neurones which project to the thalamus and habenula, GABA must be a prime candidate.

The corpus striatum, and in particular the neostriatum, is intimately connected with the substantia nigra, the largest single nucleus in the mesencephalon. This nucleus is commonly divided into two parts, the pars compacta and the pars reticulata. The sub-division is based on gross morphology; the pars compacta has a high density of cell bodies and is composed of large pigmented neurones in man, whereas the cell bodies of the pars reticulata are more sparsely distributed. The latter are regarded as being a major source of corpus striatal efferents. Some ultrastructural studies do not support the morphological sub-division of the substantia nigra (e.g. Rinvik and Grofová, 1970). However, on neurochemical grounds, the substantia nigra can be readily divided into dopamine-containing and non-dopamine-containing cell groups. The pars compacta

is predominantly composed of dopamine-containing neurones (A9 group) whereas the pars reticulata mainly contains non-dopamine-containing neurones (Dahlström and Fuxe, 1964). There is also an adjacent extranigral dopamine-containing cell group, the ventral tegmental area (A10) which is believed to be associated with the limbic striatum and other limbic areas (Fallon and Moore, 1978; Nauta, Smith, Paul and Domesick, 1978). Although the differentiation of dopamine- and non-dopamine-containing cells on morphological grounds has always proved to be difficult, it came to be accepted that the large nigral neurones were those which contained dopamine (Gulley and Wood, 1971; Schwyn and Fox, 1974). However, it has been subsequently found that both large and small nigral neurones contain dopamine (Domesick, Stinus and Paskevich, 1983). Many investigators have commented on the ultrastructural similarity of the pallidum and the substantia nigra pars reticulata (Kemp, 1970; Rinvik and Grofova, 1970; Fox et al, 1974). It has been proposed that the pallidum and nigra represent one structure which has become separated during development by part of the internal capsule (DeLong and Georgopoulos, 1979).

The largest projection to the substantia nigra is from the striatum. Although this projection has been recognised for many years (Rundles and Papez, 1937) it was not until several decades later that any knowledge of the topography was acquired (Voneida, 1960; Szabo, 1962). Such studies revealed that the head of the caudate nucleus

projects to the rostral one-third of the substantia nigra and fibres from the putamen terminate in the caudal two-thirds of the nigra. Dorsal parts of the putamen project to lateral parts of the nigra and ventral portions project to medial parts of the nigra. Neuronal tracing studies with horseradish peroxidase and autoradiographic techniques have confirmed these findings (Bunny and Aghajanian, 1976; Nauta and Domesick, 1979). From an analysis of fibre diameters in the striatofugal fascicles, it has been suggested that most, if not all, striato-nigral fibres are branches of the striato-pallidal projection (Fox, Rafols and Cowan, 1975). This is supported by observations that the synaptic morphology within the pallidum and nigra is very similar (Schwyn and Fox, 1974) and also by electrophysiological evidence (Yoshida, Rabin and Anderson, 1974). However, studies using immunohistochemical techniques do not fully support this proposal as the pallidum (at least, the external pallidal segment) has numerous enkephalin-positive striato-pallidal fibres and very few substance P-positive fibres, whereas the reverse is true of the substantia nigra (Haber and Nauta, 1983). There is always the possibility that some of the GABA-containing striato-pallidal fibres have collaterals which terminate within the substantia nigra (Fonnum et al, 1978; Nagy et al, 1978).

Striato-nigral fibres have been shown to synapse directly upon nigro-striatal neurones (Somogyi, Bolam, Totterdell and Smith, 1981) as well as on the non-

dopamine-containing neurones of the pars reticulata (Somogyi, Hodgson and Smith, 1979). The neurotransmitters believed to be associated with the striato-nigral projection are, GABA (Fonnum et al, 1978), substance P (Davies and Dray, 1976; Gale, Hong and Guidotti, 1977) and dynorphin (Vincent, Hokfelt, Christensson and Terenius, 1982). Caudate nucleus and putamen efferent fibres have not been shown to project to any more caudal nucleus than the substantia nigra. However, the nucleus accumbens does project to the mesencephalic tegmentum and the central grey (Nauta et al, 1978).

Evidence for the existence of pathways from the pallidum and subthalamic nucleus to the substantia nigra has already been described. An axonal tracing study using horseradish peroxidase suggests that the subthalamic nucleus projects to both pars compacta and pars reticulata, with the greater part of the projection terminating in the pars compacta (Kanazawa et al, 1976). The neurotransmitter of this pathway is unknown. There is evidence that the raphe nuclei project to the substantia nigra as lesions of these nuclei reduce the histofluorescence of the pars reticulata (Kuhar, Aghajanian and Roth, 1972). A subsequent autoradiographic study suggested that the raphe projection terminates in the pars compacta (Conrad, Leonard and Pfaff, 1974) although an electrophysiological study has demonstrated that raphe neurones may synapse on pars reticulata neurones (Dray, Gonye, Oakley and

Tanner, 1976). Therefore, it appears that the midbrain raphe nuclei project to both sub-divisions of the substantia nigra. It is believed that this projection arises mostly in the dorsal raphe nucleus although some fibres may originate in the median raphe nucleus (Bobillier, Sequin, Petitjean, Salvert, Touret and Jouvet, 1976; Bunny and Aghajanian, 1976). Like most projections from the raphe nuclei, the major neurotransmitter in the raphe-nigral projection is believed to be 5-hydroxytryptamine (Kuhar et al, 1972; Dray et al, 1976).

Other projections to the substantia nigra have been described in sub-primates. These include projections from the central amygdaloid nucleus and nucleus accumbens (Bunny and Aghajanian, 1976; Krettek and Price, 1978; Nauta et al, 1978), the anterior hypothalamus (Nilaver, Hoffman and Zimmerman, 1979; Sofroniew, 1980) and the pedunculo-pontine nucleus (Graybiel, 1978; McGeer et al, 1984). However, retrograde axonal transport studies have failed to demonstrate projections from the ventral striatum, the habenula or the hypothalamus to the substantia nigra in the primate (Mehler, 1981).

There are three principal efferent pathways from the substantia nigra. They project to the striatum, thalamus and superior colliculus. The pathway to the striatum has been described previously. The nigro-thalamic pathway arises from the large non-dopamine-containing neurones of the pars reticulata. The cells of origin occupy a specific region of the pars reticulata

of the rat (Faull and Mehler, 1978), the cat (Rinvik, 1975) and the monkey (Carpenter and Strominger, 1967). These neurones project to the ventromedial nucleus of the rat (Clavier, Atmadja and Fibiger, 1976; Faull and Mehler, 1978), to the ventromedial nucleus and medial VA-VL of the cat (Afifi and Kaelber, 1965; Rinvik, 1975) and to the medial ventrolateral nucleus, magnocellular part of the ventroanterior nucleus and the dorsomedial paralaminar nucleus of the monkey (Carpenter, Nakano and Kim, 1976). Since the dorsomedial paralaminar nucleus has reciprocal connections with the frontal eye field, this projection may be involved in eye movements (Künzle and Akert, 1977).

With regard to the thalamic afferent projections from the cerebellum, the pallidum and the nigra, fibres from the three sources terminate in different regions of the thalamus in both the monkey (DeVito and Anderson, 1980; Asanuma, Thach and Jones, 1983; Graybiel, 1984) and the cat (Ilinsky and Kultas-Ilinsky, 1984; Yamamoto, Noda, Miyata and Nishimura, 1984). In contrast, it appears that the output of the nigra converges with those from the cerebellum and superior colliculus in the ventromedial thalamic nucleus of the rat (Herkenham, 1979). Whilst the ventromedial thalamic nucleus is known to project widely across the ipsilateral cortex in the rat (Herkenham, 1976), in the monkey the output of the thalamic neurones receiving nigral afferents has not been determined precisely although it seems that the ventral anterior thalamic nucleus has a widespread cortical distribution with the magnocellular part of

the ventral anterior nucleus projecting to the orbito-frontal cortex (Carmel,1970). Therefore in both the rat and monkey the substantia nigra pars reticulata and the medial segment of the pallidum are in a position to influence a large area of the cortex via their thalamic relays. A nigral input to the parafascicular nucleus in the rat has been demonstrated (Clavier et al,1976; Beckstead, Domesick and Nauta,1979) and this thalamic nucleus is also believed to have a wide cortical projection (Herkenham,1978).

Stimulation of the nigro-thalamic pathway produces monosynaptic inhibition of neurones within the ventro-medial thalamic nucleus of the rat (Deniau, Lackner and Féger,1978; MacLeod, James, Kilpatrick and Starr,1980). This, together with other electrophysiological, biochemical and behavioural studies suggests that GABA is the neurotransmitter in the nigro-thalamic pathway (Kilpatrick, Starr, Fletcher, James and MacLeod,1980; Macleod et al,1980).

The degeneration of nerve terminals within the deep and middle layers of the superior colliculus following lesions of the substantia nigra were initially attributed to the interruption of cortico-tectal fibres (Afifi and Kaelber,1965; Carpenter and Strominger,1967). With the application of horseradish peroxidase retro-grade axonal transport techniques it has become clear that the substantia nigra pars reticulata does project to the superior colliculus (Hopkins and Neissen,1976; Rinvik, Grofová and Ottersen,1976). This projection

appears to be topographical in the monkey with medial and lateral parts of the nigra projecting to medial and lateral portions of the colliculus respectively (Jayaraman, Batton and Carpenter, 1977). In the rat the neurones giving rise to the nigro-tectal projection are located ventral and ventrolateral to those originating the nigro-thalamic projection (Faull and Mehler, 1978). This arrangement is similar in the cat and monkey although some differences in detail occur (Beckstead, Edwards and Frankfurter, 1981). In the cat the nigro-tectal fibres terminate in a characteristic banding pattern running in the mediolateral plane in the caudal two-thirds of the intermediate grey layer of the superior colliculus (Graybiel, 1978). Such terminal patterns have not been described in the monkey or rat.

The predominant nigro-tectal projection in the rat is ipsilateral but electrophysiological studies have suggested the existence of a small contralateral component (Deniau, Hammond-Le Guyader, Féger and McKenzie, 1977; Chevalier, Deniau, Thierry and Féger, 1981). Electrophysiological studies have also suggested that some neurones of the substantia nigra pars reticulata project to both the superior colliculus and the thalamus in the rat (Anderson and Yoshida, 1977; Deniau, Hammond, Ritzk and Féger, 1978). This observation has been supported from neuroanatomical studies using double labelling retrograde axonal tracing techniques (Bentivoglio, van der Kooy and Kuypers, 1979). Evidence from electrophysiological, lesion and biochemical

experiments has suggested that the nigro-tectal pathway contains GABA as its neurotransmitter (Vincent, Hattori and McGeer, 1978; Chevalier, Thierry, Shibasaki and Féger, 1981; Kilpatrick, Collingridge and Starr, 1982).

Observations of experiments involving axonal transport of horseradish peroxidase and radiolabelled amino acids have demonstrated that the substantia nigra also projects to the pedunculopontine nucleus (Beckstead et al, 1979). The neurotransmitter involved in this projection has not been identified but is suggested to be GABA (Childs and Gale, 1983). The functional relationships between the pedunculopontine nucleus and the corpus striatum are unknown but this is now an area of intensive research. However, it is now recognised that the pedunculopontine nucleus may occupy an important position in the neuronal circuitry of the corpus striatum as it receives afferents from the medial pallidal segment (Jackson and Crossman, 1981), the subthalamic nucleus (Nauta and Cole, 1978) and the substantia nigra pars reticulata (Beckstead et al, 1979). This nucleus also receives afferents from other brain areas known to be involved in motor control, including the motor cortex and red nucleus (Moon-Edley and Graybiel, 1983).

Evidence has been presented suggesting the existence of a dopamine-containing nigro-spinal pathway in the rat (Commissiong, Gentleman and Neff, 1979). However, autoradiographic neuronal tracing studies have failed to confirm such a projection (Beckstead et al, 1979) but a dopamine-containing spinal projection from the A11

catecholamine cell group has been demonstrated (Björklund and Shagerberg, 1979; Hökfelt, Phillipson and Goldstein, 1979). The most caudal projection of the substantia nigra found with axonal tracing techniques appears to originate in the pars reticulata and project to the reticular formation of the midbrain, pons and medulla (Hopkins and Niessen, 1976; Rinvik et al, 1976).

Despite the vast wealth of anatomical, biochemical, pharmacological and physiological knowledge of the basal ganglia, the function of these nuclei remains a matter of conjecture and speculation. However, evidence from animal experiments and clinical observations show that these nuclei play a central role in coordination of posture and voluntary movements. Single unit recordings in various basal ganglia nuclei during conditioned behaviour in the monkey have proved to be very rewarding in understanding the functioning of the basal ganglia. In most nuclei, with the exception of the caudate nucleus and substantia nigra pars compacta, a clear correlation between neuronal discharge patterns and active movements of contralateral body parts has been found (DeLong, 1971; DeLong and Georgopoulos, 1979). These studies also found that the efferents as well as the afferents are somatotopically organised. The electrophysiological data has also provided an insight into the varying roles of some of the nuclei suggesting that the pallidum may be more related to limb movements while the substantia nigra pars reticulata may be more related to orolingual, head and axial movements. This

is based on the observation that many neurones within the internal pallidal segment are active in relation to limb movements (DeLong, 1971) whereas the nigral neurones have been found to modulate their discharge in relation to licking and chewing movements but only rarely to limb movements (Mora, Mogenson and Rolls, 1977; DeLong and Georgopoulos, 1978). In reaction-time tasks, changes in neuronal discharge of basal ganglia neurones commonly occur before a movement, but this is often after the first change in the electromyographic recording (Anderson and Horak, 1981; Georgopoulos, DeLong and Crutcher, 1983). Similar observations have been made regarding the motor cortex (Evarts, 1966) and cerebellum (Thach, 1970). In general, it appears that changes in neuronal discharge occur later in the basal ganglia than in the motor cortex. Approximately 50% of the neurones in the motor cortex were found to have their activity modulated before the earliest electromyographic changes (Evarts, 1974) in contrast to only 10-20% in the basal ganglia (Georgopoulos et al, 1983).

Many studies have been made using specific brain lesions in experimental animals to try to mimic the pathological damage associated with basal ganglia dysfunction in man. However, such lesions in experimental animals do not always produce the same deficits as seen in humans with pathological lesions of the same areas. For example, patients with atrophy of the pallidum exhibit a variety of motor deficits which include akinesia, dystonia and rigidity (Jellinger,

1968) whereas unilateral and bilateral pallidal lesions in monkeys only produce mild transient motor impairments in untrained animals (Kennard, 1944; Lausen, 1955; MacLean, 1978). Lesions and temporary cooling of the pallidum only causes the movements involved in a reaction-time task to be slowed, having no effect on the reaction time per se (Horak and Anderson, 1980; Hore and Villis, 1980). Such differences between the human symptoms and the observed animal motor deficits may be attributed to two factors, (1) the "human" lesions may not be well circumscribed and (2) there may be some species variation. In contrast, some experimental lesions in monkeys can mimic the human disorder. Lesions of the subthalamic nucleus produces hemiballismus in both man and monkey (Whittier, 1947; Carpenter, Whittier and Mettler, 1950) which can be alleviated by lesions of the medial pallidal segment, the ventral lateral nucleus of the thalamus or the motor cortex (Carpenter et al, 1950; Carpenter and Mettler, 1951; Martin and McCaul, 1959).

Much of our current understanding of the basal ganglia has been obtained by studying the movement disorders resulting from basal ganglia dysfunction. In general, lesions of the basal ganglia produce positive (e.g. Huntington's disease) or negative symptoms (e.g. Parkinson's disease) and may involve complex motor deficits. Huntington's disease was first described in 1872. It is an hereditary disease with the gene responsible being located on chromosome 4 (Harper, 1984). The disorder is fully expressed in the heterozygote. The

cardinal clinical symptoms of this disease are disordered movement, dementia and psychotic disturbances all of which usually become apparent in the fourth and fifth decades of life (Paulson, 1979). The most distinctive symptom is the movement disorder which usually starts with fleeting movements of the extremities or grimacing tics of the face, lips and eyelids. The choreic movements gradually become worse and evolve into a choreoathetotic pattern. The choreic movements have been described as being as complex and coordinated as those of a normal voluntary movement but being objectively purposeless (Wilson, 1929, cited in Penny and Young, 1983). In later stages of the disease, concomitant dystonia and symptoms of Parkinson's disease is often observed. The mean life expectancy is approximately 16 years from the initial onset of the symptoms, and the cause of death is usually due to the respiratory disturbance associated with progressive dysphagia (Shoulson and Chase, 1975). A small percentage (5-10%) of Huntington's disease patients develop muscular hypertonicity and bradykinesia rather than chorea. This rigid-hypokinetic (Westphal) variant of Huntington's disease can develop in adults but occurs most often in childhood (Dewhurst and Oliver, 1970). Patients suffering from this Westphal variant of the disease have symptoms of cerebellar ataxia, pyramidal tract signs and often mental retardation, indicating a very widespread disorder. This type of Huntington's disease usually follows a more rapid course than adult-onset

Huntington's disease.

Pathological studies of this disease have demonstrated a loss of neural elements, particularly within the striatum and frontal and occipital cortical lobes, resulting in a brain mass loss of approximately 200g (Bruyn, Bots and Dom, 1979). The neuronal loss of the striatum, which is particularly marked in the rostral portions of the putamen and caudate nucleus, predominantly involves the small (Golgi type II) neurones (Dom, Baro and Bruchner, 1973). Neurochemical studies have shown that GABA and its synthetic enzyme glutamic acid decarboxylase, together with acetylcholine and substance P are all reduced in the striatum of Huntington's diseased brains (Bird and Iversen, 1974; McGeer and McGeer, 1975, 1976; Gale, Bird and Spokes, 1978). The cortical damage occurs mainly in the third and fifth layers and may involve a volume loss of 20%, reducing the thickness of the cortex to 4mm (Bruyn et al, 1979). Other basal ganglia nuclei (globus pallidus, subthalamic nucleus, substantia nigra) and non-basal ganglia nuclei (cerebellum and related brainstem nuclei) also show neuronal loss (Bruyn et al, 1979; Jeste, Barban and Parisi, 1984).

Huntington's disease has been considered to be the mirror image of Parkinson's disease. This has been based on two observations. First, Huntington's disease is associated with increased movement whereas Parkinson's disease is associated with reduced movement and secondly, L-DOPA (L-dihydroxyphenylalanine) tends to exacerbate

involuntary movements whereas this is the treatment of choice for Parkinson's disease. Also, agents known to reduce the activity of the dopamine system such as α -methylparatyrosine, tetrabenazine and butyrophenones are modestly successful in suppressing the motor symptoms of Huntington's disease (Shoulson and Chase, 1975) whereas these agents are known to aggravate the symptoms of Parkinson's disease. GABA-mimetic drugs have been reported to have some beneficial effects in Huntington's disease and in another hyperkinetic-dystonic syndrome, hemiballismus (Becker and Lal, 1983; Gonce, Schoenen, Charlier and Delwaide, 1983; Scigliano, Giovannini, Girotte, Grassi, Careceni and Schechter, 1984).

As with other basal ganglia disorders, a major line of research into the possible cause and treatment of Huntington's disease concerns the use of an animal model of the disorder. At present the best animal model of Huntington's disease is produced in the rat by the intrastriatal administration of the neurotoxin kainic acid (Coyle and Schwarcz, 1976; McGeer, McGeer, Hattori and Vincent, 1979). This procedure produces many of the neuroanatomical and neurochemical changes associated with the disease but behavioural studies have shown that no choreic movements are induced (McGeer et al, 1979). This is a common difficulty associated with attempting to produce a human disorder in the rat. The actual mechanism whereby the various neural elements are lost in Huntington's disease remains unknown, but the ability of kainic acid to mimic the neuroanatomical and neuro-

chemical profiles observed in Huntington's disease has encouraged speculation that a kainic acid-like substance may be responsible for the disease (Coyle, London, Biziere and Zacek, 1979). An alternative suggestion is that Huntington's disease may be due to a membrane abnormality (Appel, 1979). These two views are not necessarily mutually exclusive.

Although the movement disorders of Huntington's disease have been attributed to the basal ganglia, the widespread damage to motor and non-motor areas of the brain suggest that this disease may not be the best model of dysfunctioning basal ganglia. Therefore, a great deal of interest has been taken in the hypokinetic-rigid syndrome of Parkinson's disease. This disease was first described by James Parkinson in 1817, and is characterised by three major clinical signs. These are, rigidity of the musculature in either widespread or isolated areas of the body, tremor at rest of the involved areas (in most cases), and akinesia. In addition, increased salivation and perspiration may be present which have been attributed to a relative increase in the activity of the parasympathetic division of the autonomic nervous system. The major pathological feature of Parkinson's disease is a marked depletion of the pigmented neurones of the substantia nigra and locus coeruleus (Greenfield and Bosanquet, 1953). The neurochemical deficits resulting from these lesions have been well described following the observation that the levels of dopamine within the striatum and substantia

nigra of Parkinsonian brains were lower than in controls (Ehringer and Hornykiewicz, 1960). This, together with the finding that experimental lesions of the substantia nigra produced a decrease of dopamine within the striatum, were important evidence which led to the acceptance of dopamine as a neurotransmitter in its own right (Anden, Stromborn and Svensson, 1964). Although the nigro-striatal dopamine loss is believed to be the major lesion in Parkinson's disease, there is currently evidence that other brain areas, such as the cerebral cortex, may have a relative dopamine deficiency (Scatton, Rouquier, Javoy-Agid and Agid, 1982). Other neurotransmitters are also affected in Parkinson's disease. Tyrosine hydroxylase (an enzyme involved in the synthesis of catecholamines) has been shown to be reduced not only in dopamine-rich areas such as the striatum but also in dopamine-poor areas such as the hippocampus (McGeer and McGeer, 1976). However, the hippocampus is rich in noradrenaline and the loss of tyrosine hydroxylase activity is probably due to the established loss of noradrenergic neurones of the locus coeruleus (Greenfield and Bosanquet, 1953). There is also widespread loss of glutamic acid decarboxylase activity which is especially marked in the globus pallidus, substantia nigra and subthalamic nucleus (Fahn, Libsch and Cutler, 1971; McGeer and McGeer, 1976). This may reflect a loss of striatal and pallidal output neurones as these are believed to contain GABA (Fonnum et al, 1978; Nagy et al, 1978). The neurotransmitter 5-hydroxytryptamine has been shown

to be reduced in the midbrain raphe nuclei, thalamus, striatum and pallidum (Fahn et al, 1971) as have peptides such as substance P (Mau Jorge, Javoy-Agid, LeGrand, Agid and Casselin, 1983) and cholecystokinin-8 (Studler, Javoy-Agid, Cesselin, LeGrand and Agid, 1982) in the substantia nigra. It is not known whether these non-dopamine neurochemical losses are a result of the nigro-striatal pathway lesion or are due to concurrent processes of the disease.

With the discovery that dopamine is reduced in the striatum of Parkinsonian brains (Ehringer and Hornykiewicz, 1960) it was suggested that dopamine replacement therapy might be beneficial. The most effective drug was found to be the immediate precursor of dopamine, L-DOPA (Cotzias, van Woert and Schiffer, 1967) and this has become the mainstay of Parkinsonian treatment ever since. Before the advent of L-DOPA, stereotaxic lesions of the ventrolateral thalamic nucleus were commonly used to relieve some Parkinsonian symptoms, particularly the resting tremor (Siegfried, 1980). Following the introduction of L-DOPA this practice declined. However, it is now realised that tremor is the most resistant symptom of Parkinson's disease to L-DOPA (Kelly and Gillingham, 1980) whereas this symptom is the most responsive to surgery (Laitinen, 1972). This, together with the observation that L-DOPA becomes less effective with time (Papassailiou, Cotzias and Duby, 1972) has led to the suggestion that a combination of surgery and L-DOPA therapy may be the best treatment of certain

forms of Parkinson's disease (Laitinen,1972; Gildenberg, 1984).

In contrast to Huntington's chorea, the aetiology of Parkinson's disease remains unknown. The possibility of a genetic factor has often been suggested (Mjones, 1949; Barbeau and Pourcher,1982) but it is now realised that what is termed Parkinson's disease embraces a group of disorders which are not readily distinguished by clinical, pathological or biochemical means. This realisation has led to the conclusion that the majority of Parkinsonian cases have no crucial genetic factors (Calne, Duvoisin and McGeer,1984). A second hypothesis of the cause of Parkinson's disease, which is currently receiving a great deal of interest, concerns the involvement of toxins or toxic products. The neurotoxin 6-hydroxydopamine is known to cause degeneration of catecholamine-containing neurones and its mechanism of action may involve the formation of free radicals (Heikkila and Cohen,1971). It has been suggested that the degeneration of the substantia nigra in Parkinson's disease may be caused by free radicals, as a deficiency of glutathione (a compound known to be involved in the elimination of free radicals) has been observed in the substantia nigra of Parkinsonian brains (Perry, Godin and Hansen,1982). However, the major reason for the recent interest in toxins is the discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can produce a Parkinsonian syndrome in man (Davis, Williams, Markey, Ebert, Caine, Reichert and Kopin,1979; Langston,

Ballard, Tetrad and Irwin,1983). It is reasoned that an endogenous or exogenous MPTP-like compound may be responsible for at least some cases of Parkinson's disease.

The compound MPTP has been heralded as a major breakthrough in the research into Parkinson's disease and the functioning of the corpus striatum in general, as this compound also produces a Parkinsonian syndrome in monkeys (Burns, Chiueh, Markey, Ebert, Jacobowitz and Kopin,1983; Langston, Forno, Rebert and Irwin,1984). Curiously, MPTP has no such effect in rodents (Kolata, 1983) with only slight, temporary changes in dopamine turnover being observed in rats and mice (Markstein and Lahaye,1984; Hallman, Lange, Olson, Stromberg and Jonsson,1985). This emphasises once again the difficulties in finding animal models which faithfully mimic human disorders.

From animal studies and clinical observations of basal ganglia dysfunction several hypotheses have been advanced concerning the functioning of the basal ganglia, and in particular the striatum. From early studies it became accepted that the basal ganglia served more or less as a link or funnel from association areas to motor cortex (Evarts and Thach,1969; Kemp and Powell,1970). However, in the light of current knowledge of the highly topographic neuronal interconnections of the cortex, basal ganglia and thalamus, this has had to be reviewed. It now appears that the cortical inputs to the basal ganglia (particularly the striatum) from the sensori-

motor and premotor cortical areas are segregated from those arising in the association areas (DeLong and Georgopoulos, 1981; Evarts and Wise, 1984). There appears to be little convergence of these pathways within the basal ganglia, but how much may occur within the thalamus or cortex is, at present, unknown. In support of this segregation, the caudate nucleus and putamen have been suggested to be involved in cognitive and motor activities respectively (Öberg and Divac, 1979). Additional supporting evidence has been obtained from experiments in rats which have shown that striatal lesions lead to learning deficits (Divac, Markowitsch and Pritzel, 1978) and also from electrophysiological recordings in the primate where few movement-related neuronal discharge patterns have been found in the caudate nucleus (DeLong and Georgopoulos, 1979). There is neurochemical evidence that the striatum may not be homogeneously organised with the demonstration of "striosomes" with acetylcholinesterase histochemistry (Graybiel and Ragsdale, 1978). An increasingly complex picture has been built up with further striosomal patterns being found with other histochemical techniques (Graybiel, Ragsdale, Yoneoka, and Elde, 1981; Graybiel, Chesselet, Wu, Eckenstein and Joh, 1983). It has been suggested that these striosomes may represent specialised channels within the basal ganglia circuitry (Graybiel, 1984).

A common hypothesis concerning the function of the striatum suggests that it can select and maintain motor,

and possibly non-motor, behaviours (Cools, 1980; Groves, 1983; Penny and Young, 1983). It is suggested that the recently described lateral inhibition of striatal output neurones is the means by which this selection can occur (Park, Lighthall and Kitai, 1980; Somogyi, Bolam and Smith, 1981). In fact Groves (1983) has taken this a step further with hypothesised "spiny I cell matrix" and "spiny II cell clusters". It may be just possible that the spiny II cell clusters may correspond to the striosomes of Graybiel (1984). However, although neuro-anatomical and histochemical studies of the basal ganglia have produced very complex and technically highly advanced morphological and biochemical maps, our understanding of the function controlled by the basal ganglia still remains primitive.

1.4 LIMB RIGIDITY AND TREMORINE

There have been few studies on muscle rigidity in experimental animals. This is probably due to the lack of a suitable animal model of Parkinson's disease and other motor disorders involving muscle hypertonia. The studies which have been performed in rodents have been predominantly on drug-induced rigidity, produced by compounds which reduce dopamine activity, enhance acetylcholine activity or stimulate opiate receptors. Early studies often used subjective ranking of rigidity or measured the duration of catalepsy produced by drugs such as haloperidol, a dopamine receptor antagonist, and arecoline, a centrally-acting muscarinic cholinomimetic (Costall and Olley, 1971a). Other studies have

assessed the "bracing" rigidity, determined by measuring the distance the animal could be pushed along before it made a stepping movement (Schallert, DeRyck, Wishaw, Ramirez and Teitelbaum, 1979). More quantitative methods include recording electromyographic changes during drug treatment (Wand, Kuschinsky and Sontag, 1973) and mechanographic recording methods (Dickinson, Longman and Slater, 1982; Johnels and Steg, 1982). The latter methods are generally accepted to give the most reliable and convenient quantitative index of drug-induced muscle rigidity in conscious animals (Johnels and Steg, 1982). The mechanographic method in use in this laboratory (Dickinson et al, 1982) was found to be unsuitable to measure rat hindlimb rigidity induced by tremorine due to the concurrent production of tremor (see section 2.1). Therefore another limb tone measuring apparatus was constructed which was less sensitive to the tremor induced by tremorine.

Of the compounds which can produce muscular rigidity in rats, reserpine, morphine, arecoline and tremorine have been studied in most detail, and it has been shown that the effects of all these drugs can be modified by neurochemical manipulations or lesions of the basal ganglia. Reserpine-induced rigidity has been shown to be prevented by striatal lesions (Arvidsson, Jurna and Steg, 1967) and reversed by the intrastriatal administration of the dopamine receptor agonist, apomorphine (Andén and Johnels, 1977). Unilateral intrastriatal administration of reserpine produces rigidity which is most marked

in the contralateral hindlimb (Johnels, 1983). All this evidence supports the hypothesis that reserpine-induced rigidity is caused by the depletion of dopamine within the striatum. Morphine, an agonist at the opiate receptor (particularly at the μ subtype) produces a state termed "catatonia", which is a combination of muscular rigidity and catalepsy (Havemann, Winkler, Genç and Kuschinsky, 1981). It has been demonstrated that modulation of neuronal activity in the striatum and the substantia nigra is intimately involved in the expression of this condition as striatal lesions abolish the muscular rigidity (Havemann et al, 1981) and rigidity can be elicited by application of morphine to the pars reticulata but not the pars compacta of the substantia nigra (Turski, Havemann and Kuschinsky, 1982). In addition, intrastriatal administration of morphine produces rigidity (Havemann, Winkler and Kuschinsky, 1980). The mechanism by which morphine causes rigidity is unclear but it has been suggested that morphine may act on presynaptic inhibitory receptors, thereby reducing the release of neurotransmitters from corticostriatal or nigro-striatal fibres (Turski et al, 1982).

Muscular rigidity produced by the centrally-acting muscarinic cholinomimetic, tremorine, is the subject of this study. Tremorine (1,4-dipyrrolidino-2-butyne) was introduced in 1956 (Everett, Blockus and Shepperd) as a potent tremorogenic agent. Further studies indicated that tremorine was in fact a pro-drug, being converted to an active metabolite in the liver (Welch

and Kocsis,1961). This metabolite was identified as oxotremorine (1- (2-oxopyrrolidino) -4-pyrrolidino-2-butyne) by Cho, Haslett and Jenden (1961). Systemic administration of tremorine induces rigidity, tremor and akinesia in a variety of species from rats to monkeys and its effects can be antagonised by anti-Parkinsonian drugs (Everett et al,1956; Everett, Morse and Borcharding,1971; Korczyn and Eshel,1979). These findings led to the use of tremorine in experimental screening procedures for drugs with potential therapeutic value for extrapyramidal motor disorders (Jenden,1968).

The mechanism by which tremorine produces its motor effects has been the subject of research for many years. Early studies demonstrated that the levels of acetylcholine were increased in some brain areas following the administration of oxotremorine (Holmstedt,1967). This effect was attributed to the stimulation of inhibitory presynaptic muscarinic receptors by oxotremorine, thereby decreasing acetylcholine release from nerve terminals (Jenden,1968; Cox and Hecker,1971; Ringdahl and Jenden,1983). However, pharmacological studies of pre- and post-synaptic muscarinic receptors have found little (Kilbinger and Wessler,1980) or no difference (Bowen and Marek,1982) in the affinity of oxotremorine for these receptors. Oxotremorine has been reported to have a direct effect at the neuromuscular junction (Elmqvist and McIsaac,1967; Ganguly and Chaudhuri,1970). These studies demonstrated that oxotremorine could cause a contracture of both innervated (Ganguly and Chaudhuri,

1970) and denervated (Elmqvist and McIsaac, 1967) rat hemidiaphragm preparations. Ganguly and Chaudhuri (1970) also reported that a high concentration of oxotremorine in the bathing solution (10 µg/ml) could cause a blockade, similar to a depolarising blockade on the same preparation, and that the effects of oxotremorine were enhanced by the cholinesterase inhibitor, physostigmine. The same authors also described similar effects on the cat sciatic nerve - tibialis anterior muscle preparation but not on the frog rectus abdominis preparation. In contrast, tremorine per se had no effect and the authors suggest that the actions of oxotremorine may be via the release of acetylcholine from the neuromuscular junction. This suggestion does not explain the results obtained with a denervated nerve-muscle preparation (Elmqvist and McIsaac, 1967) so the possibility remains that oxotremorine may be able to stimulate skeletal muscle directly.

Despite the controversy over the relative importance of pre- and postsynaptic muscarinic receptors in the mechanism of action of oxotremorine at the neuromuscular junction, there is little doubt that the major motor effects of oxotremorine are mediated by the stimulation of central muscarinic cholinceptors (Ringdahl and Jenden, 1983). This is supported by the fact that, in intact animals, pretreatment with atropine or scopolamine can prevent all the observable effects of oxotremorine whereas quaternary atropine prevents only the peripheral parasympathomimetic effects

(Cho and Jenden, 1964; Jenden, 1968). Tremorine and oxotremorine have two other central actions which are not related to motor control, an antinociceptive action (Pleuvry and Tobias, 1971) and a hypothermic action (Doggett and O'Farrell, 1976), both of which are mediated via central muscarinic cholinceptor stimulation.

The involvement of supraspinal motor areas in the actions of cholinomimetic compounds have been evaluated in several ways. Transection of the spinal cord, brainstem and midbrain were all found to abolish tremorine-induced tremor in the cat (Kaelber and Hamel, 1960) but tremor remains after decerebration in rats, mice and rabbits (Everett et al, 1956). One way of indicating which brain areas might be involved is from the determination of local cerebral glucose utilisation (Sokoloff, Reivich, Kennedy, Des Rosiers, Patlak, Pettigrew, Sakurada and Shinohara, 1977). Such a study has revealed that many motor areas may be involved in the effects produced by oxotremorine (Dow-Edwards, Dam, Peterson, Rapoport and London, 1981). The greatest changes in glucose utilisation were observed in sensorimotor cortex, globus pallidus, subthalamic nucleus, striatum, substantia nigra pars compacta and ventrolateral thalamic nucleus, and the cerebellar vermis, fastigial nucleus, interpositus nucleus and red nucleus. This study indicates that the motor cortex, the basal ganglia, the cerebellum or some combination of the three may be responsible for the motor effects elicited by oxotremorine.

Lesion studies of the basal ganglia have produced variable and controversial results. Such studies have been concerned with the rigidity and catalepsy produced following the administration of tremorine and arecoline respectively. It has been reported that a unilateral lesion of the striatum abolishes the tremorine-induced rigidity of both the ipsilateral and contralateral hindlimbs of the rat (Dickinson and Slater, 1982), whereas a unilateral lesion of the substantia nigra has no effect on the tremorine-induced rigidity but decreases the resting tone of the ipsilateral hindlimb (Slater and Dickinson, 1983). In contrast, Costall and Olley (1971b) have found that lesions of the striatum increase the duration of arecoline-induced catalepsy and also found that bilateral lesions of the substantia nigra reduce the duration of arecoline-induced catalepsy (Costall and Olley, 1971a). These results may indicate that the mechanisms underlying rigidity and catalepsy may be different. However, a study on the intranigral administration of muscarinic cholinomimetics and scopolamine suggests that this is not so (DeMontis, Olinas, Serra, Tagliamonte and Scheel-Krüger, 1979). This study reports that the bilateral intranigral microinjection of scopolamine abolishes the rigid catalepsy and tremor produced by the systemic administration of arecoline and also that the intranigral microinjection of carbachol mimics the effects of systemic arecoline.

In the light of the confused picture concerning the involvement of basal ganglia nuclei in the motor effects

produced by centrally-acting muscarinic cholinomimetics, this study was undertaken to re-evaluate the role of the basal ganglia in tremorine-induced rigidity in the rat.

SECTION TWO : MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (Manchester University colony) weighing 170 to 190g were used in these studies. All animals were caged individually from day one of the experiment. They were housed in a room maintained at $21 \pm 3^{\circ}\text{C}$ with a normal light-dark cycle; lights on from 08.00 to 20.00h. Food and water were available ad libitum.

Drugs. The drugs and compounds used in these studies are shown in Table 1. All were made up in sterile 0.9% saline unless otherwise stated in the text. The quantities and concentrations quoted refer to the salt as shown in Table 1. Throughout this thesis atropine methylnitrate has been abbreviated to atropine MN whilst atropine sulphate is referred to as atropine.

2.1 LIMB TONE STUDIES

As previously mentioned in section 1.4, mechano-graphical techniques are considered to be the most reliable and convenient methods to measure drug-induced muscle rigidity in conscious animals (Johnels and Steg, 1982). One such method used in this laboratory to measure the hindlimb rigidity of the rat involves the measurement of air pressure within a closed system which varies in direct proportion to the hindlimb rigidity (Dickinson et al, 1982). However, this was found to be unsuitable for the measurement of the rigidity induced by tremorine because the system was so sensitive that the concurrently induced tremor was also recorded. This made the interpretation of the records obtained very difficult. Therefore another rigidity

Table 1. Drugs and compounds used in the investigation

Compound	Supplier
3-Acetylpyridine	Sigma
Apomorphine hydrochloride	Sigma
Atropine methyl nitrate	Sigma
Atropine sulphate	Sigma
‡ Baclofen (Lioresal)	Ciba-Geigy
Bicuculline methiodide	Pierce
Carbachol	BDH
Desipramine hydrochloride (Pertofran)	Ciba-Geigy
Haloperidol (Seranace)	Searle
Harmaline hydrochloride	Sigma
6-Hydroxydopamine hydrobromide	Aldrich
Ibotenic acid	CRB
Isoguvacine	CRB
Kainic acid	Sigma
Lignocaine hydrochloride (Xylocaine)	Astra
Methohexitone (Brietal)	Lilly
Methysergide bimaleate	Sandoz
Morphine sulphate	MacFarlan Smith
Muscimol	Fluka
Naltrexone hydrochloride	Endo
Neostigmine bromide	K and K
Nicotinamide	Sigma
Oxotremorine	Sigma
Picrotoxin	Sigma
Procaine hydrochloride	Sigma
Temazepam (Normison)	Wyeth
Tremorine hydrochloride	Sigma
Urethane	Sigma

measuring apparatus was constructed which was less sensitive to tremor.

2.1.1 Construction of the limb tone measuring apparatus

The apparatus is shown in Fig. 1. It had three major components: an animal restraining cage; a pivoted strain sensitive device; and an electric motor; all held within a steel frame. The general principle of the method was that the electric motor pushed the central strain sensitive section which in turn caused the hindlimbs of a rat to flex. The force required to cause the flexion was measured by the strain sensitive gauges attached to the pivoted section. The strain sensitive section was constructed from 3 strips of aluminium alloy, 3mm thick and 15mm wide. Two strips were formed into U-shapes and the third was used to join them together. Perspex extensions were attached to the front of each U-shaped aluminium strip as shown in Figs. 1 and 2. Two foil strain gauges (R.S.Components) were glued onto the front of each aluminium strip just above the perspex extension. The strain gauges consisted of accurately made grids of a copper nickel alloy (with a temperature coefficient similar to that of the aluminium alloy), the electrical resistance of which changed when they were physically deformed. Thus, when the aluminium strips encountered a resistance, they became slightly strained or deformed and this caused a change in the electrical resistances of the strain gauges that was proportional to the strain. Each pair of strain gauges

Fig 1. Schematic diagram of the apparatus for recording hindlimb tone in the rat. The electric motor drove a cam back and forth against the strain-sensitive element which was pivoted such that the perspex extensions flexed and extended the hindlimbs of a rat held in the restraining cage.

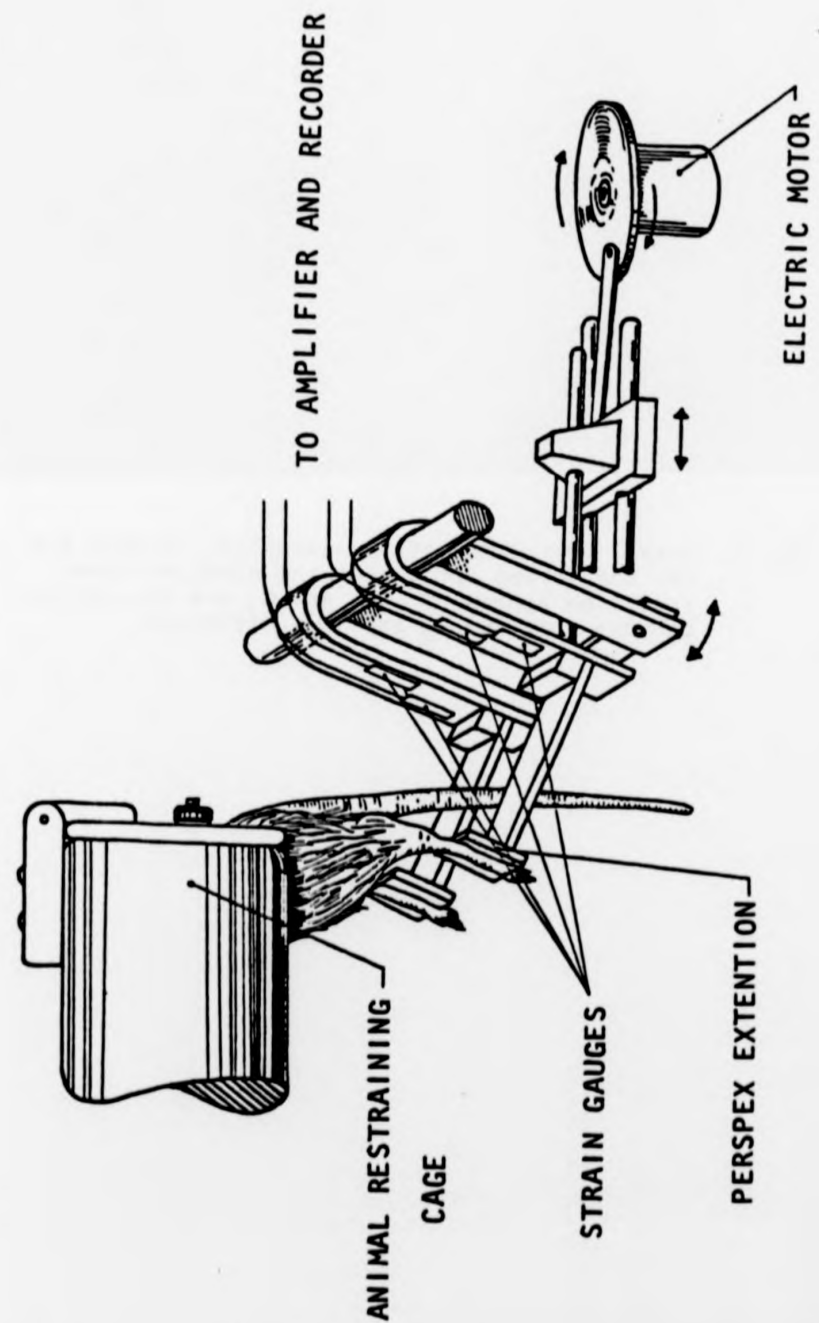
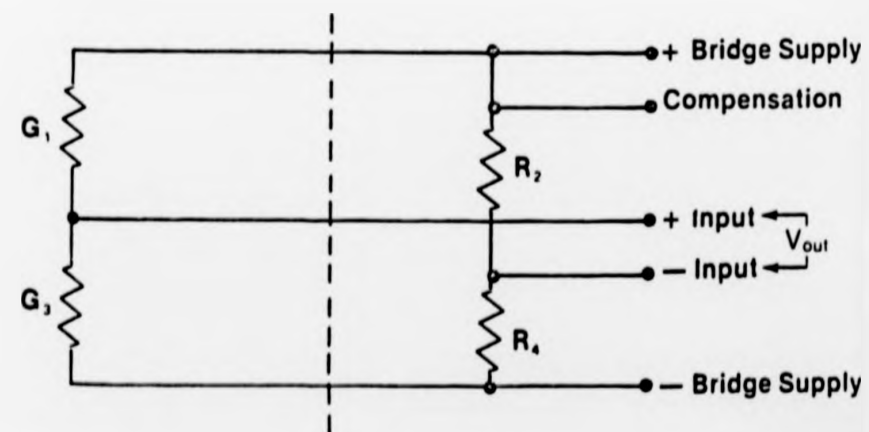
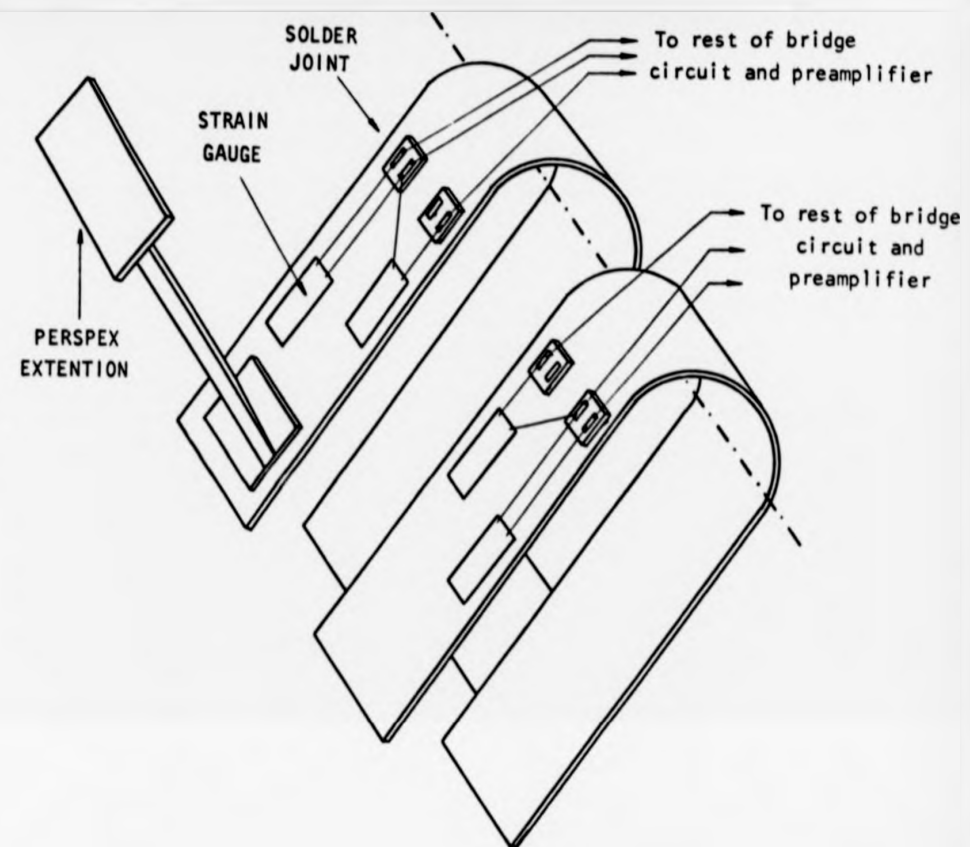


Fig 2. Detail of (a) the strain-sensitive element and (b) the wiring circuit of the hindlimb tone recording apparatus. G_1 and G_2 are the strain gauges; R_2 and R_4 are $1K\Omega$ resistors.



were incorporated in a bridge circuit with two $1K\Omega 0.1\%$ wire wound resistors (R.S.Components) as shown in Fig.2. The bridge circuits were supplied by the 12V bridge power supply of a Grass 7P1B amplifier and the return signal was amplified and displayed on a Grass pen oscillograph via a 7DA amplifier.

The electric motor drove a cam that pushed against the back of the strain sensitive component. This was pivoted such that the perspex extensions moved almost vertically up and down for each forward and backward movement of the cam. The whole cycle had a frequency of 0.5Hz and the vertical displacement of the perspex extensions was 13mm. When a rat was held in the restraining cage with its hindlimbs lightly attached to the perspex extensions the movement caused the hindlimbs to flex and extend. During the flexion the paws of the rat were pushed upwards towards its body causing flexion at all three major joints of each hindlimb. The extension of the hindlimbs was a passive event under the weight of the pivoted section (a force of approximately 20g) because the cam of the electric motor was not attached to the strain sensitive section. This mechanical isolation helped to protect the motor from sudden movements of the rat.

The restraining cage consisted of a metal and plastic cylinder (length 20cm, diameter 7cm). Two slots 15mm wide, 47mm long and 15mm apart were made in the bottom of the back of the restraining cage to allow the hindlimbs of a rat to protrude. These permitted the

legs of the rat to be flexed and extended freely. A series of holes (4mm in diameter) in the bottom of the cage permitted the intraperitoneal administration of drugs to an animal in the cage. To accommodate rats of varying size, the effective height of the restraining cage could be reduced by lowering a piece of perspex held in the top of the cage. Access to the head of the rat, to permit intracerebral administration of drugs, could be gained through a removable top section of the cage.

The apparatus was calibrated for each experiment by hanging weights from each perspex extension. The weights were placed in a lightweight sling which was suspended (via a pulley wheel) from a small hook attached to the perspex extension in such a way that the weights exerted a force in the same plane as the hindlimbs during the experiments. The deflections of the pen recorder caused by a series of weights (10 - 90g) were used to produce a calibration curve by linear regression. Each side of the apparatus was calibrated and treated independently.

2.1.2 Evaluation of the limb tone measuring apparatus

Initial experiments were conducted to (1) determine how consistently the apparatus responded with time, and (2) ensure that the apparatus was sufficiently sensitive to record changes in the resistance to flexion of the hindlimbs of rats under the influence of pharmacological agents known to affect muscle tone.

Responsiveness with time. A period of 30min. was

invariably allowed between switching on the apparatus and pen recorder and the start of any experiment to allow the electrical equipment to stabilise. The responsiveness of the apparatus was followed for 3h by carrying out a calibration procedure (described in section 2.1.1) every 30min on each side of the apparatus. A slow pen recorder chart speed was selected so that any drift in the baseline position could be readily observed.

Drug-induced changes in hindlimb tone. Morphine is known to produce catalepsy with an accompanying muscular rigidity (Wand et al, 1973), whereas general anaesthesia is known to produce a decrease in muscle tone (Bowman and Rand, 1980). A submaximal dose of morphine (15mg/Kg i.p. - see Wand et al, 1973) was chosen to produce an increase in muscle tone. The short acting barbiturate anaesthetic methohexitone (40mg/Kg i.p.) was used to cause muscle relaxation coincident with anaesthesia. The same group of rats was used for both experiments (with several days interval between) and the experiments were carried out as described in section 2.1.6.

2.1.3 Surgical procedures

Standard stereotaxic procedures were used to accurately place intracerebral cannulae and electrodes within the rat brain. The method used was in accordance with the stereotaxic atlas of König and Klippel (1963) and all stereotaxic coordinates quoted are based upon this atlas. Each animal was anaesthetised with sodium

methohexitone (50mg/Kg i.p.) with additional anaesthetic being administered as required throughout the course of the surgery. The fur over the dorsal surface of the head was trimmed away and the animal was placed in a stereotaxic frame (Narishige) which was set up according to the method of König and Klippel (1963). The body temperature of the animal was maintained throughout by supporting it on a heated table. Ethanol (70%) sterilisation of the instruments together with the prophylactic use of antibiotics were routinely used to guard against infection. The dorsal surface of the head of the animal was swabbed with ethanol before exposing the surface of the cranium with an antero-posterior incision. The overlying tissues were displaced laterally and the surface of the skull was cleaned and dried. A 3mm diameter hole was drilled immediately above the target area and the underlying dura mata was pierced with a sterile hypodermic needle.

Lesions. Discrete lesions of brain areas were made either by electrocoagulation or by the microinjection of neurotoxic compounds. Electrocoagulation lesions were performed with a monopolar tungsten electrode insulated to within 0.5mm of the tip with epoxy resin (overall diameter approximately 1mm). The electrode was lowered through a previously drilled hole in the skull to the calculated stereotaxic coordinates of the target area using a micromanipulator (Narishige). The lesion was made by passing an anodal DC current through the electrode. The electrical current was generated by

a stimulator (Grass, S8) connected in series with a stimulus isolation unit (Grass, SIU 478A), a constant current unit (Grass, CCU1) and the electrode. The return current passed through the ear bars of the stereotaxic frame. Lesion currents of 2mA with a duration of 10s were typically used. After the lesion had been made, the electrode was removed and the hole in the skull was plugged with sterile bone wax (Ethicon, W810).

Chemical lesions were made with the neurotoxic compounds kainic acid, ibotenic acid and 6-hydroxydopamine. Kainic acid was dissolved in sterile 0.9% saline and adjusted to pH7 by the addition of sodium hydroxide solution. Ibotenic acid was dissolved in a phosphate buffer solution with a resulting pH of 7. 6-Hydroxydopamine was dissolved in sterile 0.9% saline containing the antioxidant ascorbic acid (0.5mg/ml). Animals receiving 6-hydroxydopamine lesions were pretreated with the noradrenaline uptake inhibitor desipramine (20mg/Kg i.p.) 20min prior to the surgery. This procedure is known to protect the noradrenergic neurones from the actions of 6-hydroxydopamine and therefore make the lesion more selective for the dopaminergic neurones (Tassin, Thierry, Blanc and Glowinski, 1974). Intracerebral injections of the neurotoxic compounds were made through a 28 gauge stainless steel cannula approximately 4cm long placed in the required stereotaxic coordinates with a micro-manipulator. One end of the cannula was connected to a

syringe (Hamilton, 10 μ l) with a small length of flexible, rigid walled, transparent tube (Portex, 800/100/120). The compounds were injected at the rate of 0.5 μ l/min. The passage of the drug solution was monitored by introducing a small air bubble into the plastic tube and observing its movement during the injection. The cannula was held in place for a further 3min following the completion of the injection to allow the compound to diffuse away from the cannula tip. The cannula was removed and the hole in the skull was plugged with sterile bone wax.

Animals lesioned with kainic acid were treated with a benzodiazepin (Temazepam, 0.5mg/Kg i.p.) 2.5h after the injection of kainic acid. This is because kainic acid is known to produce lesions both at its site of application and at more distant sites (Krammer, 1980) and it has been reported that the administration of a benzodiazepin can reduce the distant site lesions (Ben-Ari, Tremblay, Ottersen and Naquet, 1979). The benzodiazepin also reduced some of the excitatory behaviour induced by the kainic acid (see 3.3.3).

Intracerebral cannulae. For pharmacological manipulations of neurotransmitters in selected brain regions, accurately placed, fine bore cannulae are required. Those employed in this study were made in two parts; - a chronically implanted, stereotaxically placed guide cannula, and a removable injection cannula. The guide cannulae were made from 23 gauge stainless steel tube (Stainless Tube and Needle Co.Ltd.). They were 12mm

long and had small plastic cuffs glued to one end. The outer surface of the guide cannulae was roughened with a needle file, which together with the plastic cuffs, formed a key for the dental acrylic cement used to hold the cannulae permanently in place. The injection cannulae were made from 31 gauge stainless steel tube and had a length of 23 gauge stainless steel tube glued to one end. This ensured that each time the injection cannulae were inserted into the guide cannulae they always extended 2mm beyond the ends of the guide cannulae. A small length of flexible, rigid walled, transparent tube (Portex, 800/100/120) was attached to each injection cannula using cyanoacrylate adhesive so that a syringe (Hamilton, 10 μ l) could be connected to the cannula for the microinjection of drugs.

Prior to the implantation of a cannula, the assembled guide and injection cannulae were secured in a specially constructed holder attached to a micromanipulator. The tip of the injection cannula was zeroed with respect to the ear bars and then removed so that only the guide cannula was implanted. Three holes were drilled in the skull adjacent to the target area and nickel plated screws 4-5mm in length (K.R.Whiston Ltd., 10BA) were inserted. The heads of the screws remained 2-3mm above the surface of the skull. The guide cannula was lowered to the required stereotaxic coordinates and dental acrylic cement (Simplex rapid; Howmedica) was used to anchor the cannula to two of the screws. When the cement had dried (3-5min) a second guide cannula was implanted on the other side of the

brain. The micromanipulator and cannula holder were removed and more cement was used to entirely cover the screws and surround both cannulae. A stylet made from a short L-shaped length of 31 gauge stainless steel tube was inserted into each guide cannula. These were made so that when they were inserted they ended flush with the ends of the guide cannulae. The stylets helped to maintain the patency of the implanted guide cannulae.

Following each of the above procedures, the wound was treated with sulphanilamide powder (May and Baker) and the skin was sutured together with sterile surgical suture (Ethicon, W102). The animal was removed from the stereotaxic frame, given an identifying pen mark on the tail and was kept on a heated table until it had recovered from the anaesthetic. Chlortetracycline (Aureomycin; Cynamid) was added to the drinking water for 5 days after the surgery, and a further 5 days of recovery were usually allowed before the animals were used in an experiment.

2.1.4 3-Acetylpyridine-induced lesions

It is well established that 3-acetylpyridine causes lesions within motor and non-motor structures of the rat brain when administered systemically (Hicks, 1955; Balaban, 1985). It has been reported that the administration of 3-acetylpyridine together with harmaline and nicotinamide restricts the central lesion to the inferior olive (Llinas et al, 1975). This latter method was used in this study to produce lesions

of the inferior olive. All three substances were administered systemically in volumes of 1ml/Kg. 3-Acetylpyridine (75mg/Kg i.p.) was administered at time (T) = 0, harmaline (15mg/Kg i.p.) at T = 3.5h, and nicotinamide (300mg/Kg i.p.) at T = 4.5h. The effect of this treatment on the motor behaviour of the rats was observed for 15 days. The extent of the lesions were evaluated from histological sections stained according to the method of Fink and Heimer (1967) - see section 2.3.

2.1.5 Turning behaviour

The neural basis of turning, or circling, behaviour has been attributed largely to the basal ganglia since Ferrier (1873 - in Pycock, 1980) caused contralateral turning behaviour in the dog by unilateral stimulation of the corpus striatum. Current experimental evidence indicates that the balance of striatal dopamine on the two sides of the brain is a major determinant of circling behaviour (Pycock, 1980). Such evidence has been obtained from experiments involving basal ganglia lesions and dopamine receptor agonists. Thus, when apomorphine, a dopamine receptor agonist, was administered to rats with unilateral electrocoagulation lesions of the striatum, ipsiversive turning behaviour was produced (Costall, Marsden, Naylor and Pycock, 1976). This behaviour was attributed to the asymmetric stimulation of brain dopamine receptors due to the preponderance of dopamine receptors in the striatum on the unlesioned side of the brain. Also,

when apomorphine was administered to rats with unilateral 6-hydroxydopamine lesions of the nigro-striatal pathway, contraversive turning behaviour was produced (Costall et al, 1976). This was because the 6-hydroxydopamine lesion produced a supersensitivity to dopamine in the striatum of the lesioned side of the brain and therefore once again produced an asymmetric stimulation of the two striata (Ungerstedt, 1971). Therefore, in the present study, turning behaviour induced by apomorphine was used as evidence of a unilateral lesion in the striatum or nigrostriatal pathway. Additional evidence was obtained from histological examination of the brains.

Each animal evaluated for turning behaviour received an injection of apomorphine (1mg/Kg i.p.) dissolved in sterile 0.9% saline containing a small amount of the antioxidant sodium metabisulphite. They were placed in an empty cage (40 x 25cm) and the direction and rate of turning behaviour were recorded for 30min.

2.1.6 Limb tone method

All the animals used for observations on limb tone were first acclimatised to the experimental procedure by being held in the apparatus for at least 30min two days prior to the start of the experimental studies. On subsequent occasions, an increase in limb tone was produced by a standard dose of either tremorine (20mg/Kg i.p.) or morphine (15mg/Kg i.p.). Peripheral stimulation of muscarinic receptors by the

active metabolite of tremorine (oxotremorine) was prevented by pretreating the rats with atropine MN (1mg/Kg i.p.). From preliminary experiments (see section 3.2) it was decided that the increase in hindlimb tone produced by the second administration was to be used as the control response. Central microinjections were performed while each animal was hand-restrained. The stylet was removed from the implanted guide cannula, the injection cannula was inserted, and the microinjection was made over a period of 30s. The passage of the drug solution was monitored by observing the movement of a small air bubble introduced into the plastic tube connected to the injection cannula. The injection cannula was left in place for a further 30s to allow the drug to diffuse away from the cannula tip. Bilateral injections of equal volumes were always made and the stylets were replaced into the guide cannulae after the completion of the microinjections. Where unilateral drug administrations were carried out (with an equal volume of vehicle applied to the contralateral side) they were randomised within the group so that half had the treatment to the right hand side and the other half to the left hand side. This was to prevent any bias within the recording apparatus having any effect on the results. The animal was placed in the restraining cage and transferred to the limb tone recording apparatus. Each hindlimb was lightly attached to the perspex extensions of the apparatus with adhesive strapping. The relative positions of the

restraining cage and the perspex extensions were adjusted so that movement of the apparatus produced a consistent unimpeded flexion and extension movement of the hindlimbs. The motor of the apparatus was switched on and the limb resistance was recorded for 10min before the administration of tremorine or morphine. These agents were administered, without removing the rat from the apparatus, by making the injection through a hole in the bottom of the restraining cage. Limb tone was monitored for a further 30min, during which time the position of the rat within the restraining cage was frequently checked.

A similar procedure was adopted to measure the effect of intracerebral administration of cholinomimetic agents on hindlimb tone. In these experiments the animals were not pretreated with atropine MN. The central microinjections were performed before the animal was placed in the restraining cage and the effect of the treatment on hindlimb tone was followed for 30min. No tremorine or morphine was used in these studies.

At the end of the recording period, the rat was removed from the apparatus and returned to its home cage. At least 48h were allowed to elapse before any further experimental procedures were performed on the same animal. Control and test responses were obtained over a period of eight days to reduce the possible effect of weight gain on the recorded limb tone. The total number of central microinjections was restricted

to a maximum of five per animal. At the conclusion of the experimental observations the rats were sacrificed and all the lesion and cannula sites were examined macroscopically or microscopically as described in section 2.3.

2.2 ELECTROPHYSIOLOGICAL STUDIES

2.2.1 Surgical procedures

Each animal was anaesthetised with urethane (1.3 - 1.5g/Kg i.p.) using a 25% solution. The urethane was administered as a split dose, two-thirds immediately and the remainder as required 10min later. This procedure produced a reproducible and controlled level of anaesthesia. Throughout the experiment the body temperature of the rat was maintained at 37°C with a heated blanket. A small diameter flexible cannula (Portex, 100/800/100) was inserted into a lateral tail vein to allow intravenous administration of drugs. Initially, local venous dilation was achieved by immersing the tail in warm water (approximately 35°C) for 30s. An incision, 1.5 - 2cm long, was made over and following the course of a lateral tail vein. The exposed tissues were bathed with a local anaesthetic (lignocaine, 2%) while the vein was dissected away from its connecting tissues over approximately 10mm of its length. The local anaesthetic helped minimise the venoconstriction resulting from the surgical trauma. The peripheral end of the vessel was occluded with a cotton tie while a second cotton thread was loosely looped around the

central portion. The vein was supported on an aneurysm needle while the cannula (completely filled with sterile 0.9% saline) was inserted through a hole previously made with a fine pair of iris scissors. The cannula was held in place by two cotton threads around the vessel and a piece of adhesive strapping around the tail.

A short antero-posterior incision was made in the skin overlying the trachea. The overlying tissues were displaced laterally to expose the trachea which was dissected free of its connecting tissues over approximately 2cm of its length. A short, rigid walled cannula (Portex, 100/800/460) was inserted into the trachea through a hole previously made with a fine pair of scissors and was held in place with a cotton thread. The wound was closed with a single cotton suture. The fur over the dorsal surface of the head was trimmed and the rat was mounted in a stereotaxic frame (L.P.C.) set up according to the method of König and Kippel (1963). The dorsal surface of the skull was exposed, cleaned of connective tissue and dried before a hole (approximately 3 x 4mm) was made with a dental drill immediately above the required recording site. The broken edge of the bone was plugged with bone wax to prevent bleeding and a small well was constructed around the exposed brain surface using dental acrylic cement. After the removal of the dura mata the well was filled with paraffin oil.

Micropipettes. Glass micropipettes were used to record

single unit activity and to infuse compounds into the striata of rats. These were made from glass capillary tubes with internal diameters of 1mm and external diameters of 2mm (Clark Electromedical Instruments) using a vertical microelectrode puller (S.R.I.). Each capillary tube contained a single glass fibre running through its entire length which aided movement of fluid to the tip of the micropipette when made. The micropipettes used to record extracellular single unit activity had tip diameters of less than 1 μ m. These were filled with filtered (Milipore, 0.2 μ m filter) 0.5M sodium ethanoate (containing 2% pontamine sky blue; Gurr) and had electrical resistances of 7-20M Ω . The shank of each pipette was approximately 7mm in length when measured from the tip to the barrel. This length was chosen so that the slender taper caused minimum damage to the brain tissue through which it was lowered whilst having sufficient rigidity to prevent the tip from being diverted away from the target coordinates by fibre bundles.

Each micropipette used to infuse compounds into the striatum had the tip broken back until the diameter was 15-20 μ m. The pipette was filled completely with the drug solution which also contained 1% pontamine sky blue. The micropipette was connected to a nanolitre pressure ejection pump (WPI), with precautions being taken to exclude air bubbles from the system. The pump and micropipette were attached to a micromanipulator (Kopf) adjusted so that the micropipette was at an

angle of 30° from the vertical in an anterior direction of the antero-posterior plane. This was necessary to allow sufficient space for the simultaneous insertion of the recording electrode into the globus pallidus or the substantia nigra pars reticulata. The infusion micropipette was zeroed on the ear bars for the stereotaxic placement within the striatum. The micropipette was placed within the striatum through a small hole drilled in the skull and remained in place throughout the experiment.

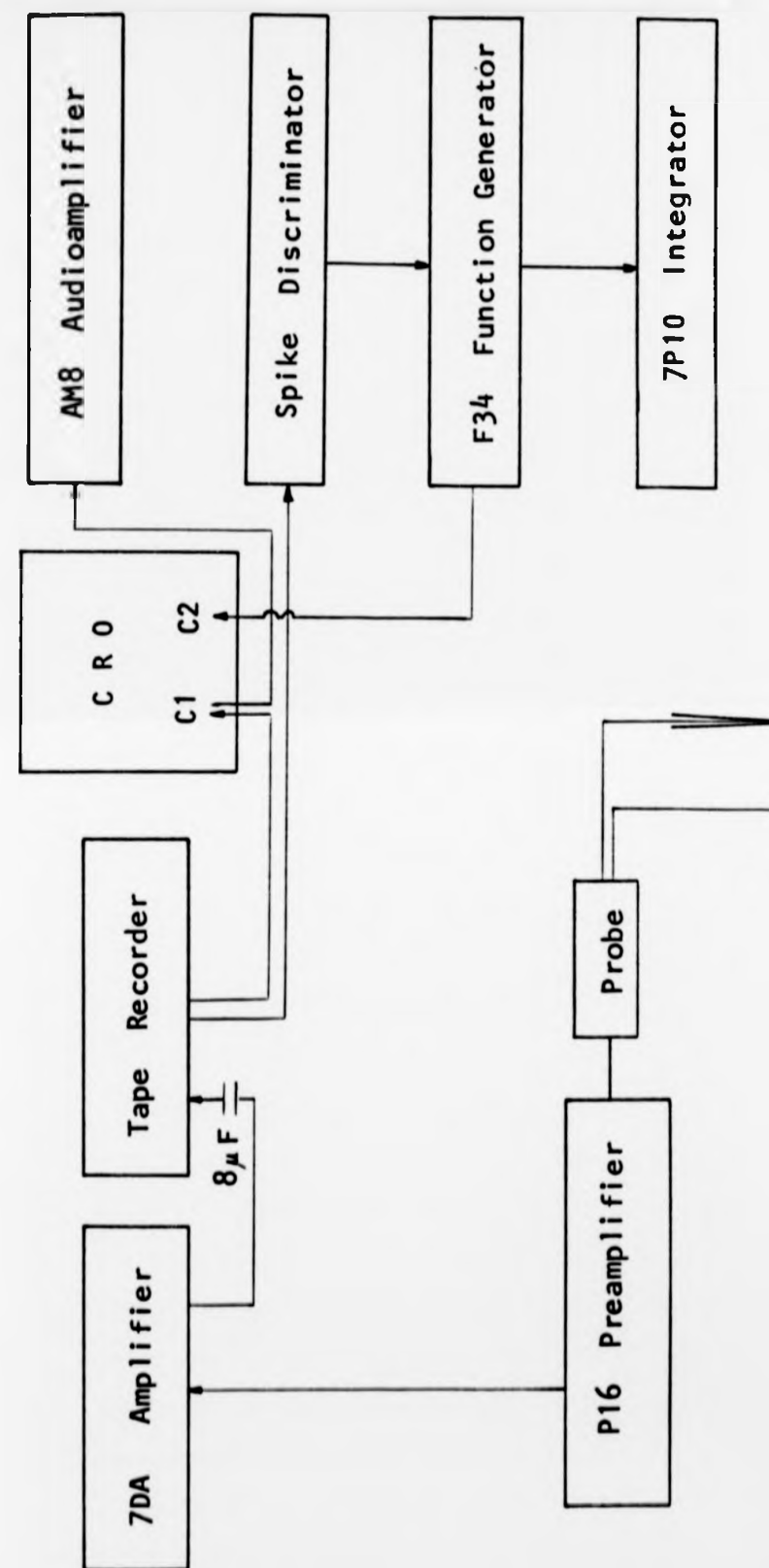
The recording micropipette was held in a perspex-block electrode holder attached to a micromanipulator (L.P.C.) which had a fine (μm) vertical movement. Because of the small size of the micropipette tip (less than $1\mu\text{m}$) it could only be accurately zeroed in the antero-posterior and medio-lateral planes. Accurate measurements against the ear bars in the dorso-ventral plane were not possible so the horizontal coordinate was measured as a distance down from the surface of the brain. This could be accurately measured on the micromanipulator as there was a distinct "click" together with the sound of neuronal activity from the audioamplifier when the micropipette touched the surface of the brain completing the amplification circuit (see section 2.2.2). The micropipette was lowered into the brain at a rate of approximately $1\text{mm}/\text{min}$ until the dorsal boundary of the desired nucleus had been reached.

2.2.2 Electrophysiological recording circuit

The recording circuit used is summarised in Fig. 3. The recording micropipette was connected to a DC preamplifier (Grass, P16) via a length of silver wire and a high impedance probe. The reference (ground) connection was made from the probe to the neck muscle of the rat with a platinum needle electrode. All the implanted micropipettes and electrode, together with their connections to the high impedance probe were surrounded by copper gauze to reduce outside electrical interference.

The AC output of the P16 preamplifier was taken to a Grass 7DA driver amplifier for further amplification. This amplified signal was fed into a DC tape recorder (Racal), an oscilloscope (CRO; Tektronik), an audioamplifier (Grass, AM8) and a spike discriminator. The discharge frequency of the single unit was displayed on a pen recorder (Grass, pen oscillograph). This record was obtained by feeding the amplified signals through the spike discriminator, which in turn triggered a function generator (IEC, F34). The latter produced a calibrated, constant sized square wave for each trigger pulse. The output from the function generator, which was continuously monitored on the CRO together with the "raw" recorded signal, was fed into an integrator (Grass, 7P10). The integrator was set to produce a 10s ramp integrated display which was recorded on paper via a 7DA amplifier and Grass pen oscillograph (not shown in Fig. 3).

Fig 3. Schematic diagram of the circuit used to record single unit activity. C1, channel one; C2, channel two; CRO, cathode ray oscilloscope. The $8\mu\text{F}$ capacitor was used to remove a standing DC voltage produced by the P16 preamplifier. See text for further details.



The integrator was calibrated by directly triggering the function generator (while it was still connected to the integrator) with a stimulator (Grass, S88). The frequency of the stimulator output was monitored on a CRO so an accurate calibration of the integrator could be made.

2.2.3 Electrophysiology method

Only spontaneously active single units which met the following criteria were used in these experiments:-

- a) The action potentials were biphasic with durations of 3ms or longer. Such action potentials were adjudged to be from cell bodies rather than from fibres of passage.
- b) The firing pattern was not obviously in phase with the ventilatory rhythm, did not change if the recording micropipette was withdrawn a few micrometers, and did not change significantly over a 15min recording period
- c) The signal to noise ratio was sufficiently large to permit the spike discriminator to give an accurate reproduction of the firing pattern

Similar protocols were used for each experiment. After a control period of approximately 15min duration, atropine MN (1mg/Kg i.v.) was administered in those instances where an intravenous muscarinic cholinomimetic agent was subsequently being injected to prevent stimulation of peripheral muscarinic receptors. A further period of 10min was allowed to elapse in order for the atropine MN to be absorbed fully. Two routes of drug

administration were used, systemic (via the tail vein cannula) and central (via the intrastriatal micropipette). When the systemic route was used the total volume injected was always less than 1ml. When the central route was used, the drug was infused for 5min at a rate of 50nl/min. The single unit activity was subsequently monitored for up to 2h. In some cases, when there was no change in the activity of the unit, a second unit was located and the striatal infusion was repeated. This was performed at least 1h after the initial infusion. In experiments where there was a change in the frequency of discharge of the recorded unit, either the duration of the change in activity was monitored, or a pharmacological receptor antagonist was administered (i.v.) to determine if the response could be reversed.

At the end of each experiment, the recording site was marked by ejecting some pontamine sky blue from the recording micropipette. This was achieved by connecting the micropipette to a stimulator (Grass, S88) via a stimulus isolation unit (Grass, SIU 478A) and a constant current unit (Grass, CCU1) and passing a negative DC current of approximately 20 μ A for 10min. The return current passed through the ear bars. Histological evaluation of the infusion and recording sites were made as described in section 2.3.

2.3 HISTOLOGY

At the conclusion of each experiment the sites of

recording electrodes, lesions and cannulae were examined either macroscopically or microscopically. Macroscopic evaluation was restricted to only a few randomly selected animals in any particular group (only those with cannulae implanted into readily identified nuclei) while the remainder were examined microscopically.

Animals selected to have their cannulae sites verified macroscopically had 0.5 μ l of dilute Indian ink microinjected down each guide cannulae as previously described. The animal was then killed by cervical dislocation and the brain removed. The brain was sliced vertically down the cannulae tracks with a scalpel blade. The location of the ink could be easily made by eye.

Animals selected for microscopic evaluation were deeply anaesthetised with sodium pentobarbital 60mg/Kg i.p. (Sagatal; May and Baker). If cannulae placings were involved, 0.5 μ l of dilute Indian ink was microinjected down each guide cannula. Just as spontaneous ventilation ceased, the chest cavity was opened and an intracardiac injection of heparin 5000U/Kg (Evans Medical) was made. After approximately 90s the blood was displaced through the venae cavae by the transcardial perfusion of 100ml of heparinised saline (0.9% saline containing heparin 50U/ml) made through the left ventricle. The perfusate was administered from a 50ml syringe attached to a cardiac needle via a two way tap and a length of rubber tubing. Following this, the

animal was perfused with 100ml of 10% neutral buffered formalin (pH 7.2, buffering agent calcium carbonate in the form of marble chips) in a similar manner. The animal was left for 5min to allow the tissues to fix before the brain was removed and placed in 10% neutral buffered formalin.

Prior to sectioning, the brains were transferred to a sucrose solution (1% gum acacia in 30% sucrose containing thymol which prevented bacterial growth) until they no longer floated. This usually required 2-3 days. Serial 20 μ m sections of the required areas were made using a freezing microtome (Pelcool; MSE). The sections were mounted and dried on glycerine/albumen subbed slides. They were then stained with Luxol fast blue and Feulgens basic fuschin, modified from Culling (1974). The sections were enclosed and observed under a microscope. In the case of sections from brains which had received intrastriatal drug infusions, the extent of the infusion, as defined by the staining with pontamine sky blue, was marked by damaging the brain sections with a needle prior to staining. This was because the pontamine sky blue was removed by the Luxol fast blue/basic fuschin staining procedure.

The suppressed silver staining method for degenerating neurones (method II) of Fink and Heimer (1967) was employed to evaluate the extent of the lesion induced by 3-acetypyridine. The animals were perfused and the brains were stored as described above. A

freezing microtome was used to cut 20 μ m sections which were collected in 2% formalin. Staining was carried out on free floating sections according to the schedule shown in Table 2. Stained sections were mounted and dried onto glycerine/albumen subbed slides. They were then dehydrated, enclosed, and observed under a microscope.

2.4 STATISTICAL ANALYSIS

It is important in experiments involving biological systems to determine whether the changes seen in experimental samples reflect changes in the populations from which they are drawn. This is a particularly difficult problem if the sample sizes are small, as is the case in this study. However, statistical analyses based on certain assumptions have been devised in order to solve this problem. Those used in this study have been of the parametric type. A significance level of 0.05 was adopted throughout. This means that if the calculated probability of the observed result occurring by pure chance was less than 5% then it was taken to be caused by the treatment the samples received.

Parametric statistical tests are based on two assumptions which must be satisfied for the results of the analyses to be valid. These assumptions are that the populations the samples are drawn from are Normally distributed and that these distributions have similar variances. This latter assumption can be checked by using the individual sample variances as estimates of the population variances, and comparing

Table 2. Summary of the Fink and Heimer (1967)
staining procedure (method II)

Step number	Solution composition	Time in solution (min)
1.	Water	1
2.	0.025% potassium permanganate	3
3.	Water	1
4.	1:1, 1% oxalic acid:1% hydroquinone	2
5.	Water	1
6.	2.5% uranyl nitrate	7
7.	Water	1
8.	0.2% silver nitrate + pyridine	110
9.	1.5% silver nitrate 20ml 95% ethanol 12ml 0.88 ammonia 2ml 2.5% sodium hydroxide 1.7ml	3
10,11	Water 9.1ml 95% ethanol 0.9ml 10% formalin 2.7ml 1% citric acid 2.7ml	3
12	Water	1
13	0.5% sodium thiosulphate	1
14	Water, mount, dehydrate, enclose	

the groups with a variance ratio test (F test). If this ratio proves to be significant then any parametric test is invalidated because the two samples (and hence the populations) do not have similar variances. In this case a non-parametric statistical analysis would be required.

For the comparison of two groups Students' "t" test was used. Due to the limb tone experimental design, the test for paired data was always used. However, if a group of animals received more than one treatment, a one factor analysis of variance was performed. If this proved to be significant, a least significant difference was calculated from the required value in the "t" distribution to determine which treatments were significantly different. All statistical evaluations carried out in this study were as described in Steel and Torrie (1980).

2.5 ASSAY OF OXOTREMORINE IN BRAIN

2.5.1 Guinea pig isolated ileum preparation

A guinea pig isolated ileum preparation was used to bioassay the atropine-sensitive contractile substances extracted from the brains of rats treated with tremorine. Guinea pigs of either sex were sacrificed by cervical dislocation and exanguination after being starved overnight. The intraperitoneal cavity was opened from the ventral side and a length of late ileum was removed and placed in a bicarbonate buffered physiological saline (Krebs' solution, composition shown in Table 3). Intraileal contents were displaced

Table 3. Composition (mM) of the physiological salt solution used to bathe the guinea pig isolated ileum preparation

Glucose	11
NaHCO ₃	25
NaCl	118
KCl	4.75
KH ₂ PO ₄	0.93
MgSO ₄ ·7H ₂ O	1.17
CaCl ₂	2.54

The solution was continuously bubbled with O₂95%, CO₂5%.

with Krebs' solution and the remaining mesenteric connective tissue was dissected away. Lengths of ileum (2-3cm) were prepared by attaching cotton threads to each end without occluding the lumen. One end of the ileum segment was secured to a glass tissue holder and the tissue and tissue holder were placed in a 30ml tissue bath containing Krebs' solution. The tissue baths had water jackets through which circulating water maintained the tissue bath at 37°C. The Krebs' solution was constantly bubbled with a gas mixture (95%O₂, 5%CO₂) to maintain its pH, buffering capacity and oxygen content. The second cotton thread was attached to an isometric strain gauge (Grass, FT03C) and an initial tension of 1g was exerted on the tissue. Adjustments were made throughout the experiment to maintain this tension. The output from the strain gauge was amplified (Grass, 7P1B, and Grass, 7DA) and dis-

played on a pen recorder (Grass, pen oscillograph). The system was calibrated by hanging weights from the strain gauge.

Brain extracts. These were prepared from freshly sacrificed rats which had previously been given tremorine (20mg/Kg i.p.), or atropine MN (1mg/Kg i.p.) + tremorine (20mg/Kg i.p.), or no treatment at all. The rats were killed by cervical dislocation (20min after tremorine administration) and the brains were rapidly removed. The brains were roughly chopped with a scalpel blade and each brain was placed in a glass:teflon homogeniser containing 3ml of Krebs' solution. The homogenate was transferred to a test tube and centrifuged at 1500g for 10min. The resulting supernatant was used in the bioassay.

2.5.2 Bioassay method

Two guinea pig isolated ileum preparations were run in parallel, one to act as a test tissue and the other as a concurrent control. After an initial equilibration period of 30min, dose-response sequences to oxotremorine and the brain extract were carried out on each tissue. Ten fold increments were used (1ng-1µg/ml bath) for oxotremorine. The oxotremorine solutions were made up in Krebs' solution and the volume added to the tissue bath at any one time was always less than 1ml. The contact time (the period from adding the drug to washing the drug away) was varied as required (30-50s) to allow a maximum tension to develop. A cycle time (the period from one drug administration to the next) of 4min was

used. Two concentrations of oxotremorine which gave approximately 30% and 70% of the maximum response, together with one volume of brain extract which gave a response of approximately 50%, were selected to be used in the 3 point assay. Each concentration was tested three times in a randomised order on both tissues. These responses were used to estimate the concentration of oxotremorine in the brain extract as explained in section 3.5.

The sensitivity of both oxotremorine and the brain extract to atropine was demonstrated on one tissue while the other acted as the concurrent control. Atropine (250ng/ml bath) was added to one tissue bath 2min before either the brain extract or the oxotremorine dose which produced the 70% response. An equal volume of Krebs' solution was added to the control preparation in place of the atropine.

A 3-point assay was also performed to estimate the recovery rate of oxotremorine from the brain. This was carried out by microinjecting 10 μ g of oxotremorine (in 1 μ l) into the striatum of a urethane anaesthetised rat (as described for neurotoxins, section 2.1.3) and preparing an extract from the brain 15min later. The bioassay was performed as described above.

SECTION THREE : RESULTS

3.1 EVALUATION OF THE LIMB TONE MEASURING APPARATUS

Stability. The stability of the limb tone apparatus over a 3h period was evaluated as described in section 2.1.2. The individual calculated linear regression slopes and intercepts, along with the corresponding linear correlation coefficients are shown in Table 4. Analysis of covariance showed that there were no significant differences between the calculated slopes or intercepts for either side of the apparatus. During the 3h period there was a slight drift in the position of the baseline on both recording channels but this did not exceed 5% of a full scale pen deflection (equivalent to less than 3g of limb resistance). Similar results were obtained when this evaluation procedure was carried out on subsequent occasions during this study.

It was found that there was some cross-channel interference which occurred when the apparatus was asymmetrically loaded. This only occurred when the difference in loading was in excess of 50g, and amounted to a pen deflection equivalent to up to 20g on the more lightly loaded side when the load differential was 90g. This was found to be due to vibration caused by the cam from the electric motor (Fig.1). The introduction of a piece of foam rubber between the electric motor mountings and the steel frame reduced this vibration so that cross-channel interference did not occur until the asymmetrical loading exceeded 70g and was only equivalent to 10g when the load differential was 90g.

Table 4. Results of an investigation to determine the variation in the responsiveness of the limb tone measuring apparatus

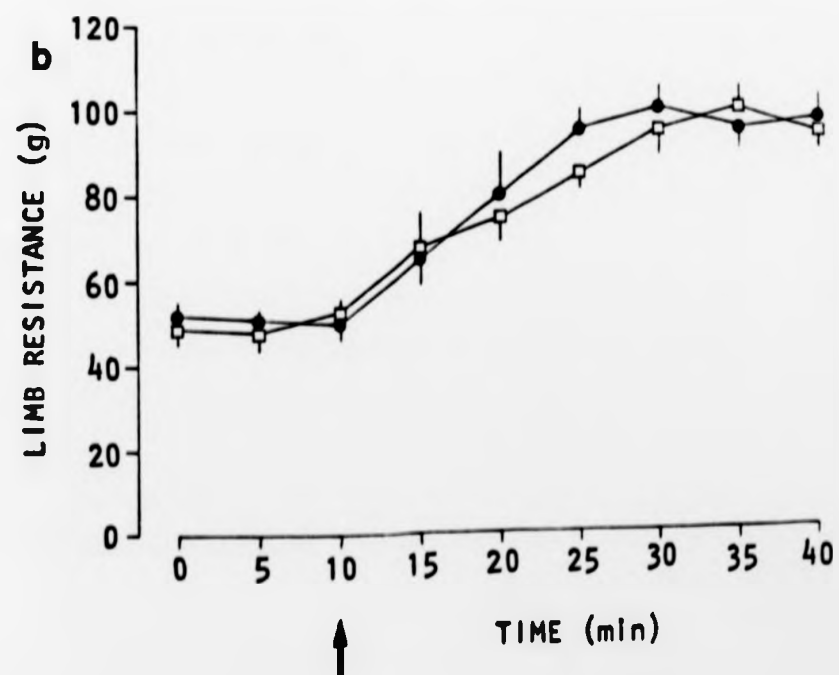
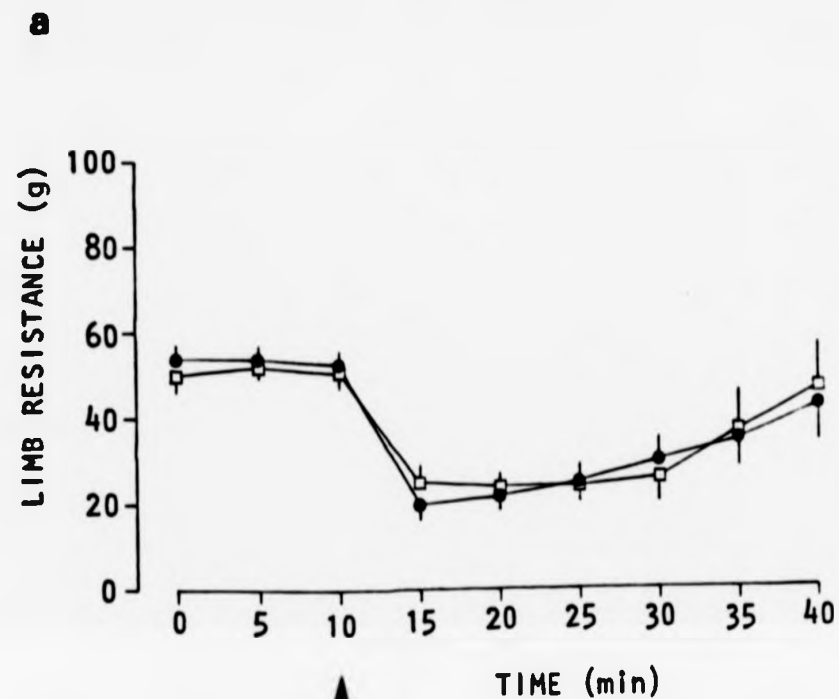
Time (min)	Left side			Right side		
	Slope	Intercept	Correlation coefficient	Slope	Intercept	Correlation coefficient
0	0.328	-0.265	0.998	0.333	0.273	0.999
30	0.308	0.607	0.999	0.340	0.259	0.999
60	0.318	0.208	0.999	0.342	0.080	0.999
90	0.310	-0.105	0.996	0.338	0.105	0.998
120	0.315	0.250	0.998	0.344	0.200	0.999
150	0.312	-0.102	0.997	0.331	0.210	0.998
180	0.316	0.110	0.998	0.339	0.201	0.998
Mean	0.315	0.100	0.998	0.338	0.190	0.999
SEM	0.002	0.110	0.0004	0.002	0.027	0.0002

However, this cross-channel interference was not a problem in the experimental procedures used to measure rat hindlimb tone because the maximum left-right difference in hindlimb tone measured was never greater than 30g in any experiment. Thus, all hindlimb tone measurements were made when the two sides of the limb tone apparatus were independent of each other.

In summary, these results indicate that a calibration curve obtained either at the start or end of an experimental period reliably represents the responsiveness of the apparatus during the experiment, and also that the two sides of the limb tone apparatus can be regarded as independent of each other.

Drug-induced changes in hindlimb tone. The ability of the limb tone apparatus to record changes in rat hindlimb tone induced by the administration of methohexitone or morphine was evaluated as described in section 2.1.2. The administration of the general anaesthetic methohexitone (40mg/Kg i.p.) caused the rats to become heavily sedated almost immediately. This was accompanied by a reduction in the recorded hindlimb resistance of both hindlimbs of approximately 30g (Fig.4). In contrast, morphine (15mg/Kg i.p.) caused a gradual increase in the recorded hindlimb resistance of both hindlimbs (Fig.4). The maximal effect (an increase of approximately 50g) was produced 20min after administration. During the period of increased limb tone (which lasted 1-1.5h) the rats were cataleptic, taking

Fig 4. Effect of intraperitoneal administration of (a) methohexitone (40mg/Kg) and (b) morphine (15mg/Kg) on the resistance to flexion in the left (●—●) and right (□—□) hindlimbs of conscious rats. Drugs were administered at 10min (arrowed). Each point is the mean obtained with 6 rats. Vertical bars show the SEM.



up a characteristic posture when held in the restraining cage.

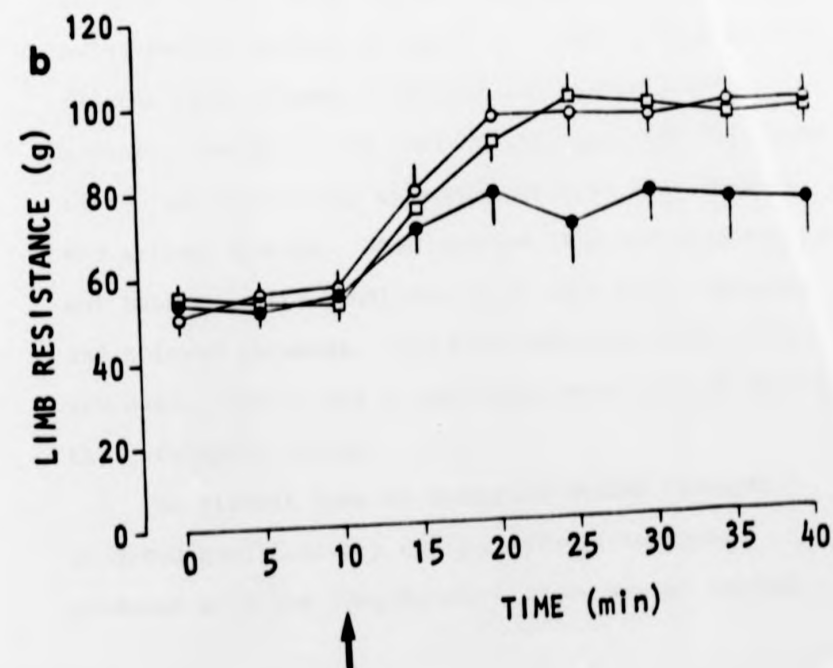
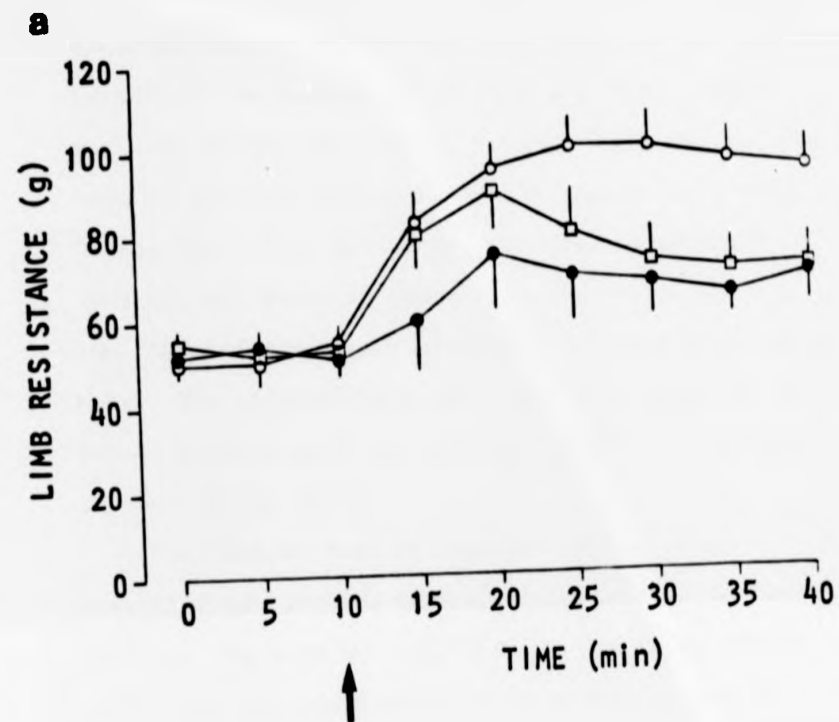
These results indicate that the limb tone apparatus is sufficiently sensitive to record and quantify drug-induced changes in rat hindlimb tone. Also, these results demonstrate that there is no bias in the recording system as the recorded limb resistances of both hindlimbs were similar to each other following either morphine or methohexitone administration.

3.2 PRELIMINARY STUDIES OF THE EFFECTS OF TREMORINE ON LIMB TONE

Preliminary experiments were carried out to select a dose of tremorine which gave a significant and reproducible increase in rat hindlimb tone. Three doses of tremorine (5, 10, and 20mg/Kg i.p.) were evaluated on separate occasions in the same group of rats. Each rat was pretreated with atropine MN (1mg/Kg i.p.) 10min prior to the tremorine administration to prevent peripheral muscarinic receptor stimulation. The method used was as described in section 2.1.6. and the results are shown in Fig.5.

With the lowest dose of tremorine the rats became excited, as evidenced by an increase in voluntary movement. This lasted throughout the recording period and made the interpretation of the recorded limb tone traces difficult. Therefore it is not possible to say that the increase in limb resistance shown in Fig.5 was an accurate estimate of the limb tone at this dose. Tremor and piloerection were also present throughout

Fig 5. Effect of intraperitoneal administration of tremorine on hindlimb resistance to flexion in conscious rats. (a) Effect of 3 doses of tremorine : 5 (●—●), 10 (□—□) and 20 (○—○) mg/Kg in a group of 6 rats. (b) Reproducibility of tremorine (20mg/Kg) response. ●—●, 1st administration; □—□, 2nd administration; ○—○, 3rd administration to a group of 6 rats. Tremorine was given at 10min (arrowed). Left and right hind-limb responses were pooled and each point represents the mean value. Vertical bars show the SEM. All rats were pretreated with atropine methylnitrate (1mg/Kg) at time zero. A minimum period of 48h was allowed to elapse between successive drug administrations on the same animal.



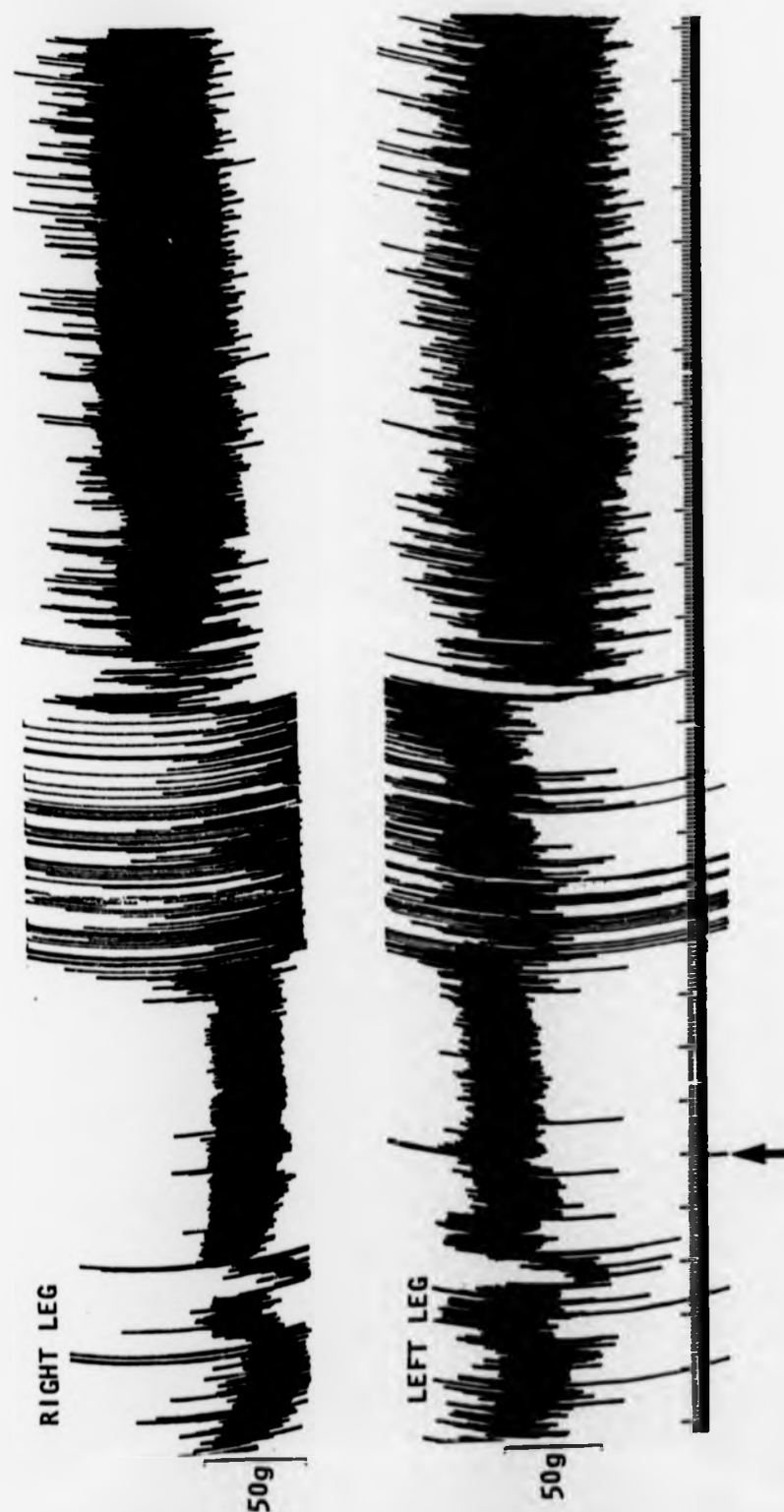
the recording period. The tremor occurred in all the skeletal muscles of the body and limbs but was most marked in the muscles of the head and neck. This muscular tremor was clearly visible while the rat was held in the restraining cage, and lasted for 30-45min. During the tremor period all the muscles appeared to contract and relax in phase with each other and the amplitude of the tremor increased with voluntary movement. The piloerection, which was most apparent on the dorsal surface near the midline, had a similar duration to that of the tremor.

The 10mg/Kg dose of tremorine produced an elevation of hindlimb tone which lasted for 10-15min (Fig.5). As with the 5mg/Kg dose, the first effect seen after the administration of tremorine was an increase in voluntary movement which lasted for approximately 10min. This was soon followed by a prolonged quiet period lasting at least 1h. During this period all the rats assumed a similar and characteristic posture. While in the restraining cage, the forelimbs of the rat were fully extended forwards and the back was arched upwards. When removed from the cage the rat sat back onto its hindlimbs which were fully extended and splayed outwards. The forelimbs were also fully extended. Tremor and piloerection were present during the cataleptic period.

The highest dose of tremorine tested (20mg/Kg) produced qualitatively similar effects to those produced with the 10mg/Kg dose: there was an initial

Fig 6. Pen recorder trace showing tremorine (20mg/Kg i.p.)-induced hindlimb rigidity in a rat. The rat was pretreated with atropine methylnitrate (1mg/Kg i.p.) 10min before the administration of tremorine (at arrow). Note the movement artefacts 4-8min after the tremorine injection and their reduced occurrence during the period of rigidity.

Time trace : small divisions, 5s; large divisions, 1min.



excitatory period which lasted for 5-10min, followed by a prolonged immobile phase with tremor and pilo-erection. With this dose of tremorine, the increase in hindlimb tone was greater than that produced by the other doses of tremorine tested, and was sustained throughout the recording period (Fig.5). A typical recording of hindlimb resistance changes produced with tremorine (20mg/Kg i.p.) is shown in Fig.6. The sharp drop in limb resistance 2min prior to the tremorine administration coincided with a short period of grooming, something that often occurred during pre-drug periods. The movement artefacts produced during the excitatory period (4-9min post tremorine administration) can be clearly seen and obviously made it difficult to take accurate readings from these records. However, once the rat became still (9min post tremorine administration) the absence of movement artefacts made the measurement of hindlimb tone much easier.

Two decisions were taken on the basis of these preliminary studies: (1) the standard dose of tremorine would be 20mg/Kg, and (2) measurements of limb tone would be made in the 10-30min period after tremorine administration.

The reproducibility of the quantitative estimations of the tremorine-induced increase in limb tone was evaluated in a second group of rats. The effect of the standard dose of tremorine (20mg/Kg i.p.) was tested on three separate occasions as described in section 2.1.6. The results are shown in Fig.5. Although characteristic

signs of tremorine intoxication were present following every administration of tremorine, there were quantitative differences in hindlimb tone. It can be seen that the magnitude of the hindlimb tone response to the initial administration of tremorine was less than on a subsequent occasion (Fig.5). However, the hindlimb tone responses obtained following the second and third injections of tremorine were quantitatively very similar. Therefore a third decision was taken - that the data from the second injection of tremorine were a much more reliable indication of the changes in limb tone. Thus, throughout this whole study, the control responses shown and quoted are those obtained following the administration of the second dose of tremorine to each of the animals.

The effect of the tremorine metabolite oxotremorine (0.75mg/Kg i.p.) on the behaviour of the rat was investigated to determine whether it was similar to tremorine. The effect on hindlimb tone was evaluated as described in section 2.1.6. and the results are shown in Fig.7. Immediately after the administration of oxotremorine there was a period of increased voluntary movement (duration 3-5min) followed by a short period of immobility (duration 7-10min). During this period there was an increase in hindlimb tone together with muscular tremor and piloerection similar to that observed with tremorine. Following the period of immobility there was a second period of increased voluntary movement which continued to the end of the recording period,

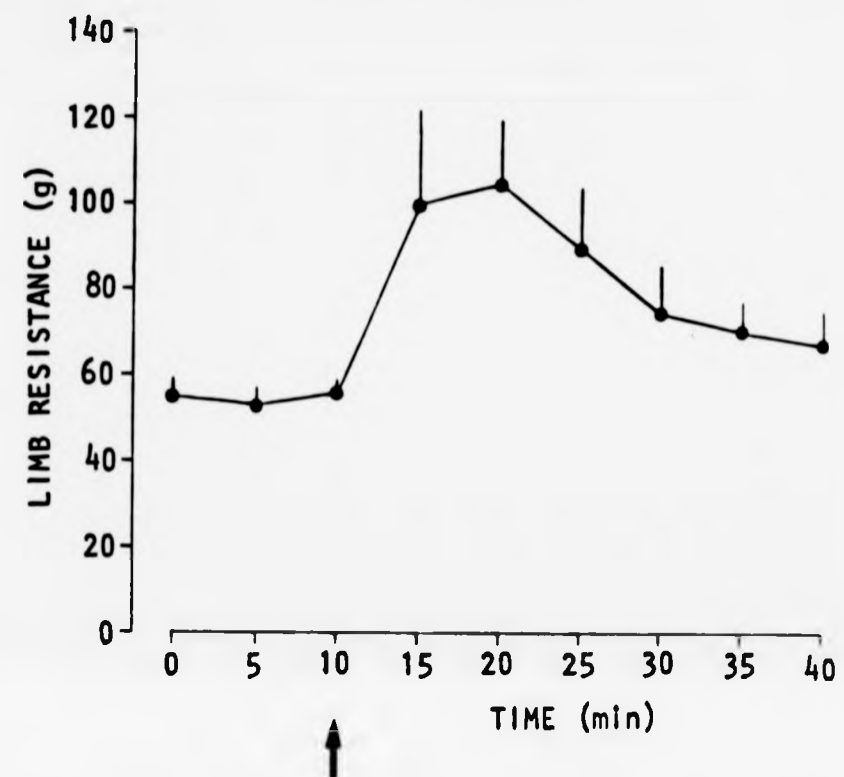


Fig 7. Effect of intraperitoneal administration of oxotremorine (0.75mg/Kg) on hindlimb resistance to flexion in conscious rats. The rats were pretreated with atropine methylnitrate (1mg/Kg) at time zero and oxotremorine was administered at 10min (arrowed). Left and right hindlimb responses were pooled and each point is the mean value from 6 rats. Vertical bars show the SEM.

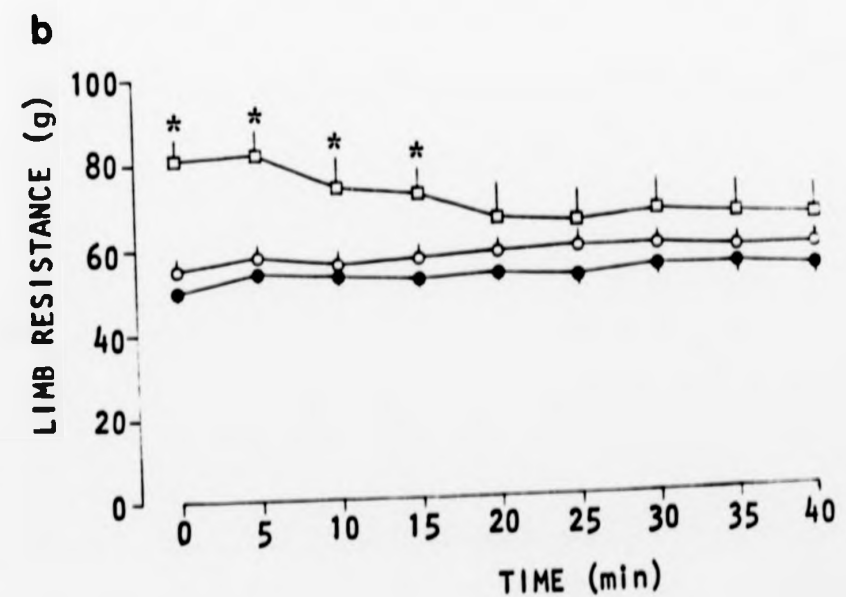
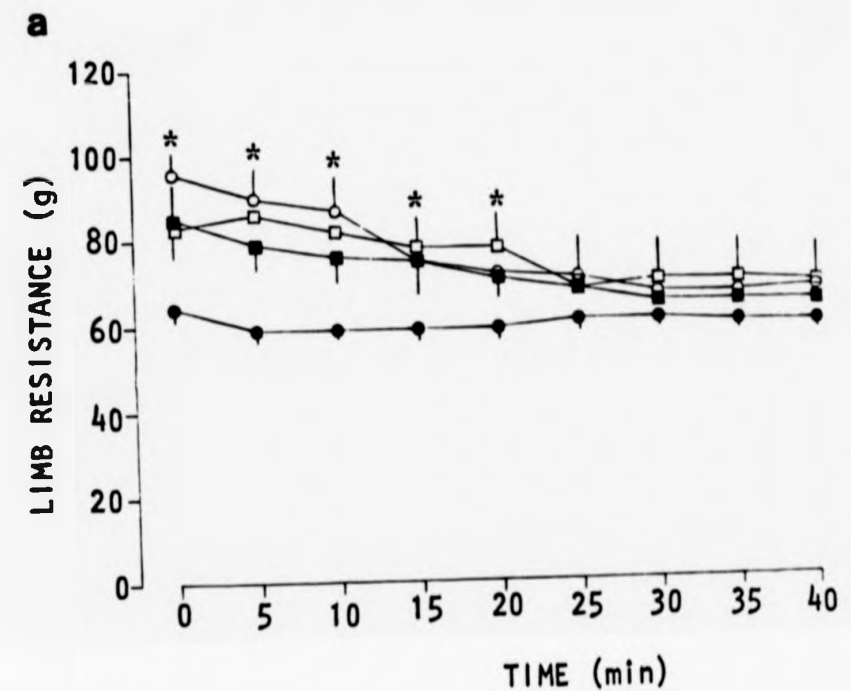
during which time the hindlimb tone returned to almost control values. Within 15min of being removed from the restraining cage, the appearance of the rat had returned to normal. Therefore, this dose of oxotremorine produced qualitatively similar effects to those produced with tremorine but of a very much shorter duration.

3.3 LIMB TONE RESULTS

3.3.1 Effects of intrastriatal cholinomimetic compounds on limb tone

To determine if tremorine-induced hindlimb rigidity was mediated by the direct stimulation of muscarinic receptors in the striatum, cholinomimetic agents were locally applied to the striatum. Groups of rats fitted with chronically implanted striatal cannulae (coordinates, 8.0A, 2.6L, -1.0H) received bilateral microinjections of carbachol, oxotremorine and neostigmine. Typical placements of intrastriatal microinjections are shown in Fig.11. The effects of these agents on hindlimb tone were measured as described in section 2.1.6. Four different doses of oxotremorine (1, 10, 30, and 50 μ g, in 1 μ l volumes) were evaluated and the results are shown in Fig.8. The three higher doses of oxotremorine produced similar effects. Each dose caused a transient but statistically significant increase in the hindlimb resistance of 20-30g (lasting 15-20min). During this period the rats adopted a posture similar to that seen after systemic tremorine administration (section 3.2) with piloerection, tremor,

Fig 8. Effect of bilateral intrastratial administration of oxotremorine on hindlimb resistance to flexion in conscious rats. (a) Effects of saline (1 μ l, \bullet — \bullet) and oxotremorine 10 μ g (□—□), 30 μ g (○—○) and 50 μ g (■—■) in a group of 6 rats. (b) Effects of saline (1 μ l, \bullet — \bullet) and oxotremorine 1 μ g (○—○) and 10 μ g (□—□) in a group of 6 rats. Intrastratial injections were performed at time zero. Left and right hindlimb responses were pooled and each point is the mean value. Vertical bars show the SEM. A minimum period of 48h was allowed to elapse between successive drug administrations on the same animal. Analysis of variance, $p < 0.05$ in (a) and (b); *, $p < 0.05$ vs saline control using a two-tailed paired Student's t test.



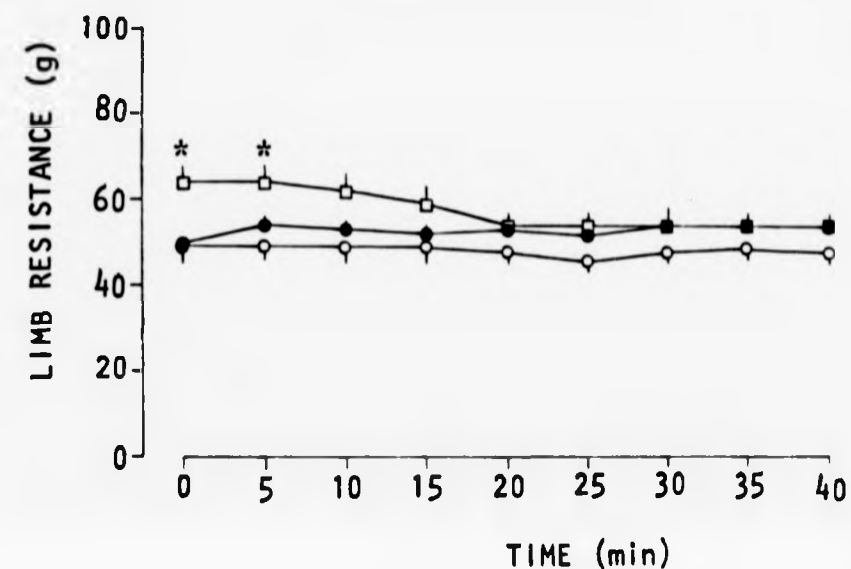


Fig 9. Effect of bilateral intraatrial administration of carbachol on hindlimb resistance to flexion in conscious rats. Saline (1µl, ●—●) and carbachol 1µg (○—○) and 10µg (□—□) were administered at time zero. Left and right hindlimb responses were pooled and each point is the mean value from 6 rats. Vertical bars show the SEM. A minimum period of 48h was allowed to elapse between successive drug administrations on the same animal. Analysis of variance, $p < 0.05$; *, $p < 0.05$ vs saline control using a two-tailed paired Student's *t* test.

and increased lacrimation and salivation also present. However, unlike with systemic tremorine administration, there was no excitatory period following intrastriatal oxotremorine, instead the rats immediately became akinetic. The lowest dose of oxotremorine ($1\mu\text{g}$) had no effect upon hindlimb tone or posture of the rats. As the $1\mu\text{g}$ dose of oxotremorine was ineffective, and the $10\mu\text{g}$ dose produced a near maximal effect on hindlimb tone, it suggests that the dose-response curve for oxotremorine rigidity lies between these two doses.

The effects of $1\mu\text{g}$ and $10\mu\text{g}$ of carbachol (in $1\mu\text{l}$ volumes) in the striatum were also tested and the results are shown in Fig.9. The higher dose of carbachol produced a short lasting increase in hindlimb tone of 15g together with tremor, lacrimation, salivation, and piloerection, while the lower dose produced no such effects. Although the magnitude of the limb tone changes induced by carbachol were smaller than those produced by oxotremorine, the qualitative effects of the two compounds were similar.

Neostigmine, an inhibitor of acetylcholinesterase, was also evaluated after bilateral administration of $40\mu\text{g}$ (in $1\mu\text{l}$) into the striatum. The effect of neostigmine on limb tone was monitored after two time periods in separate groups of rats. The first period, 30-70min post injection, is shown in Fig.10, and the second period, 60-100min post injection, is shown in Fig.10. During the first period the limb tone of the rats slowly declined although the change never became

Fig 10. Effect of bilateral intrastriatal administration at time zero of saline (1 μ l, \bullet — \bullet) or neostigmine (40 μ g, \square — \square) on hindlimb resistance to flexion in conscious rats. Left and right hindlimb responses were pooled and each point is the mean value from 6 rats. Vertical bars show the SEM. *, $p < 0.05$ vs saline control using a two-tailed paired Student's t test.

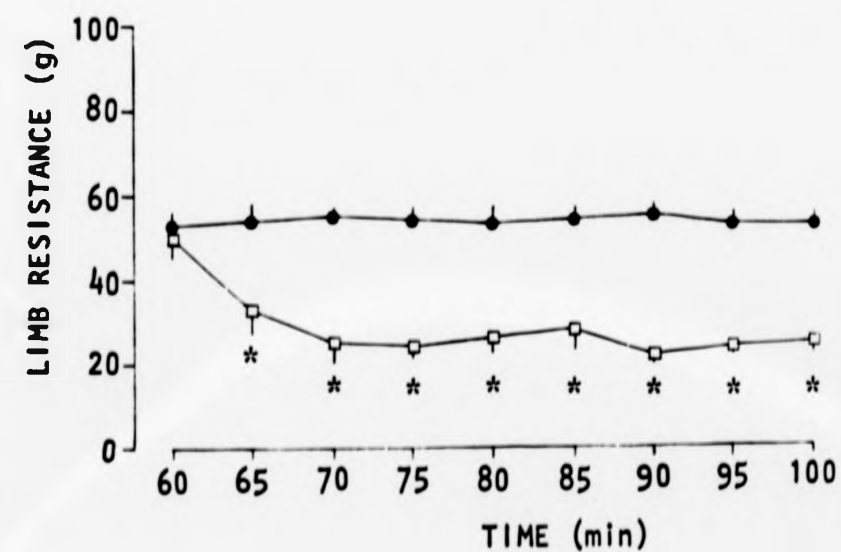
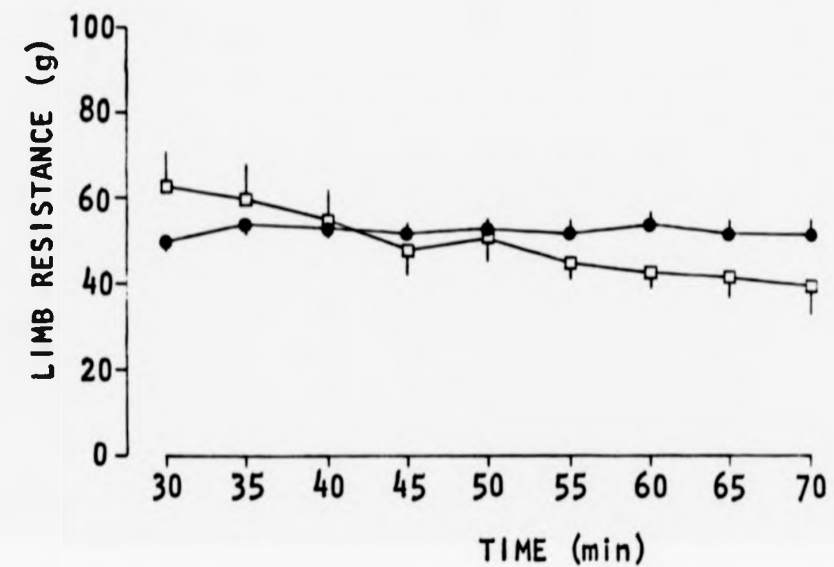
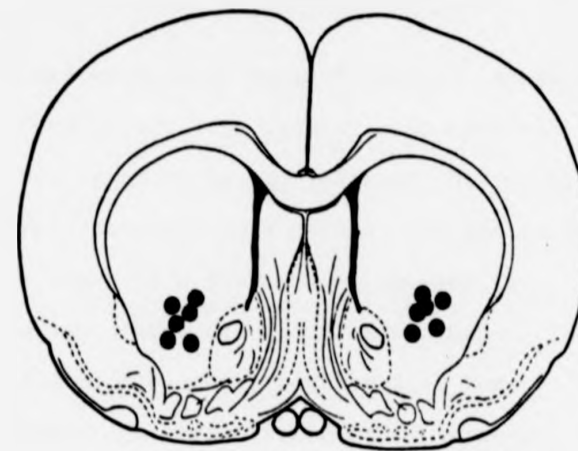
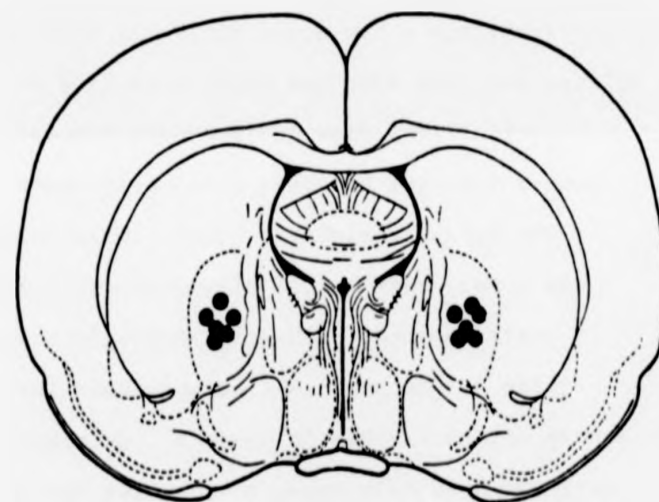


Fig 11. Diagrammatic representations of the distribution of microinjection sites. Each diagram is a composite of injection sites for a representative group of 6 rats with bilateral cannulae implanted into the striatum (A 8.38mm), globus pallidus (A 6.57mm) and substantia nigra pars reticulata (A 1.95mm).

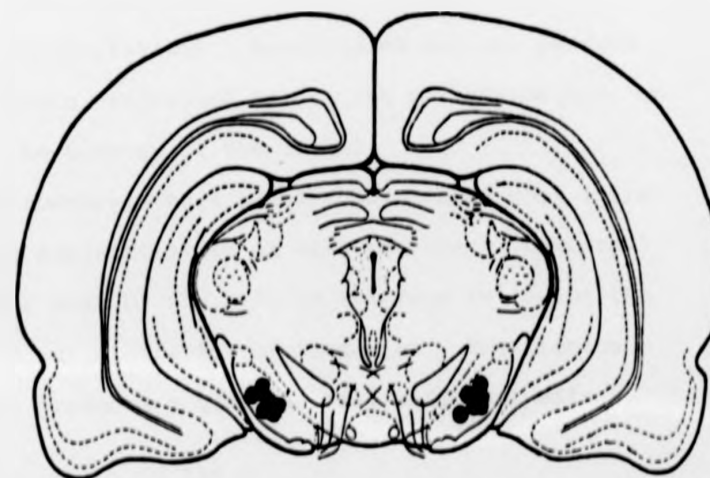
A 8.38mm



A 6.57mm



A 1.95mm



significantly different from control levels. However, during the second period, the fall in limb tone that was recorded was found to be significantly different from the control (non-injected) values. It can be seen in Fig.10 that there is a discrepancy between the results of the two groups receiving neostigmine. The second group had significantly reduced limb tone values 65min after the intrastriatal administration of neostigmine whereas the first group did not. This may be just a reflection of intergroup variation. However, the trend within the first group was a continual decline in limb tone with time which suggests that the results obtained with the second group were qualitatively correct. Neostigmine also produced muscular tremor which started within 10min of administration and continued for approximately 1.5h. This tremor was qualitatively different from that observed after intrastriatal oxotremorine and carbachol or after systemic tremorine. Instead of several muscle groups contracting and relaxing in phase with each other as seen after tremorine, individual motor units in many muscles contracted and relaxed in an apparent random order (fasciculation). Neostigmine did not produce piloerection, increased salivation or lacrimation, or affect the posture of the animal.

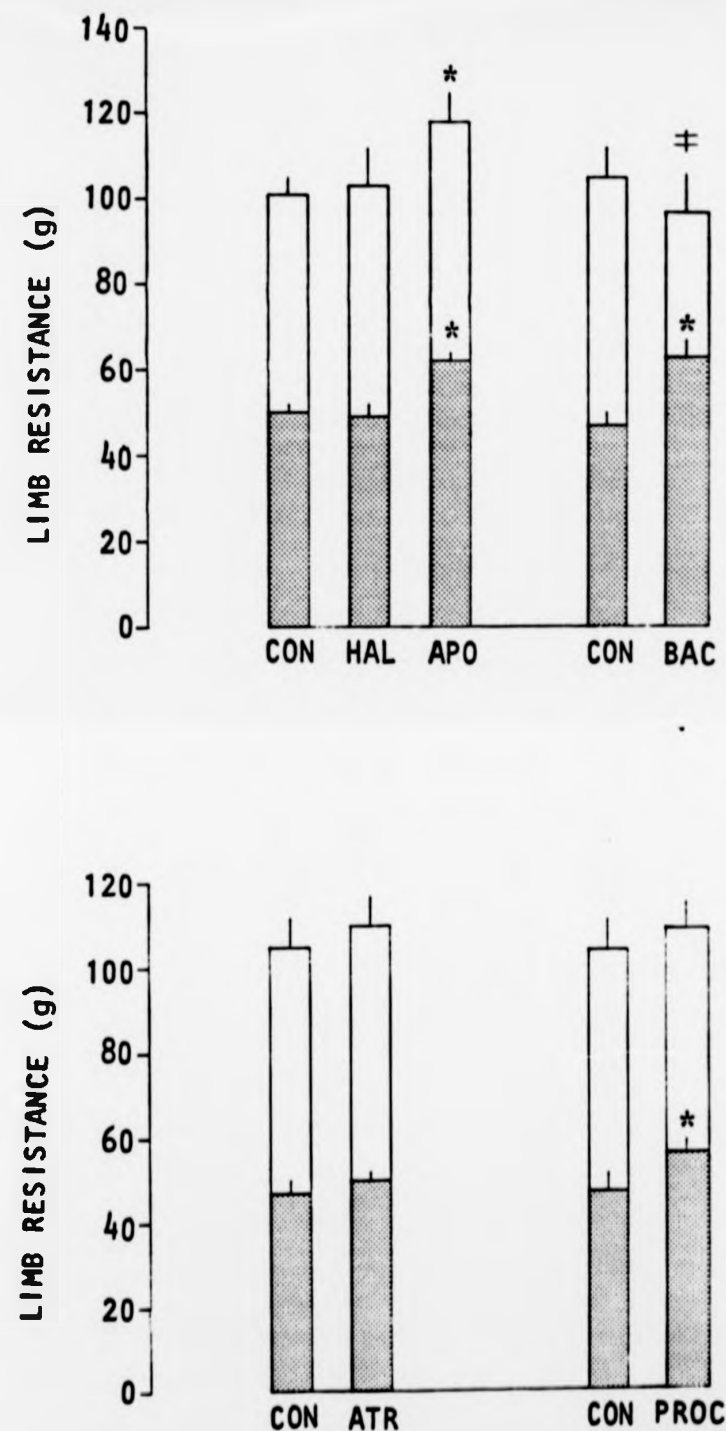
In summary, these results have shown that intrastriatal administration of oxotremorine or carbachol can mimic most of the effects observed following the systemic administration of tremorine. Neostigmine however, produced a qualitatively different effect.

3.3.2 Effects of intrastriatal administration of pharmacological agents on tremorine-induced rigidity

Experiments were performed to determine whether pharmacological manipulation of striatal neurotransmitters affect the increase in hindlimb tone (rigidity) produced by the systemic administration of tremorine. Groups of rats had bilateral cannulae implanted in the striatum (coordinates, 8.0A, 2.6L, -1.0H) as described in section 2.1.3. Responses to the standard dose of tremorine (20mg/Kg i.p.) were recorded following bilateral micro-injection of 1 μ l of saline and again following bilateral administration of various pharmacological agents as described in section 2.1.6. The substances tested were; apomorphine, atropine, baclofen, and haloperidol. The effect of intrastriatal administration of the local anaesthetic procaine was also examined. Data from left and right hindlimbs were pooled and the results are shown in Fig.12. The resting tone was the average limb tone recorded in the 10min period before the injection of tremorine. The drug-induced rigidity was the average limb tone recorded 25-30min after the administration of tremorine. All intrastriatal microinjections were performed 10min before the systemic administration of tremorine.

The dopamine receptor antagonist, haloperidol (5 μ g in 1 μ l) had no effect on the behaviour of the rats, the resting limb tone, nor the tremorine-induced rigidity. In contrast, the dopamine receptor agonist apomorphine (3.3 μ g in 1 μ l) not only increased the

Fig 12. Effects of bilateral intrastratial administration of pharmacological agents on resting tone (stippled bars) and on tremorine-induced rigidity (open bars) in conscious rats pretreated with atropine methylnitrate (1mg/Kg i.p.). Resting tone was recorded 0-10min after intrastratial drug administration. Tremorine (20mg/Kg i.p.) was given at 10min and rigidity was recorded at 25-30min. All results are the mean of both hind-limb responses recorded in 6 rats. Vertical lines show the SEM. CON, control (1 μ l saline); HAL, haloperidol (5 μ g); APO, apomorphine (3.3 μ g); BAC, baclofen (2 μ g); ATR, atropine (8 μ g); PROC, procaine (750 μ g). All volumes microinjected were 1 μ l except for procaine (2 μ l). *, $p < 0.05$ vs saline-treated control using a two-tailed paired Student's t test. ‡, see text for further details.



resting limb tone but also increased the tremorine-induced rigidity (Fig.12). Immediately after the intrastriatal injection of apomorphine the rats became immobile. There were no stereotyped behaviours as seen following systemically administered apomorphine (see section 3.3.3). When the animals were placed in the restraining cage they remained still and quiet for the 10min until tremorine was administered. The behaviour of the rats following the injection of tremorine was the same as control (section 3.1). The actual increase in hindlimb tone produced by tremorine per se (i.e. tremorine-induced tone minus resting tone) after intrastriatal application of apomorphine was not significantly different from that of the saline controls. This suggests that the absolute magnitude of the increase in limb tone induced by tremorine was unchanged.

Baclofen (2 μ g in 1 μ l), an analogue of the amino acid neurotransmitter gamma-aminobutyric acid, produced a significant increase in resting tone. The tremorine-induced rigidity was not significantly different from the control values but the actual increase in limb tone produced by tremorine (i.e. tremorine-induced tone minus resting tone) after intrastriatal baclofen administration was significantly less than that of the saline controls. Atropine (8 μ g in 1 μ l), a potent muscarinic receptor antagonist, had no effect on either resting tone nor tremorine-induced hindlimb rigidity. There was also no noticeable effect on the behaviour of the rats.

The dose of procaine used was determined from observations of its ability to produce apomorphine-induced ipsiversive turning behaviour when micro-injected unilaterally into the striatum of a group of rats. A group of rats with chronically implanted striatal cannulae were used. They had been previously given apomorphine (1mg/Kg i.p.) with no other treatment and were found to exhibit no turning behaviour. Unilateral microinjections of procaine (200, 500, 750, or 1000µg in 2µl) were made together with an equivalent volume of 0.9% sterile saline into the contralateral striatum. Turning experiments were then carried out as described in section 2.1.5 and the results are shown in Table 5. The lowest dose of procaine (200µg) produced no turning behaviour while 500µg produced occasional turning behaviour. The highest dose of procaine (1000µg) produced a period of sedation lasting for 5-10min, followed by a recovery period during which circling was observed. The 750µg dose produced turning behaviour without any period of sedation. The rats were tested again for apomorphine-induced turning behaviour 2 days later (without intrastriatal drug administration). As no turning behaviour was observed on that occasion it was concluded that the effects of procaine were reversible with time. The 750µg dose of procaine was chosen to be used to produce reversible lesions of the striatum in the limb tone experiments.

The intrastriatal administration of procaine

Table 5. Turning behaviour induced by apomorphine (1mg/Kg i.p.) in rats following unilateral intrastriatal administration of procaine

Procaine dose (in 2µl)	Time of onset (min)	Duration (min)	Direction of turning	Frequency, 10min after onset (Complete turns/min)
200µg (n = 3)	No turning behaviour			
500µg (n = 3)	No turning behaviour			
750µg (n = 3)	5 - 9	15 - 17	I/L	4 - 7
1000µg (n = 3)	10 - 15	28 - 33	I/L	4 - 9

The numerical values represent the minimum and maximum values observed for each parameter. I/L, ipsilateral.

(750µg in 2µl) was found to produce a significant increase in the resting hindlimb tone of the rat. It had no noticeable effect on the behaviour of the rats and had no effect on the tremorine-induced hindlimb rigidity. The actual increase in limb tone produced by tremorine in procaine-treated rats was the same as in saline-treated rats.

3.3.3 Effect of unilateral lesions of the striatum on limb tone

Lesions of striatal neurones and striatal dopaminergic afferents (from the substantia nigra) were produced, and the effects on resting hindlimb tone and tremorine-induced rigidity were recorded. The lesions were made by (1) the intrastriatal microinjection of the neurotoxic agents kainic acid and ibotenic acid, and (2) by the injection of 6-hydroxydopamine into the medial forebrain bundle. One group of rats had striatal lesions produced by the microinjection of kainic acid (0.5µg in 1µl) into two sites within the striatum (coordinates, 7.6A, 2.6L, -1.0H and 8.6A, 2.6L, 0.0H). A second group of rats had lesions produced by a single dose of ibotenic acid (10µg in 2µl) microinjected into the striatum (8.0A, 2.6L, -1.0H). The third group of rats had a unilateral lesion of the ascending dopaminergic pathways produced by the microinjection of 6-hydroxydopamine (8µg in 4µl) into the lateral hypothalamus (4.8A, 1.6L, -2.9H). Immediately upon recovery from the anaesthetic, all the rats exhibited turning behaviour. In the case of the 6-hydroxydopamine-induced lesion, the turning was initially ipsilateral

to the lesion, whereas contralateral circling was seen in the other two groups. Kainic and ibotenic acid microinjections also produced increased lacrimation and salivation, together with piloerection, which lasted for approximately 3-4h. On the following day every group of rats exhibited ipsilateral turning behaviour irrespective of the lesion. This gradually diminished with time until, by day 10, there was little or no turning or postural asymmetry even when the rats were disturbed.

The response to the standard dose of tremorine (20mg/Kg i.p.) was examined both pre- and post-lesioning in each group of animals. In those animals with kainic acid-induced striatal lesions the effect of morphine (15mg/Kg i.p.) was also investigated. The results are shown in Figs.13 to 16. All three lesions produced a small, but statistically non-significant, increase in the contralateral hindlimb resting tone. However, each lesion had a greater effect on tremorine-induced rigidity recorded in the contralateral hindlimb. The tremorine-induced rigidity was potentiated (Figs.13, 14, 15). In contrast, no significant effects on resting tone nor tremorine-induced rigidity were recorded in the ipsilateral hindlimb. At the end of the recording period, when the animals were replaced in their home cages, a slight postural asymmetry was observed. The rats took up the characteristic posture associated with tremorine intoxication (section 3.2) but leaned towards the side ipsilateral to the lesion. This may have been related

Fig 13. Effect of a unilateral 6-hydroxydopamine-induced lesion of the medial forebrain bundle on tremorine-induced rigidity in (a) the ipsilateral and (b) the contralateral hindlimbs of conscious rats (●—●, pre-lesion; □—□, 15 days post-lesion). Rats were pretreated with atropine methylnitrate (1mg/Kg i.p.) at time zero and tremorine (20mg/Kg i.p.) was administered at 10min (arrowed). Each point is the mean value from 6 rats. Vertical bars show the SEM. *, $p < 0.05$ vs pre-lesion using a two-tailed paired Student's *t* test.

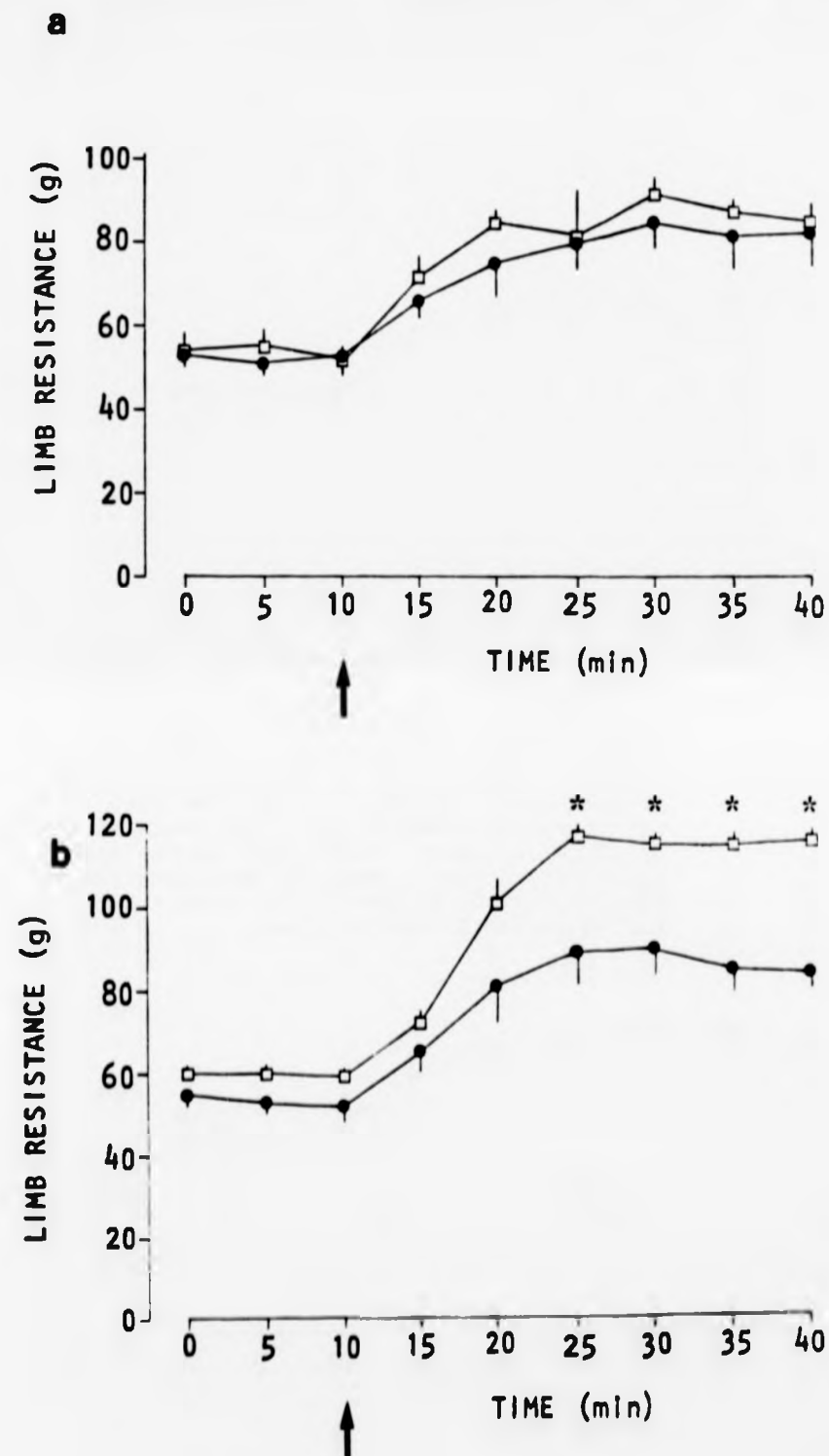


Fig 14. Effect of a unilateral kainic acid-induced lesion of the striatum on tremorine-induced rigidity in (a) the ipsilateral and (b) the contralateral hindlimbs of conscious rats (●—●, pre-lesion; □—□, 15 days post-lesion). Rats were pretreated with atropine methylnitrate (1mg/Kg i.p.) at time zero and tremorine (20mg/Kg i.p.) was administered at 10min (arrowed). Each point is the mean value from 6 rats. Vertical bars show the SEM. *, $p < 0.05$ vs pre-lesion using a two-tailed paired Student's t test.

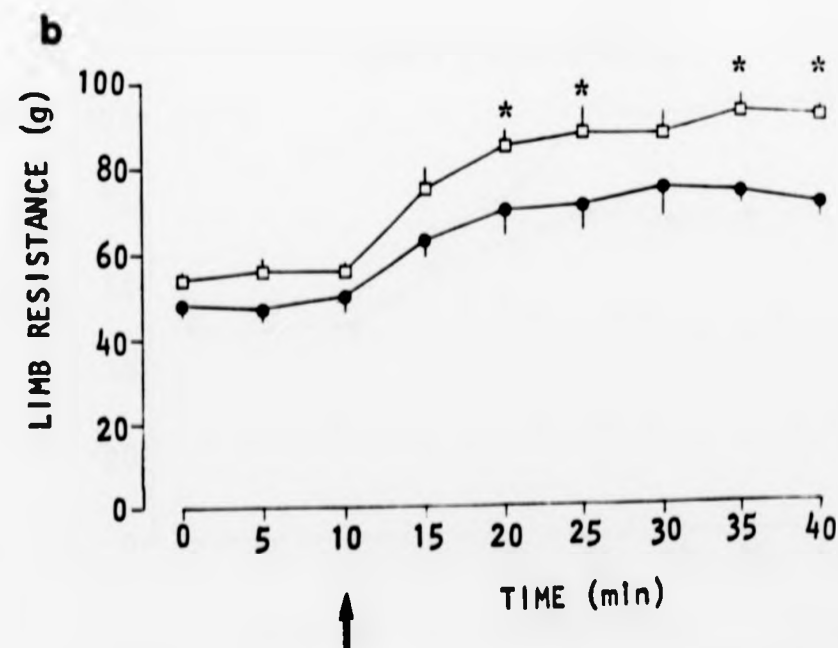
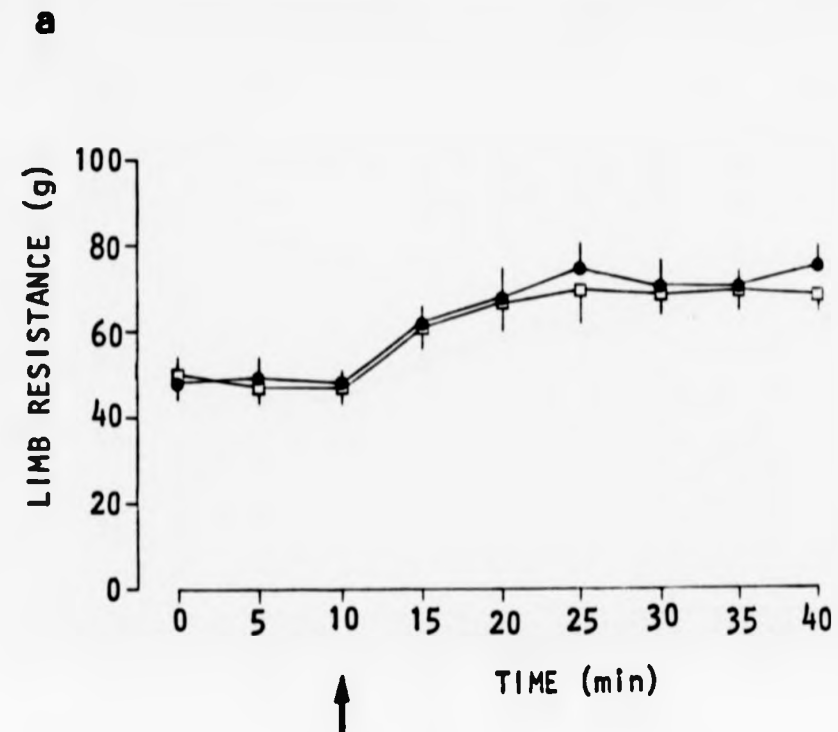


Fig 15. Effect of a unilateral ibotenic acid-induced lesion of the striatum on tremorine-induced rigidity in (a) the ipsilateral and (b) the contralateral hindlimbs of conscious rats (●—●, pre-lesion; □—□, 15 days post-lesion). Rats were pretreated with atropine methylnitrate (1mg/Kg i.p.) at time zero and tremorine (20mg/Kg i.p.) was administered at 10min (arrowed). Each point is the mean value from 6 rats. Vertical bars show the SEM. *, $p < 0.05$ vs pre-lesion using a two-tailed paired Student's t test.

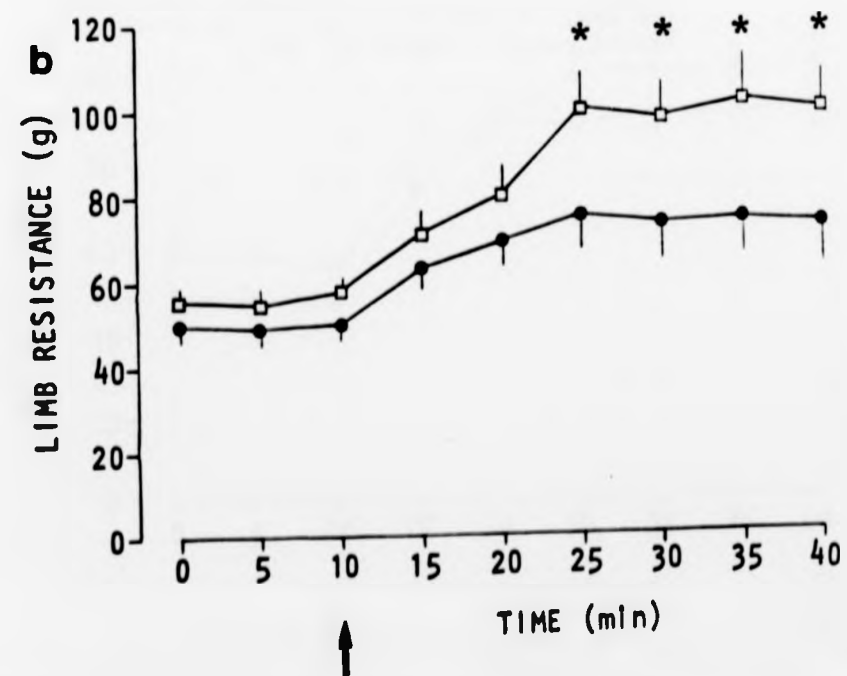
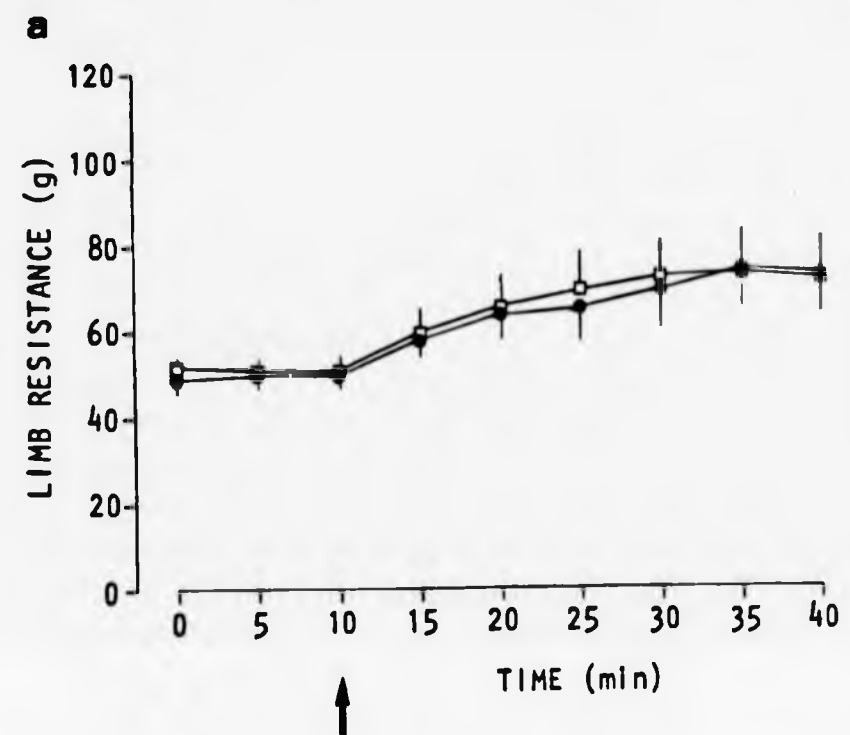
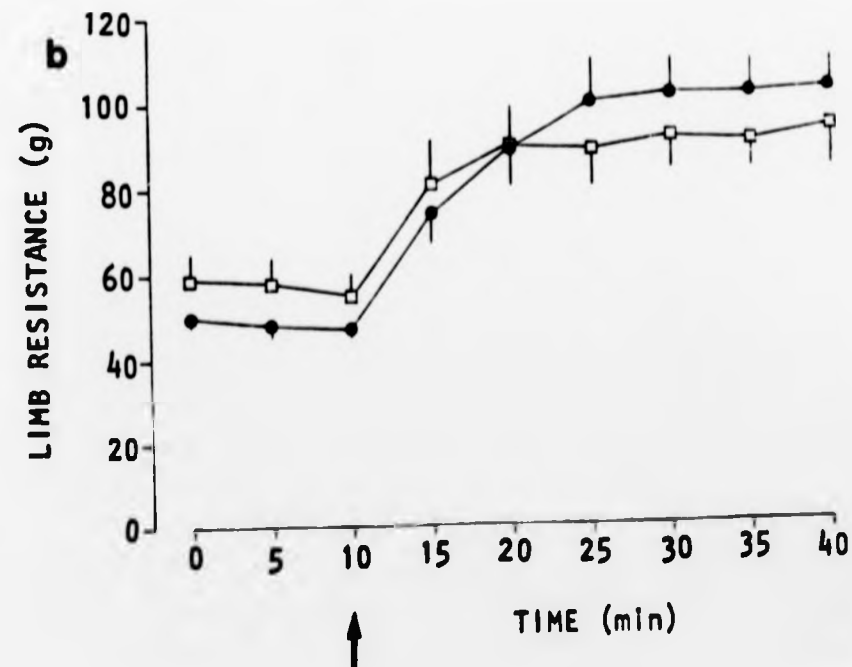
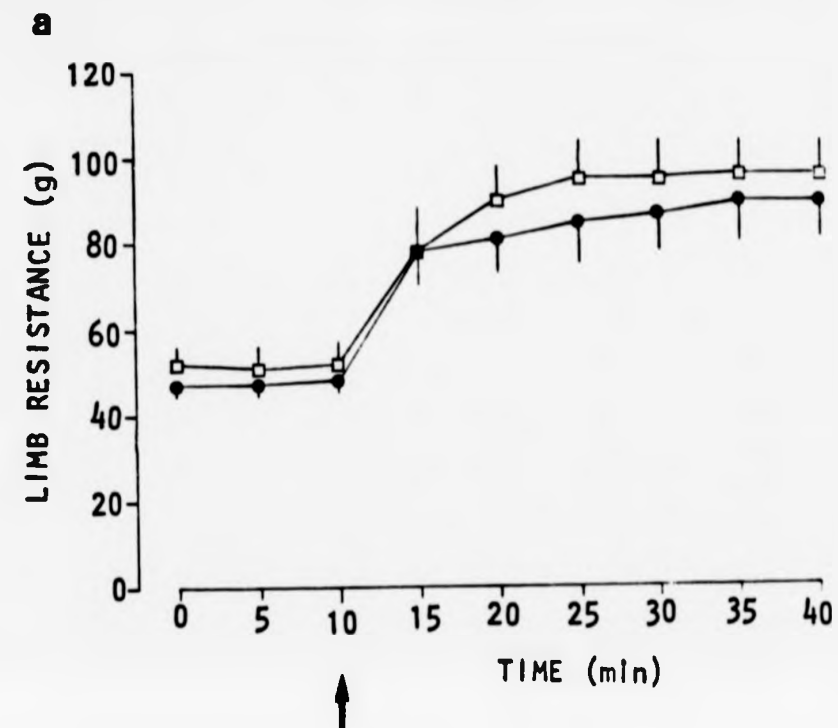


Fig 16. Effect of a unilateral kainic acid-induced lesion of the striatum on morphine-induced rigidity in (a) the ipsilateral and (b) the contralateral hindlimbs of conscious rats (●—●, pre-lesion; □—□, 18 days post-lesion). Morphine (15mg/Kg i.p.) was administered at 10min (arrowed). Each point is the mean value from 6 rats. Vertical bars show the SEM. *, $p < 0.05$ vs pre-lesion using a two-tailed paired Student's t test.



to the increased tonus of the contralateral hindlimb.

The effect of morphine on hindlimb tone was only evaluated in animals with kainic acid-induced striatal lesions (Fig.16). The lesion had no effect on resting tone or morphine-induced rigidity in the ipsilateral hindlimb. In the contralateral hindlimb, there was a small increase in resting tone and a small reduction in the magnitude of morphine-induced rigidity, although neither effect was statistically significant. When the true increase in limb tone induced by morphine (i.e. morphine-induced tone minus resting tone) was compared before and after the striatal lesion there was also no significant difference ($0.07 > p > 0.05$). No postural asymmetry was observed when these rats were removed from the restraining cage at the end of the recording period.

Evidence for the accuracy of the lesions was obtained in two ways (1) by histological examination and (2) by recording apomorphine-induced turning behaviour in the three groups of rats as described in section 2.1.5. The results of the turning experiments are shown in Table 6. It was found that apomorphine induced turning behaviour in all of the animals tested, together with the usual signs of apomorphine-induced stereotypy (compulsive sniffing and exploratory behaviour). The animals which had received the 6-hydroxydopamine-induced lesions exhibited contraversive turning behaviour whereas the rats in the other two groups demonstrated ipsiversive turning behaviour. These are the expected results from rats with successfully induced lesions (section 2.1.5).

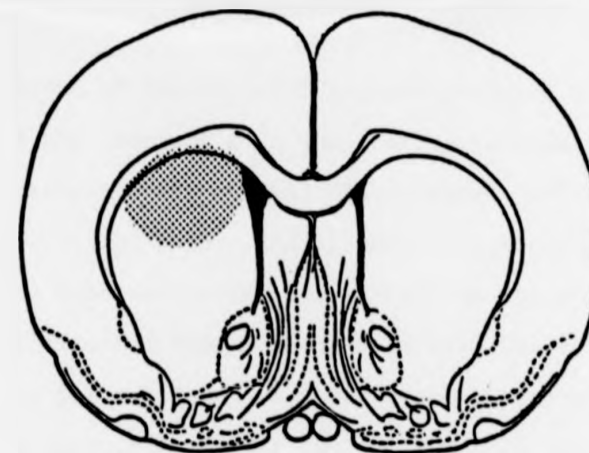
Table 6. Turning behaviour induced by apomorphine (1mg/Kg i.p.) in rats with unilateral striatal lesions

Lesion	Time of onset (min)	Duration (min)	Direction of turning	Frequency, 10min after onset (Complete turns/min)
Kainic acid (n = 6)	7 - 8	20 - 25	I/L	6 - 9
Ibotenic acid (n = 6)	7 - 9	17 - 25	I/L	5 - 9
6-Hydroxydopamine (n = 6)	3 - 4	27 - 35	C/L	11 - 14

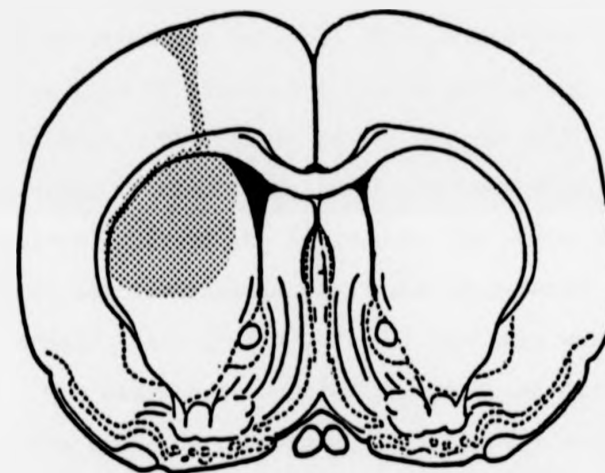
The numerical values represent the minimum and maximum values observed for each parameter. I/L, ipsilateral; C/L, contralateral.

Fig 17. Diagrammatic representation of the size of the unilateral striatal lesion induced by kainic acid. The stippled area corresponds to the area of gliosis observed at various anterior levels in all 6 lesioned rats. The increased size of the lateral ventricle on the lesioned side is clearly visible.

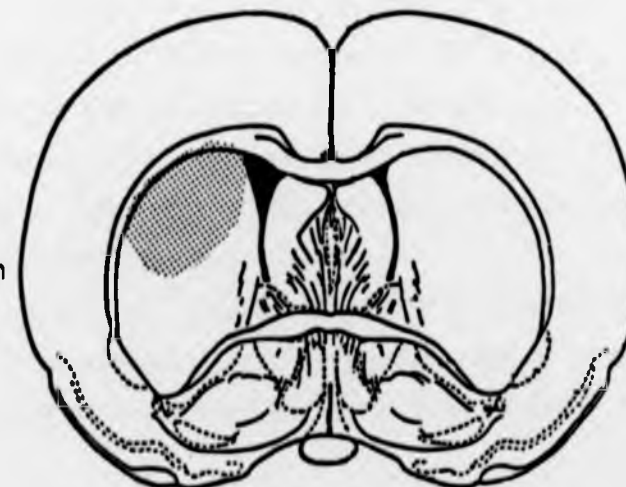
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A 7.89mm



A 7.19mm

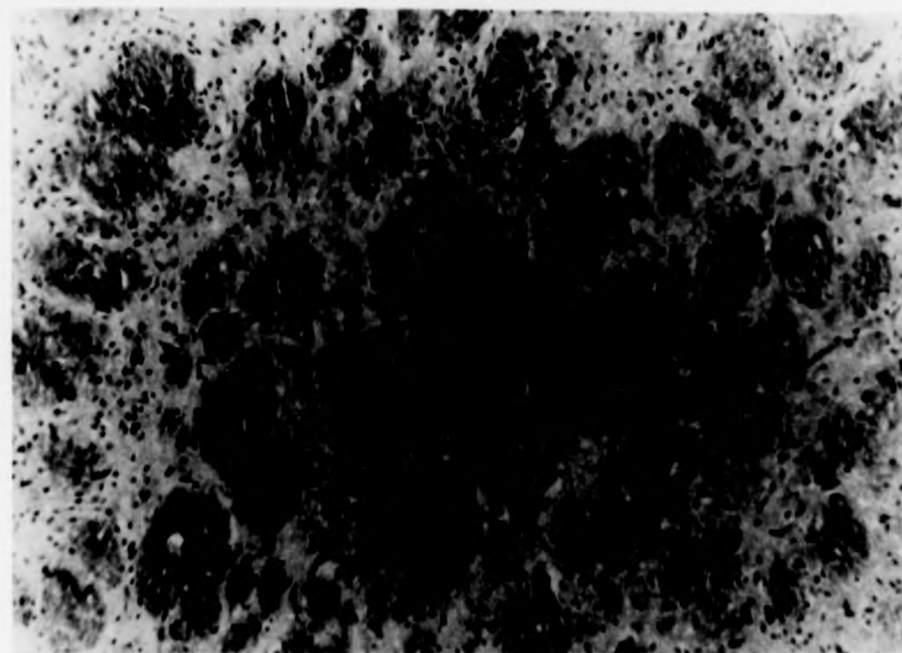


The extent of the striatal lesions produced with kainic acid and ibotenic acid were estimated from Luxol fast blue/basic fuschin stained histological sections (section 2.3). The kainic and ibotenic acid-induced lesions were confined to the dorsal half of the striatum. The lesions extended from 8.6A to 6.8A with the major lesion lying between 8.4A and 7.0A as shown in Fig.17. The lateral ventricles on the lesioned side of the brain were found to be enlarged while the diameter of the ipsilateral striatum was reduced. This was probably a result of the loss of neuronal elements within the lesioned striatum. The fibres of the corpus collosum formed a barrier to the diffusion of the neurotoxins which therefore confined the lesions to the striatum. However, some cortical damage was found associated with the cannula tract (Fig.17, 7.89A) but this was very localised. The neurotoxins produced a profound disruption of the cytoarchitecture of the striatum as shown in Plate 1. The depletion of neuronal cell bodies and increase in glial cells within the lesioned area is clearly evident in these photographs. It is also apparent that some of the fibres of passage were damaged by the neurotoxins since the individual fasciculi of fibres within the lesioned area are not as ordered as those in non-lesioned areas.

The effects of these lesions and the previously described intrastriatal drug administrations on resting and elevated hindlimb tone are summarised in Table 7.

Plate 1. Transverse sections through the striatum of the rat comparing the effect of (a) unilateral intrastriatal administration of kainic acid ($1\mu\text{g}$ in $2\mu\text{l}$) with (b) the corresponding area of the non-lesioned striatum. Sections were stained with Luxol fast blue/basic fuschin. Original magnification $\times 126$.

a



b

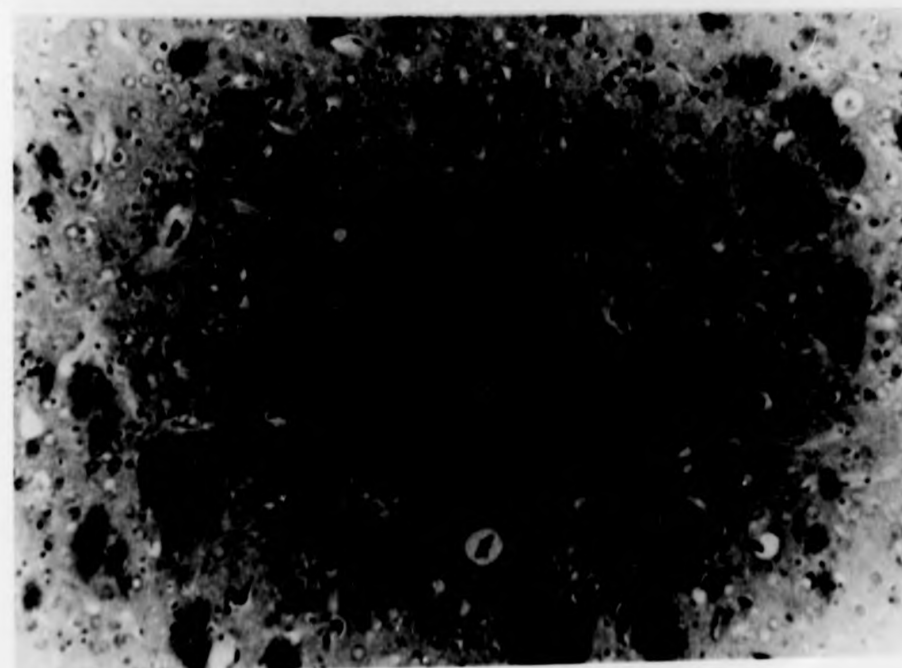
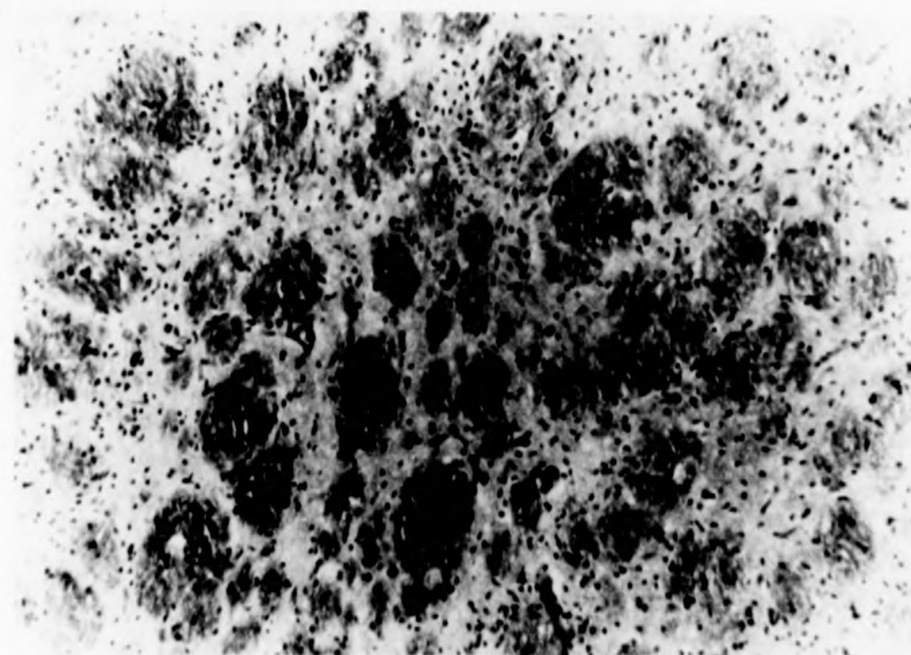


Plate 1. Transverse sections through the striatum of the rat comparing the effect of (a) unilateral intrastriatal administration of kainic acid ($1\mu\text{g}$ in $2\mu\text{l}$) with (b) the corresponding area of the non-lesioned striatum. Sections were stained with Luxol fast blue/basic fuschin. Original magnification $\times 126$.

a



b

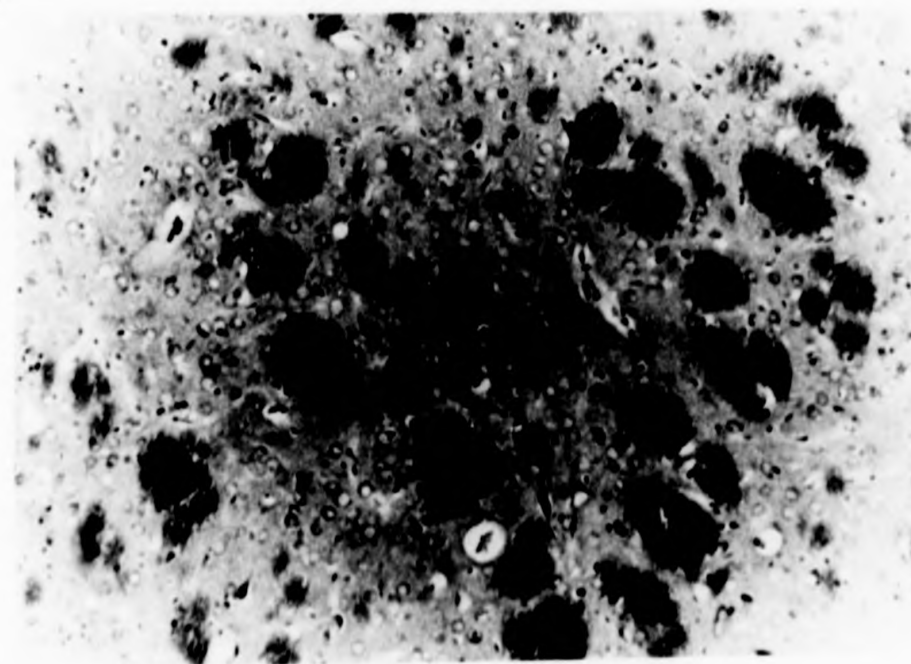


Table 7. Summary of the effects of unilateral striatal lesions and bilateral intra-striatal drug administrations on resting tone and tremorine-induced hindlimb rigidity in the rat

Treatment	Resting tone	Tremorine-induced rigidity	
		I/L	C/L
Unilateral:			
Rainic acid lesion	↑↑	↑↑	↑↑
Ibotenic acid lesion	↑↑	↑↑	↑↑
6-Hydroxydopamine lesion	↑↑	↑↑	↑↑
Bilateral:	B/L		B/L
Oxotremorine	↑		↑↑
Carbachol	↑		↑↑
Neostigmine	↑		↑↑
Apomorphine	↑		* ↑
Atropine	↑↑		↑↑
Baclofen	↑		↑↑
Haloperidol	↑↑		* ↑↑
Procaine	↑		↑↑

The arrows indicate an increase, decrease or no change compared with the paired control response. B/L, both hindlimbs pooled together; I/L, ipsilateral hindlimb; C/L, contralateral hindlimb. *, see relevant text section for further details.

3.3.4 Effects of intranigral administration of pharmacological agents on resting tone and tremorine-induced rigidity

Experiments were carried out (1) to determine whether direct stimulation of muscarinic receptors in the substantia nigra pars reticulata (SNR) could mimic the effects of systemically administered tremorine, and (2) to determine if pharmacological manipulation of SNR neurotransmitters modified tremorine-induced limb rigidity.

The rats used in these experiments had bilateral cannulae implanted into the SNR (coordinates, 2.0A, -2.6H, 1.9L) as described in section 2.1.3 and the changes in hindlimb tone were measured as described in section 2.1.6. Bilateral stimulation of muscarinic cholinceptors of the SNR was produced by two doses of oxotremorine, 2 μ g and 15 μ g (both in 1 μ l volumes), and the results are shown in Fig.18. Typical placements of intranigral microinjections are shown in Fig.11. The lower dose of oxotremorine produced no significant effects on hindlimb tone nor on the general behaviour of the rats. However, immediately following the intranigral injection of 15 μ g of oxotremorine, the rats became immobile and assumed a posture similar to that observed after systemic tremorine administration. The limb tone measurements showed that a significant increase in hindlimb tone of approximately 20g was produced which was maintained for 20min. There was no excitatory period following intranigral administration of oxotremorine as seen after the systemic administration of tremorine, although tremor and piloerection

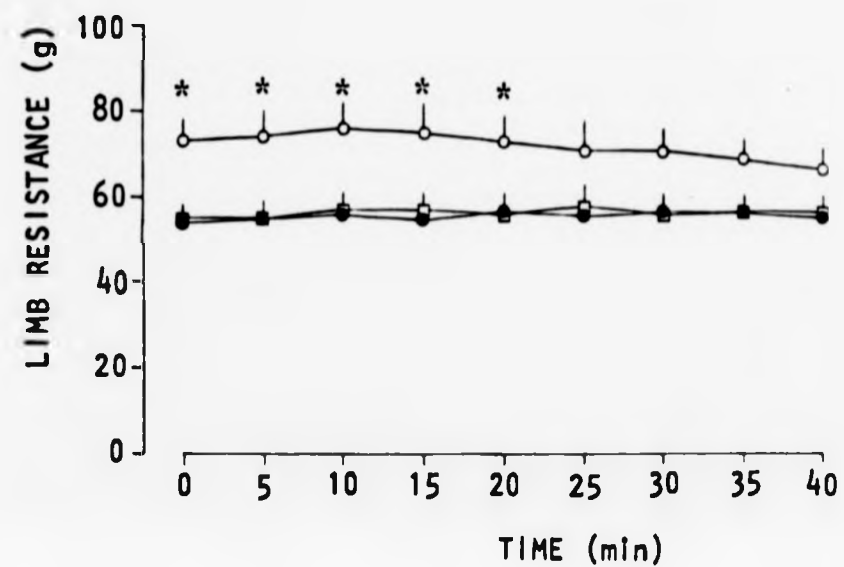


Fig 18. Effect of bilateral intranigral administration at time zero of saline (1µl, ●—●) or oxotremorine (2µg, □—□; 15µg, ○—○) on hindlimb resistance to flexion in conscious rats. Left and right hindlimb responses were pooled and each point is the mean value from 6 rats. Vertical bars show the SEM. A minimum period of 48h was allowed to elapse between successive drug administrations on the same animal. Analysis of variance $p < 0.05$; *, $p < 0.05$ vs saline-treated controls using a two-tailed paired Student's *t* test.

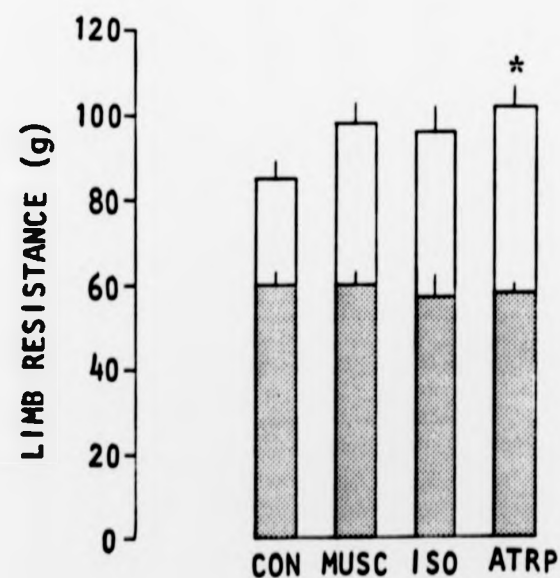
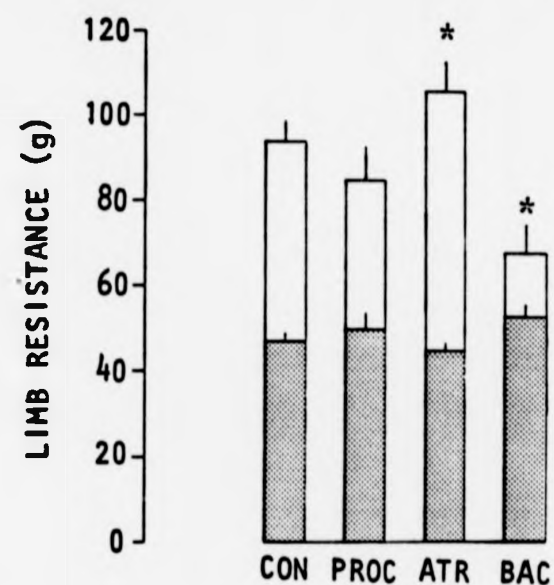
were produced. In contrast to the intrastriatal injection of oxotremorine, intranigral oxotremorine did not produce an increase in lacrimation and salivation.

These results demonstrate that intranigral administration of oxotremorine is able to mimic many of the effects produced by the systemic injection of tremorine.

The effects of bilateral intranigral application of atropine, baclofen, isoguvacine, muscimol, and procaine on the rigidity induced by the systemic administration of tremorine were compared to saline controls (1 μ l). The results are shown in Fig.19. The histograms were prepared in a similar manner to those in section 3.3.2. All intranigral microinjections were performed 10min before the systemic administration of tremorine. The effects of intranigral administration of atropine were examined using two doses, 4 μ g and 15 μ g (both in 1 μ l volumes). Neither dose produced any noticeable effect on the behaviour of the rats, nor on their resting hindlimb tone. However, both doses of atropine produced a significant increase in the tremorine-induced rigidity. There was no subjective increase in the amplitude of the tremorine-induced tremor.

Immediately following the intranigral administration of procaine (500 μ g in 1 μ l) the rats became more alert, being easily disturbed by sudden sharp noises. The resting hindlimb tone was unaltered by the intra-

Fig 19. Effects of bilateral intranigral administration of pharmacological agents on resting tone (stippled bars) and on tremorine-induced rigidity (open bars) in conscious rats pre-treated with atropine methylnitrate (1mg/Kg i.p.). Resting tone was recorded 0-10min after intranigral drug administration. Tremorine (20mg/Kg i.p.) was given at 10min and rigidity was recorded at 25-30min. All the results are the mean of both hindlimb responses recorded in 6 rats. Vertical lines show the SEM. CON, control (1 μ l saline); PROC, procaine (500 μ g); ATR, atropine (4 μ g); BAC, baclofen (2 μ g); MUSC, muscimol (50ng); ISO, isoguvacine (500ng); ATRP, atropine (15 μ g). All volumes microinjected were 1 μ l. Analysis of variance, $p < 0.05$; *, $p < 0.05$ vs saline-treated controls using a two-tailed paired Student's t test.



nigral injection of procaine, whereas tremorine-induced rigidity appeared to be reduced. However, this reduced tremorine response was not statistically significant. Tremorine-induced tremor and piloerection also appeared to be unaltered by the intranigral administration of procaine.

The intranigral administration of baclofen produced no noticeable effects on the behaviour of the rats, nor on their resting hindlimb tone. However, tremorine-induced rigidity was significantly reduced by intranigral baclofen. Also, the tremorine-induced tremor appeared to have a reduced amplitude, but as no measurements of tremor were made, this was a subjective assessment. The degree of piloerection appeared to be similar to that observed in the control situation.

The gamma-aminobutyric acid (GABA) - mimetic agents, muscimol and isoguvacine had no behavioural effects nor produced any significant changes in either resting hindlimb tone or tremorine-induced rigidity, following their bilateral intranigral administration. However, it can be seen from Fig.19 that the tremorine-induced increase in hindlimb tone of 20-30g in the control situation was rather less than recorded in previous groups. Therefore the results obtained in this group should be regarded with a little caution, but it is clear that the intranigral administration of either muscimol or isoguvacine did not produce the same effects on hindlimb tone as those observed following intranigral baclofen.

The effects of intranigral drug administration on resting hindlimb tone and tremorine-induced rigidity are summarised in Table 8.

Table 8. Summary of the effects of bilateral intranigral drug administrations on resting tone and tremorine-induced hindlimb rigidity in the rat

Drug treatment	Resting tone (B/L)	Tremorine-induced rigidity (B/L)
Oxotremorine	↑	
Atropine	—	↑
Baclofen	—	↓
*Isoguvacine	—	—
*Muscimol	—	—
Procaine	—	—

The arrows indicate an increase, decrease or no change compared with the paired control response. B/L, both hindlimbs pooled together. *, see relevant text section for further details.

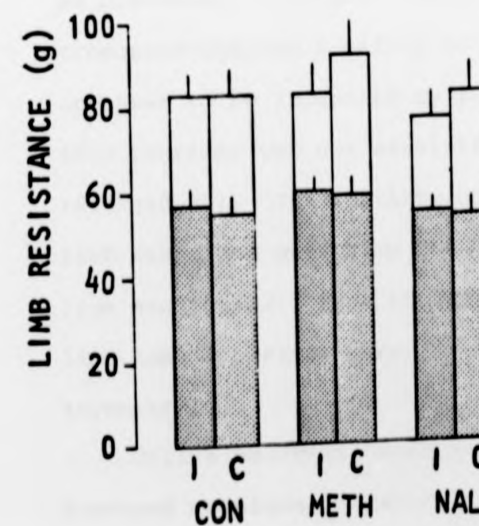
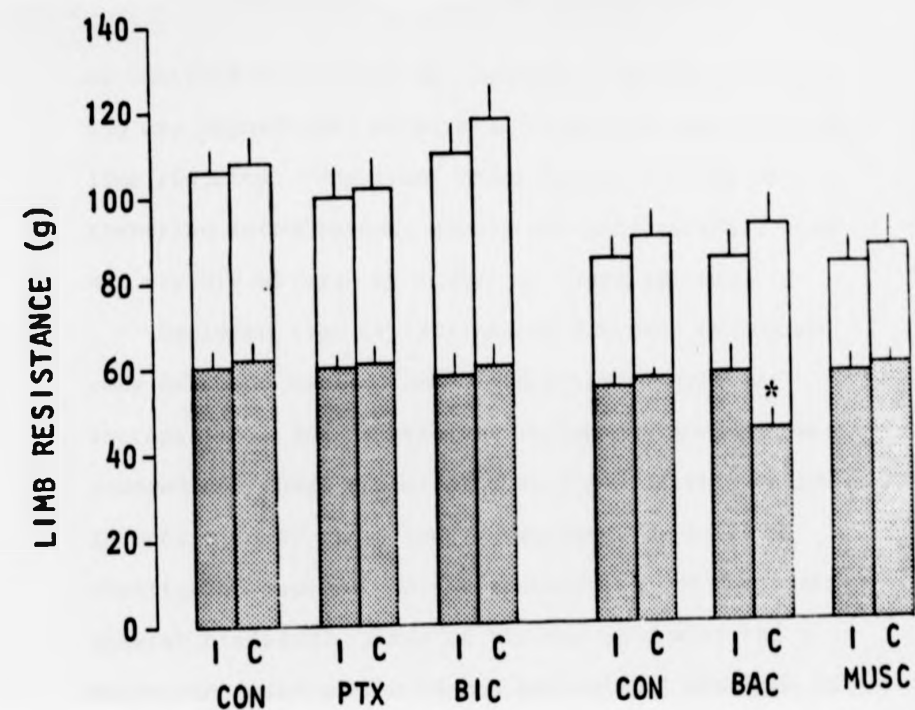
3.3.5 Effects of intrapallidal administration of pharmacological agents on tremorine-induced rigidity.

Experiments were performed to determine whether pharmacological manipulation of globus pallidus neurotransmitters could affect the increase in hindlimb tone (rigidity) produced by systemic administration of tremorine. The rats used in these experiments had bilateral cannulae implanted into the globus pallidus (coordinates, 6.6A, 1.2H, 2.5L) as described in section 2.1.3 and the changes in limb tone were measured as described in section 2.1.6. Pharmacological manipulations of the pallidum were made either unilaterally or

bilaterally. When a unilateral drug administration was performed an equivalent volume of vehicle (saline) was injected into the contralateral pallidum. All control responses to the standard dose of tremorine (20mg/Kg i.p.) were obtained following microinjection of 1 μ l of 0.9% sterile saline bilaterally into the globus pallidus. Typical placements of intrapallidal microinjections are shown in Fig.11. The effects of the unilateral administration of baclofen, bicuculline, methysergide, muscimol, naltrexone, and picrotoxin were evaluated and the results are presented in Fig.20. As with the histograms shown previously, the resting tone is the mean limb tone recorded in the 10min period before the injection of tremorine, whereas tremorine-induced rigidity is the mean limb tone recorded 25-30min after the administration of tremorine. All intrapallidal drug administrations were performed 10min before the injection of tremorine.

Immediately after the unilateral intrapallidal microinjection of picrotoxin (2 μ g in 1 μ l), a non-competitive GABA antagonist, the rats exhibited turning behaviour and postural asymmetry towards the contralateral side. Similar affects were observed after the administration of bicuculline (1 μ g in 1 μ l), a competitive GABA antagonist, into the pallidum. Some increase in spontaneous movements was observed after intrapallidal microinjection of these GABA antagonists. Neither picrotoxin nor bicuculline had any significant effect on the resting tone of either the ipsilateral

Fig 20. Effects of unilateral intrapallidal administration of pharmacological agents on resting tone (stippled bars) and tremorine-induced rigidity (open bars) of the ipsilateral (I) and contralateral (C) hindlimbs in conscious rats pretreated with atropine methylnitrate (1mg/Kg i.p.). Resting tone was recorded 0-10min after unilateral intrapallidal drug administration (1 μ l saline was injected into contralateral pallidum). Tremorine (20mg/Kg i.p.) was given at 10min and rigidity was recorded at 25-30min. All the results are the mean values from 6 rats. Vertical lines show the SEM. CON, control (1 μ l saline); PTX, picrotoxin (2 μ g); BIC, bicuculline (1 μ g); BAC, baclofen (2 μ g); MUSC, muscimol (500ng); METH, methysergide (5 μ g); NAL, naltrexone (15 μ g). All volumes micro-injected were 1 μ l. Analysis of variance only showed a significant ($p < 0.05$) difference in the baclofen and muscimol group. *, $p < 0.05$ vs saline-treated controls and vs ipsilateral leg using a two-tailed paired Student's t test.



or contralateral hindlimb. Equally, neither compound had any significant effects on tremorine-induced hindlimb rigidity. The other characteristic signs of tremorine intoxication, tremor and piloerection, were not visibly altered by either of these agents.

Baclofen (2 μ g in 1 μ l) caused the rats to become very calm and sedated immediately after unilateral intrapallidal administration. Voluntary movement was reduced and there appeared to be a generalised decrease in muscle tone. Limb tone measurement revealed a significant decrease in the resting tone of the contralateral hindlimb. Three of the six rats also had a decreased resting tone in the ipsilateral hindlimb but in the group as a whole this effect was not statistically significant. Following the administration of the standard dose of tremorine all the rats responded with increased limb tone, piloerection, and muscular tremor as previously described (section 3.2). Although tremorine-induced rigidity in the contralateral hindlimb appeared to be increased by intrapallidal baclofen, this increase was not statistically significant ($0.1 > p > 0.07$). The ipsilateral and contralateral hindlimb responses were also not significantly different from each other. When the rats were removed from the limb tone apparatus they did not show any postural asymmetry.

Unlike baclofen, muscimol (0.5 μ g in 0.5 μ l) produced no signs of sedation following its intrapallidal administration. There was some ipsilateral

turning behaviour and when placed in the restraining cage the rats had a postural asymmetry orientated to the ipsilateral side. As with the postural asymmetry induced by picrotoxin and bicuculline, it disappeared within 10min of tremorine administration. However, muscimol had no effect on resting limb tone or tremorine-induced rigidity and there were no visible effects on the tremor or piloerection. The 5-hydroxytryptamine antagonist, methysergide (5 μ g in 1 μ l), produced turning behaviour immediately after unilateral microinjection into the pallidum. The turning movements were towards the contralateral side and the rats took up an asymmetrical posture orientated towards the contralateral side when placed in the restraining cage. There were no statistically significant effects on resting hindlimb tone produced by methysergide and the small increase in tremorine-induced rigidity recorded in the contralateral hindlimb was not significantly different from the control response or the ipsilateral hindlimb response. There were no visible effects of methysergide on tremorine-induced tremor or piloerection and there was no asymmetrical posturing when the rats were removed from the apparatus.

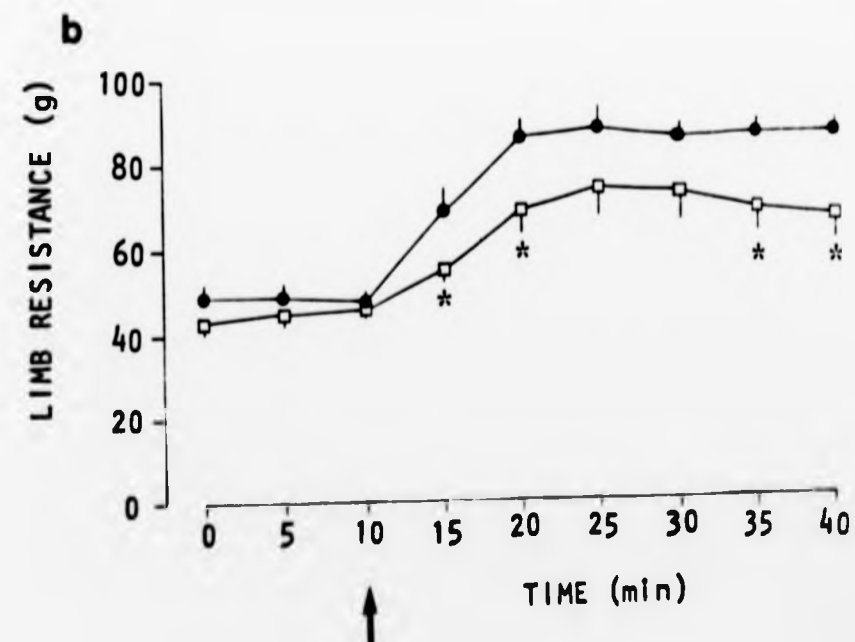
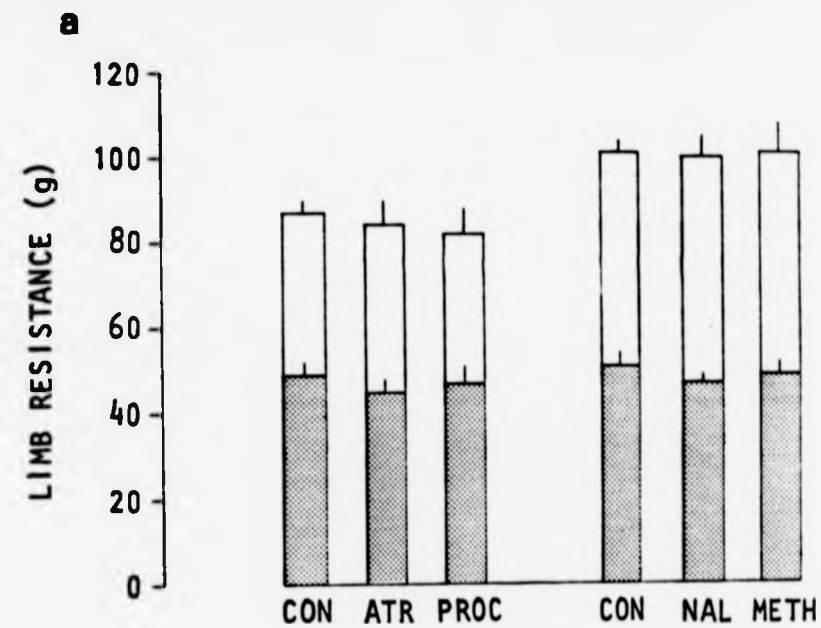
The opiate receptor antagonist naltrexone (15 μ g in 1 μ l), produced no turning behaviour, postural asymmetry, or any other changes in the behaviour of the rats following unilateral intrapallidal administration. There was no significant change in the resting hindlimb tone and the small decrease in the ipsilateral hindlimb

response to tremorine was statistically non-significant. The piloerection and tremor produced by tremorine after intrapallidal naltrexone did not appear to differ from the control response.

Bilateral pharmacological manipulations of the globus pallidus were performed in order to avoid the turning behaviour and postural asymmetry observed after some unilateral manipulations (see above) and therefore remove any effect these behaviours may have had on the recorded limb tone. Bilateral administration of atropine, baclofen, methysergide, naltrexone and procaine were evaluated and the results are presented in Fig.21. The results from both hindlimbs were pooled and the histograms were prepared in a similar manner to those in 3.3.2. Atropine (4 μ g in 1 μ l) produced no effect on the behaviour of the rats following its intrapallidal administration. There were also no significant changes in resting tone or tremorine-induced rigidity. The tremorine-induced tremor and piloerection also appeared to be unaffected by the intrapallidal micro-injection of atropine.

Immediately following the bilateral, intrapallidal administration of procaine (500 μ g in 1 μ l) the rats became calm and sedated. However, there was no reduction in the resting hindlimb tone. There was also no change in the tremorine-induced increase in hindlimb tone. The other signs of tremorine intoxication, tremor and piloerection, also appeared to be unaltered. Naltrexone (15 μ g in 1 μ l) caused no behavioural changes and did not

Fig 21. Effects of bilateral intrapallidal administration of pharmacological agents on tremorine-induced rigidity in conscious rats pre-treated with atropine methylnitrate (1mg/Kg i.p.). (a) Resting tone (stippled bars) was recorded 0-10min after intrapallidal drug administration. Tremorine (20mg/Kg i.p.) was given at 10min and rigidity (open bars) was recorded at 25-30 min. CON, control (1µl saline); ATR, atropine (4µg); PROC, procaine (500µg); NAL, naltrexone (15µg); METH, methysergide (5µg). (b) The effect of saline (1µl, ●—●) and baclofen (2µg, □—□). Tremorine was administered at 10min (arrowed). All results are the mean of both hindlimb responses recorded in 6 rats. Vertical lines show the SEM. *, $p < 0.05$ vs saline-treated control using a two-tailed paired Student's *t* test.



affect resting hindlimb tone. This was similar to the results obtained following unilateral intrapallidal administration. There was no significant change in the tremorine-induced hindlimb tone and there was also no apparent effect on tremorine-induced tremor and piloerection.

Following the bilateral intrapallidal administration of methysergide (5 μ g in 1 μ l) there were no obvious motor effects such as turning behaviour or postural asymmetry as seen with unilateral administration (see above). However, as with the unilateral microinjection of methysergide, the bilateral administration had no significant effect on resting hindlimb tone. Equally, there were no effects on tremorine-induced rigidity, tremor, or piloerection. The bilateral intrapallidal administration of baclofen (2 μ g in 1 μ l) produced different results to those obtained when it was applied unilaterally. As before, baclofen produced sedation immediately after administration but the accompanying decrease in hindlimb tone was not statistically significant from the control values. Bilateral intrapallidal baclofen also produced a significant antagonism of tremorine-induced rigidity, which was not observed after unilateral administration. However, there was no apparent change in tremorine-induced tremor or piloerection.

The effects of both unilateral and bilateral intrapallidal drug administration on resting hindlimb tone and tremorine-induced rigidity are summarised in Table 9.

Table 9. Summary of the effects of unilateral and bilateral intrapallidal drug administrations on resting tone and tremorine-induced hindlimb rigidity in the rat

Drug treatment	Resting tone		Tremorine-induced rigidity	
	I/L	C/L	I/L	C/L
Unilateral:				
Baclofen	↔	↓	↔	↔
Bicuculline	↔	↔	↔	↔
Methysergide	↔	↔	↔	↔
Muscimol	↔	↔	↔	↔
Naltrexone	↔	↔	↔	↔
Picrotoxin	↔	↔	↔	↔
Bilateral:	B/L		B/L	
Atropine	↔		↔	
Baclofen	↔		↓	
Methysergide	↔		↔	
Naltrexone	↔		↔	
Procaine	↔		↔	

The arrows indicate an increase, decrease or no change compared with the paired control response. B/L, both hindlimbs pooled together; I/L, ipsilateral hindlimb; C/L, contralateral hindlimb.

3.3.6 Effect of bilateral superior colliculus lesions on tremorine-induced rigidity

A bilateral electrocoagulation of the superior colliculus was performed on 7 rats. Three sites (1.5A, 0.5A, 0.0A, -0.8H, 2.0L) within each superior colliculus were lesioned with a DC current of 2mA for 10s as described in section 2.1.3. The effect of tremorine on hindlimb tone was measured as described in section 2.1.6, both the day before and 5 days after the production of the lesion. No changes in the general behaviour of the

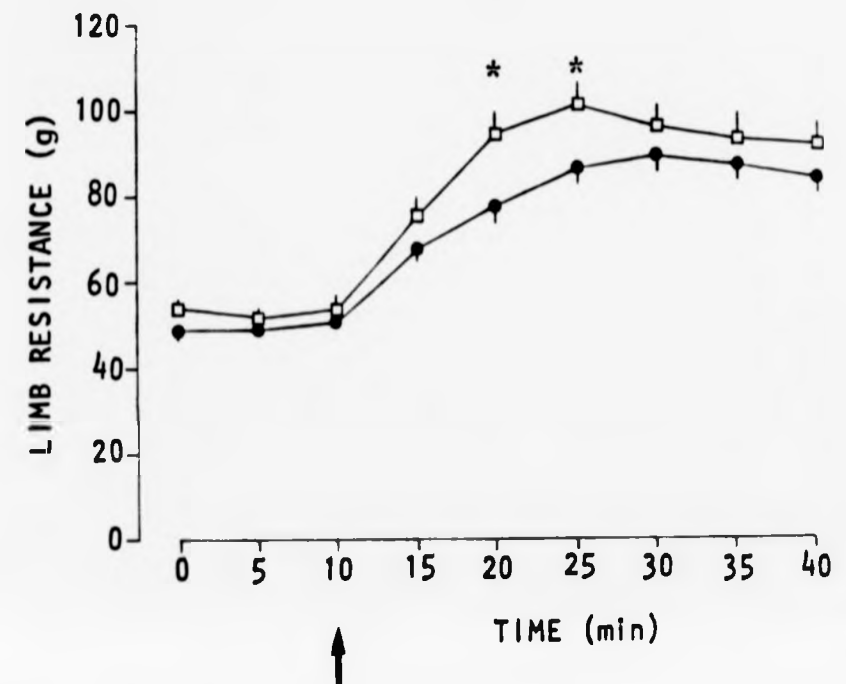


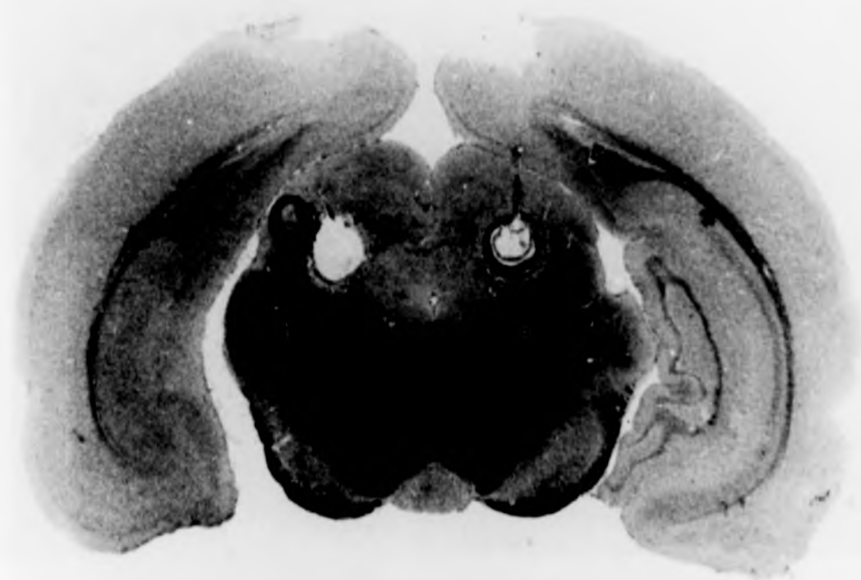
Fig 22. Effect of a bilateral electrocoagulation of the superior colliculus on tremorine-induced hindlimb rigidity in conscious rats (●—●, pre-lesion; □—□, 5 days post lesion). Rats were pretreated with atropine methylnitrate (1mg/Kg i.p.) at time zero and tremorine (20mg/Kg i.p.) was administered at 10min (arrowed). Left and right hindlimb responses were pooled and each point is the mean value from 7 rats. Vertical bars show the SEM. *, $p < 0.05$ vs pre-lesion using a two-tailed paired Student's t test.

rats were observed during the 5 post operative days. Measurements of the hindlimb tone after the production of the lesions did not reveal any significant change in the resting tone (Fig.22). However, the lesion caused an increase in tremorine-induced rigidity although this was only significantly different from control values 10-15min after tremorine administration. This period corresponds to the excitatory phase induced by tremorine (see section 3.2) which usually has the most variable measurement of limb tone of the entire recording period. However, following the superior colliculus lesion, this excitatory phase was absent although all the other characteristics of tremorine intoxication were present. There were no observed effects of the collicular lesions on tremorine-induced tremor or piloerection.

Histological observation of the lesion sites indicated that the lesions were confined to the superior colliculus in all rats, with the surrounding structures (periaqueductal grey, ventral reticular formation, inferior colliculus and overlying cortex) remaining undamaged. A typical lesion is shown in Plate 2, with the extent of the lesions being shown diagrammatically in Fig.23. The area of damage included all 6 layers of the superior colliculus with the most extensive damage being to the lateral portions of the deeper layers (the stratum griseum profundum and the stratum album profundum). The most anterior (more than 2.0A) and most posterior (more than 0.2P) parts of the superior colliculus remained undamaged.

Plate 2. Transverse sections through the rat brain showing (a) bilateral electrocoagulation of the superior colliculus and (b) recording electrode placement in the substantia nigra pars reticulata. Sections were stained with Luxol fast blue/basic fuschin. Original magnification, (a) x 64, (b) x 12.5.

a

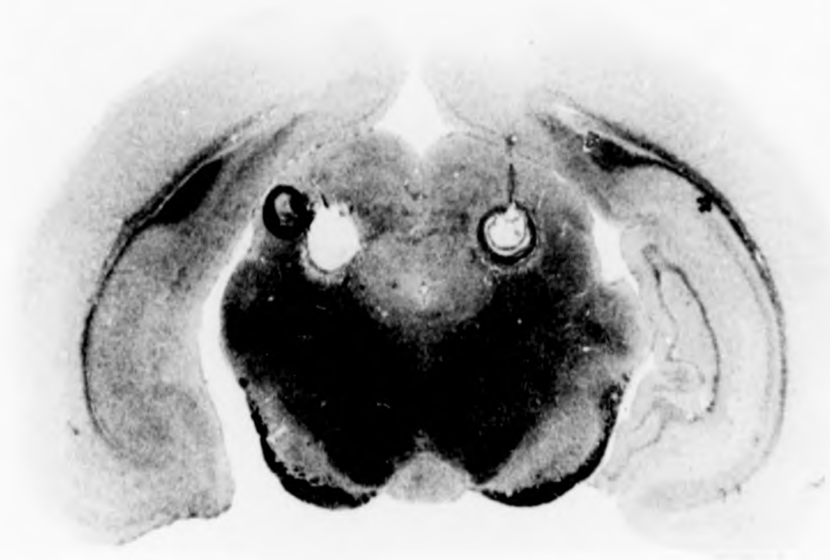


b



Plate 2. Transverse sections through the rat brain showing (a) bilateral electrocoagulation of the superior colliculus and (b) recording electrode placement in the substantia nigra pars reticulata. Sections were stained with Luxol fast blue/basic fuchsin. Original magnification, (a) x 64, (b) x 12.5.

a



b

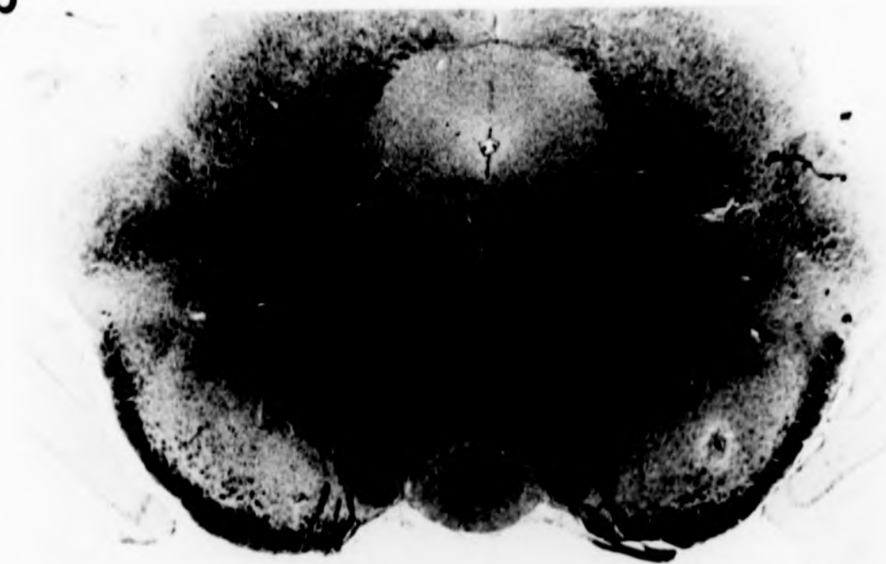
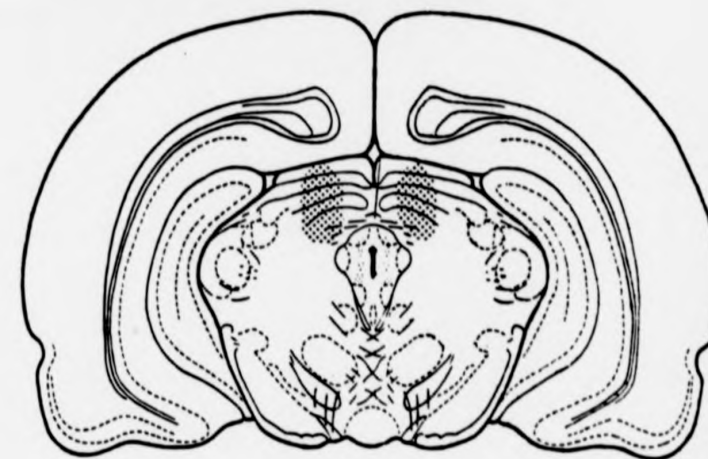
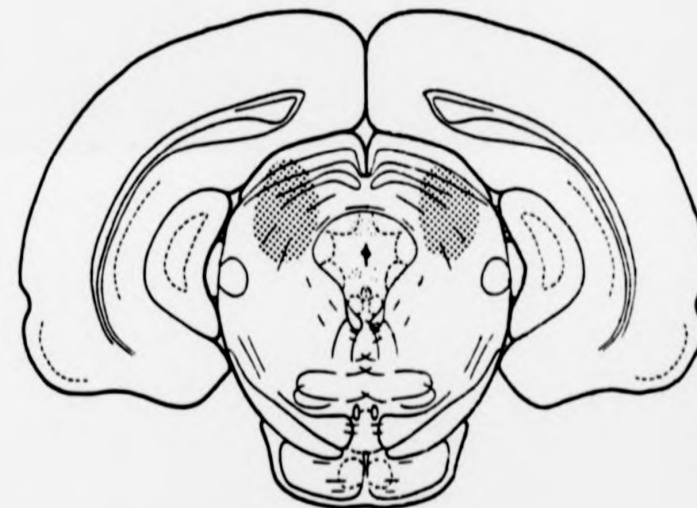


Fig 23. Diagrammatic representation of the size of the bilateral electrocoagulations of the superior colliculus. The stippled area corresponds to the area of gliosis observed at various anterior levels in all 7 lesioned rats.

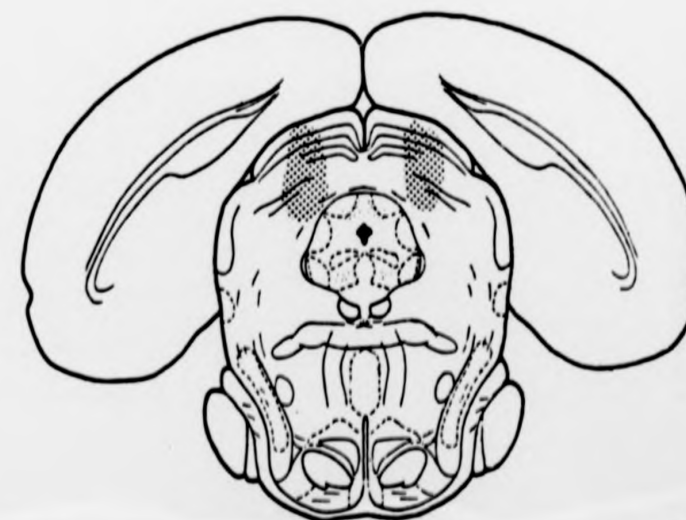
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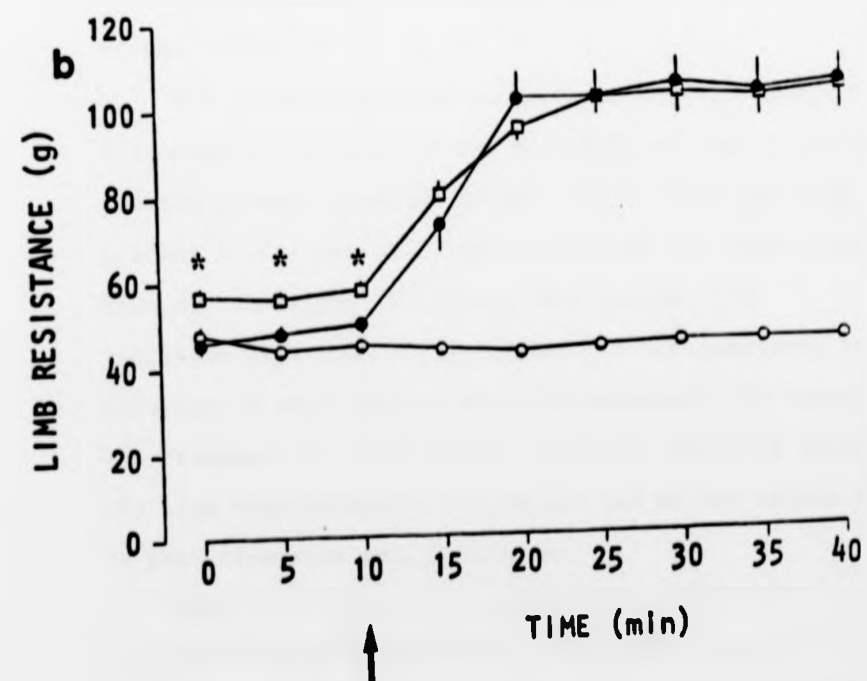
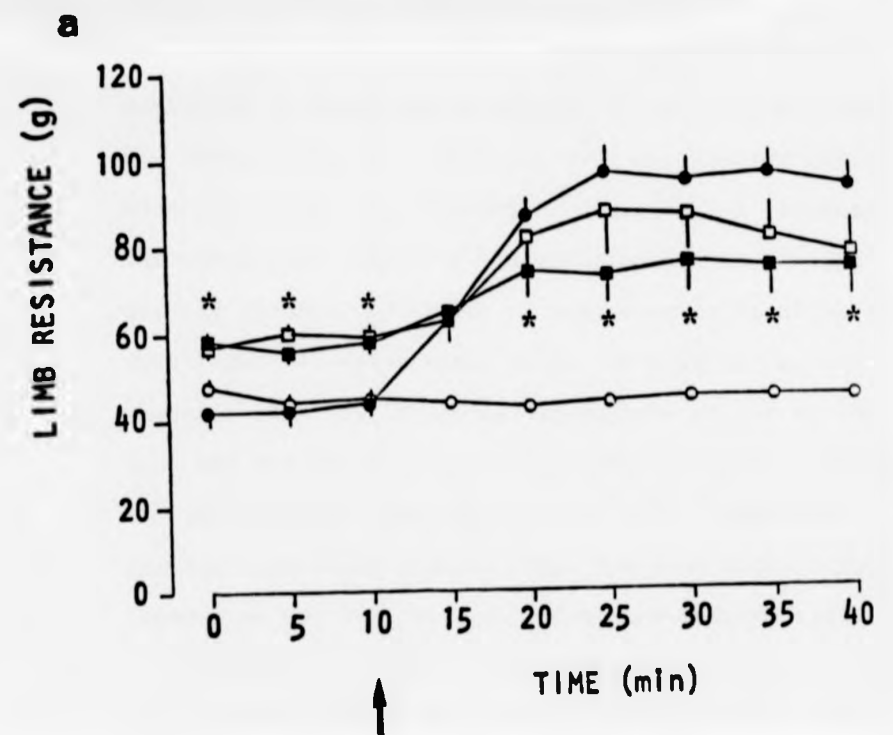


3.3.7 Effect of 3-acetylpyridine-induced lesions on limb tone

A major afferent pathway to the cerebellum from the inferior olive was destroyed by the systemic administration of 3-acetylpyridine, harmaline and nicotinamide as described in section 2.1.4. The administration of 3-acetylpyridine produced very little effect on the behaviour of the rats apart from a slight sedation which became apparent after approximately 2h. Following the harmaline injection a muscular tremor was produced. This had a lower amplitude than that produced by tremorine and the tremor was most evident in the limbs although the body musculature was also affected. The tremor gradually diminished over a period of 1h but never disappeared entirely. Voluntary movements decreased also during this period and the limbs of the rats became fully extended. During the period 6-12h after the nicotinamide injection the balance of the rats became impaired so that any corrective movements they made were too strong. However, by day 7 the motor ability of the rats had greatly improved. Voluntary walking movements with a characteristic gait were present. This has been described as "mud walking" by Llinas et al (1978), as each limb was moved in an exaggerated manner as if stuck in mud. A coarse, low frequency tremor was present and became particularly noticeable during voluntary movements.

The effect of the 3-acetylpyridine-induced lesions on elevated hindlimb tone induced by tremorine (20mg/Kg i.p.) and morphine (15mg/Kg i.p.) were

Fig 24. Effect of 3-acetylpyridine-induced lesions on hindlimb rigidity induced by the intraperitoneal administration of (a) tremorine (●—●, pre-lesion; □—□, 4 and 10 (■—■) days post-lesion) and (b) morphine (●—●, pre-lesion; □—□, 7 days post-lesion) in conscious rats. Rats receiving tremorine were pretreated with atropine methylnitrate (1mg/Kg) at time zero. Tremorine (20mg/Kg), morphine (15mg/Kg) or saline (1ml/Kg) was administered at 10min (arrowed). Left and right hindlimb responses were pooled and each point is the mean value from 6 rats. Vertical bars show the SEM. Analysis of variance, $p < 0.05$; *, $p < 0.05$ vs pre-lesion using a two-tailed paired Student's t test.



evaluated as described in section 2.1.6, and the results are shown in Fig.24. Within 4 days the resting hindlimb tone had become significantly increased, and remained so throughout the period of the experiment. The lesion greatly reduced the effect of tremorine on hindlimb tone. This reduction was greater on day 10 than on day 4 which suggests that the lesion was incomplete on day 4. Not only was the measured tremorine-induced rigidity reduced, but the increase over resting tone (i.e. tremorine-induced tone minus resting tone) was also significantly reduced on day 10. Although tremorine-induced rigidity in lesioned rats on day 4 was not significantly different from control values, the actual increase over resting tone was significantly reduced. This may have been due to the higher resting tone measured after the production of the lesion.

The 3-acetylpyridine-induced lesion also produced a pronounced increase in the amplitude of the tremorine-induced tremor, assessed subjectively. This was most evident in the period 5-15min following the administration of tremorine. Following this period, the increased amplitude of the tremor was not constant, but occurred in short bursts with approximately 10s durations. The frequency of these bursts gradually declined during the limb tone recording period and had become absent by 1h post tremorine administration.

When the effects of the inferior olive lesion on morphine-induced rigidity were evaluated on day 7, it was found that the response was essentially the same as

normal (Fig.24). An increased resting tone was recorded as before, but the morphine-induced rigidity and the incremental increase in limb tone produced by morphine (i.e. morphine-induced tone minus resting tone) were not statistically significant from control values.

Evaluation of the lesion was made both behaviourally and histologically. It has been reported that harmaline-induced tremor is abolished following the destruction of the inferior olive by 3-acetylpyridine (Simantov, Snyder and Oster-Granit, 1976). On day 12, the lesioned rats were given an i.p. injection of harmaline (15mg/Kg) and observed for 30min. Two unlesioned rats were also treated in the same way to act as controls. Within 5min of the harmaline injection, the control rats exhibited a characteristic muscular tremor. The musculature of both the body and limbs were involved in the tremor which had a lower amplitude to that induced by tremorine. The tremor induced by harmaline could best be observed in the individual digits of the rats. However, when the lesioned rats were observed following the harmaline injection, it was difficult to decide on the tremor because a low frequency high amplitude tremor was already present. The observation of the movements of the digits suggested that no additional tremor was induced by harmaline.

Microscopic evaluation of brain sections stained with either Luxol fast blue and basic fuschin or silver nitrate (Fink and Heimer, 1967) as described in section

Plate 3. Transverse sections through the hindbrain of the rat showing normal and 3-acetylpyridine-induced lesion of the inferior olive. (a) Normal inferior olive. (b) Lesioned inferior olive. The cell bodies of the inferior olive show increased silver staining and are surrounded by small black spherules. (c) Degenerating axons entering the inferior cerebellar peduncle, seen as beads of silver stain. All sections were stained according to method II of Fink and Heimer (1967). Original magnification for all sections x 160.

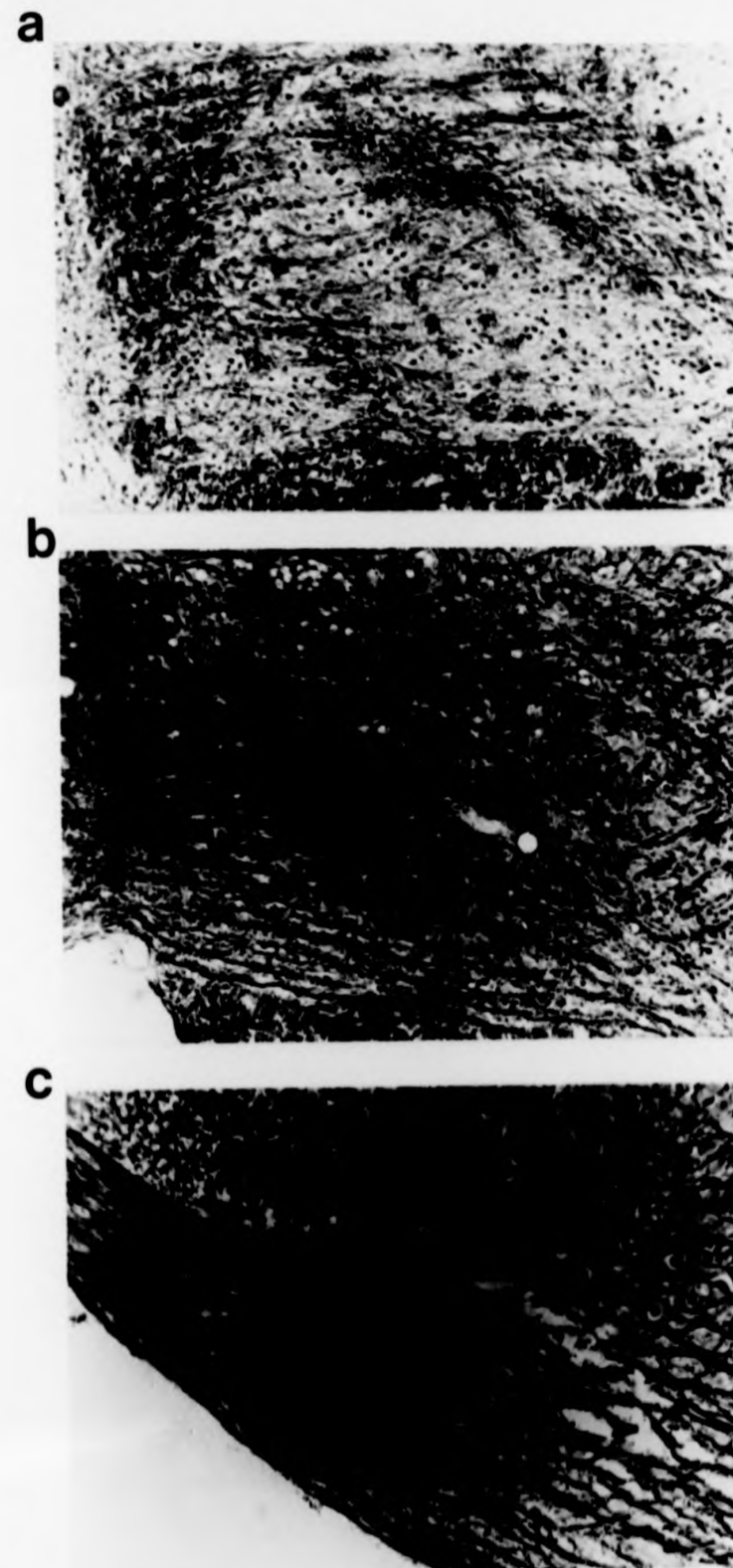
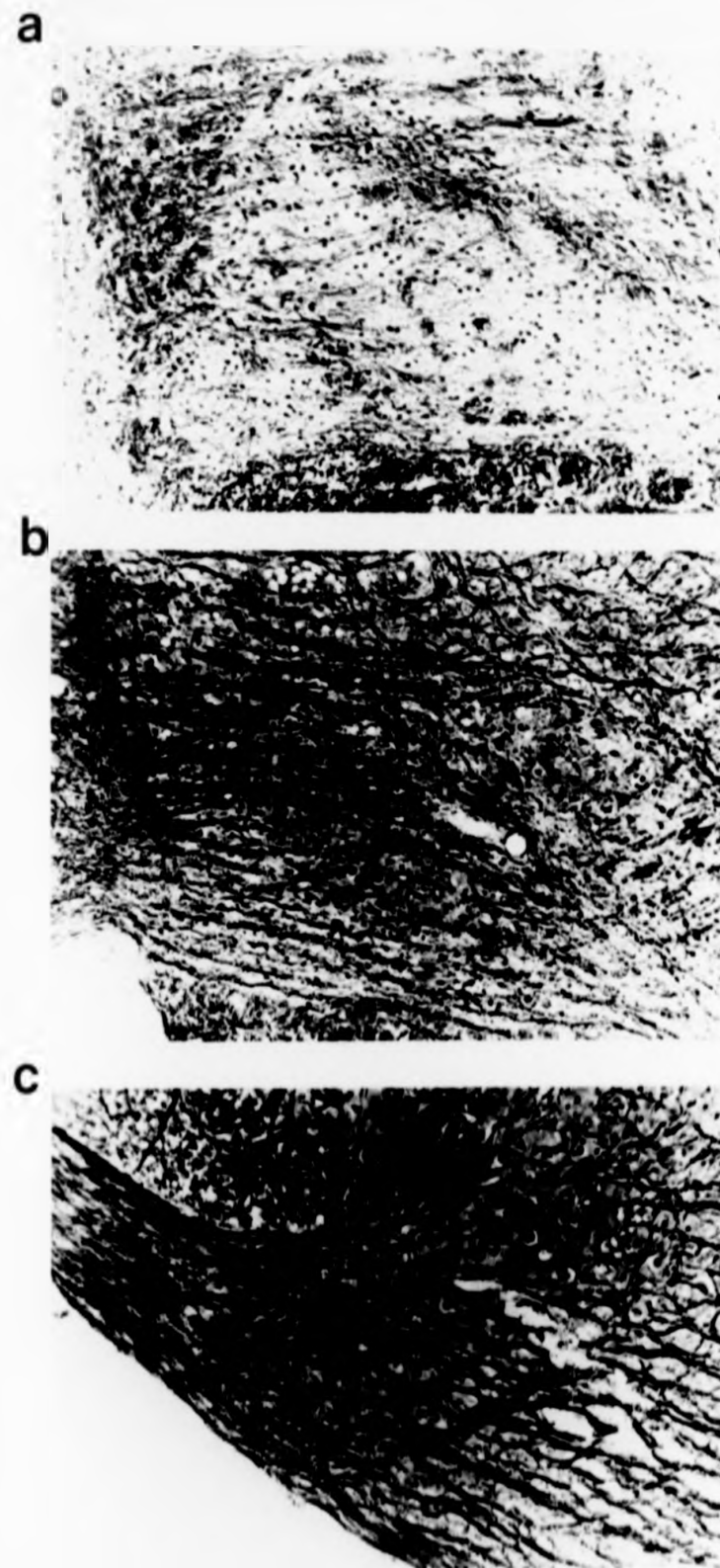


Plate 3. Transverse sections through the hindbrain of the rat showing normal and 3-acetylpyridine-induced lesion of the inferior olive. (a) Normal inferior olive. (b) Lesioned inferior olive. The cell bodies of the inferior olive show increased silver staining and are surrounded by small black spherules. (c) Degenerating axons entering the inferior cerebellar peduncle, seen as beads of silver stain. All sections were stained according to method 11 of Fink and Heimer (1967). Original magnification for all sections x 160.



2.3 were made. Examination of sections stained with Luxol fast blue and basic fuschin revealed no gliosis of the inferior olive, only a shrinkage of the neuronal cell bodies. The Fink and Heimer staining procedure, by impregnating the degenerating cell bodies and axons produced a clearer indication of the damage produced by 3-acetylpyridine. Such impregnation of cell bodies within the inferior olive are shown in Plate 3. Degenerating dendrites and axon terminals appear as scattered black spherules and can be clearly seen around the cell bodies of the inferior olive. Degenerating axons stain up as rows of black bodies, and such staining was observed entering the inferior cerebellar peduncle as shown in Plate 3. This degenerating pattern is consistent with degenerating climbing fibres from the inferior olive.

Other areas of the brain stem were also damaged. These included the nucleus ambiguus, which was most affected, and the vestibular nuclei, and the nuclei of the X and XI cranial nerves. No impregnated axons or cell bodies were found in nuclei of the mid and fore-brain such as the superior colliculus, substantia nigra, globus pallidus, entopeduncular nucleus, subthalamic nucleus, striatum and motor thalamic nuclei.

3.4 EFFECT OF INTRASTRIATAL DRUG INFUSIONS ON SINGLE UNIT ACTIVITY RECORDED IN THE GLOBUS PALLIDUS AND SUBSTANTIA NIGRA

Experiments were performed to determine the effects of pharmacological manipulations of the striatum on the recorded single unit activity of its two major output

nuclei, the globus pallidus and the substantia nigra pars reticulata (SNR). The experimental procedure was as described in section 2.2.3. Individual rats received an intrastriatal infusion of apomorphine, carbachol, oxotremorine (all 2mg/ml), or saline. All solutions contained 1% pontamine sky blue to act as a diffusional marker for the substances under test. Infusions were made at a rate of 50nl/min for 5min - thus a total of 500ng of compound was administered. Rats which initially received an intrastriatal infusion of oxotremorine or carbachol, subsequently were given tremorine (20mg/Kg i.v.) after pretreatment with atropine MN (1mg/Kg i.v.). This was done to compare the effects of systemic and local cholinomimetic administrations.

Four criteria had to be met before any changes in neuronal discharge rate were positively ascribed to the substance under test. These were; (1) changes in discharge rate were greater than 30% of the control rate, (2) changes in discharge rate lasted more than 10min and occurred within 10min of administration, (3) the discharge rate returned to control levels spontaneously unless treated with an antagonist, and (4) the infusion and recording micropipettes were successfully located within the desired structures. The results are summarised in Table 10.

The striatal infusion of carbachol or oxotremorine produced an increase in lacrimation within 5min of the start of the infusion. This was also seen after the

Fig 25. Pen recorder trace to illustrate the effect of unilateral intrastriatal infusions of (a) saline (500nl) and (b) oxotremorine (500ng) on ipsilateral substantia nigra single unit spontaneous activity. Infusions were made at 50nl/min for 5min as indicated by the bars. The arrow indicates the administration of atropine sulphate (8mg/Kg i.v.).

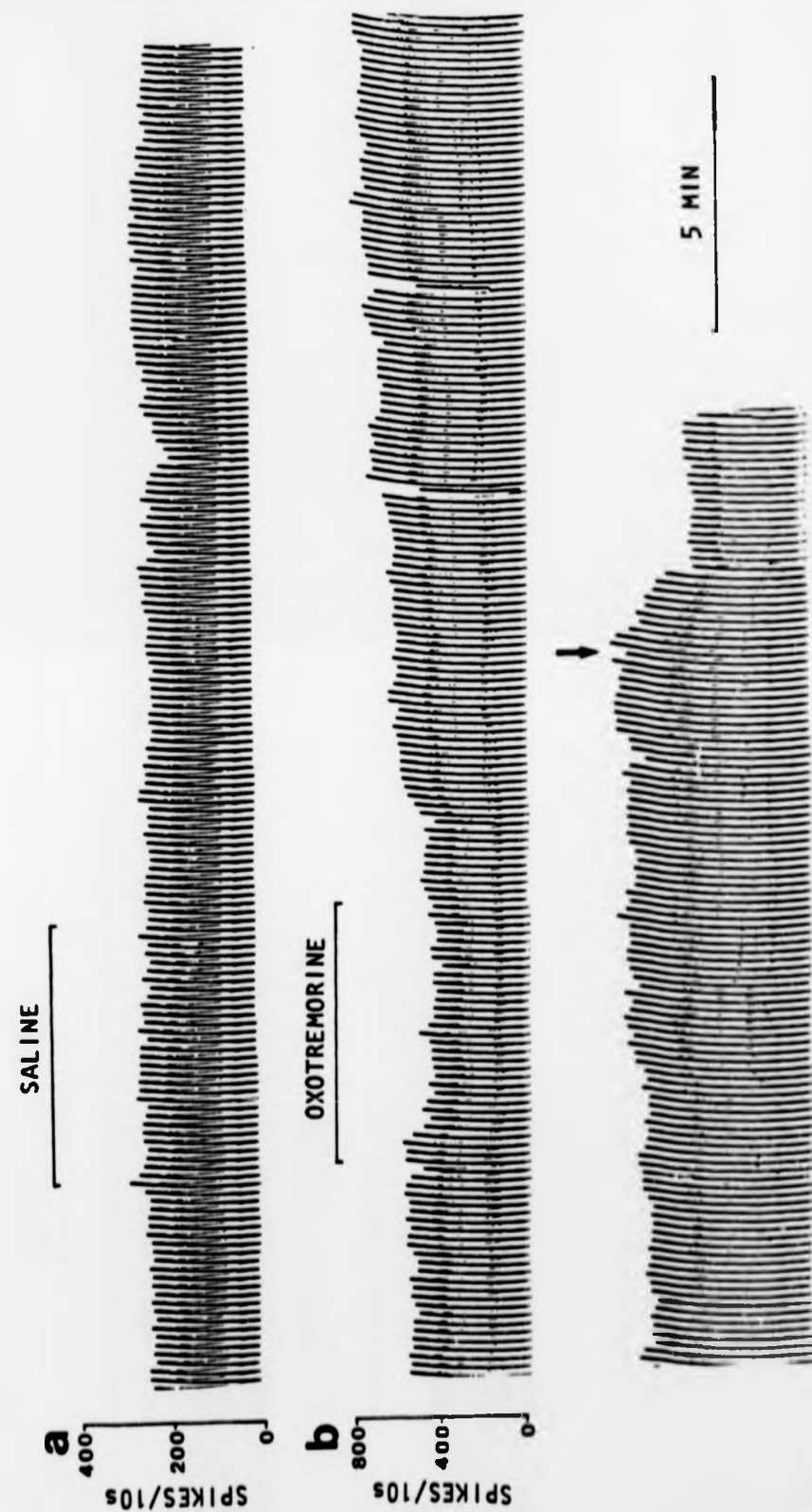


Table 10. Summary of the effects of intrastriatal drug infusions and systemically administered tremorine on the spontaneous activity (s.a.) of single units in the substantia nigra pars reticulata (SNR) and globus pallidus. See text for further details.

Drug administered	No. units with an increase in the frequency of s.a.	No. units with no change in the frequency of s.a.	No. units with a decrease in the frequency of s.a.
SNR:			
Oxotremorine (500ng)	8	9	1
Saline (500nl)	0	7	0
Tremorine (20mg/Kg i.v.)	10	1	0
Globus pallidus:			
Carbachol (500ng)	1	6	3
Oxotremorine (500ng)	2	4	2
Apomorphine (500ng)	2	6	4
Tremorine (20mg/Kg i.v.)	5	2	0

intrastriatal administration of these substances to conscious animals (see section 3.3.1). Of the 18 nigral units recorded during intrastriatal infusion of oxotremorine, 9 (50%) showed no effect, 8 (44%) showed an increase, while only 1 (6%) showed a decrease in discharge frequency. An example of a recording from a nigral unit which responded with an increase in discharge rate to intrastriatal oxotremorine is shown in Fig.25, together with a saline control. In all 7 saline control infusions no significant changes in discharge rates were recorded. In Fig.25 it can be seen that the unit firing rate was decreased slightly during the infusion of oxotremorine. This was seen on several occasions in both nigral and pallidal recording experiments. These were always of short duration and as they were seen during some saline infusions it was concluded that these were non-specific effects caused by the infusion procedure. Also from Fig.25 it can be seen that oxotremorine caused a sustained increase in discharge rate which could be quickly reversed with atropine. In experiments where the oxotremorine response was allowed to run its full course (i.e. there was no attempt at reversal with atropine) the neuronal discharge rate returned to control values within approximately 35min (range 25-55min) after the start of the infusion.

The response of nigral units to systemically administered tremorine was similar to that observed after intrastriatal oxotremorine (Table 10). Of the

11 units tested, 10 (91%) showed an increase and 1 (9%) showed no change in discharge rate. None of the units recorded responded with a decrease in discharge rate. The one unit which did not respond to tremorine was also unresponsive to intrastriatal oxotremorine. Of the 10 units which did respond to tremorine, 5 also responded in a similar manner with intrastriatal oxotremorine while the remainder were insensitive to intrastriatal oxotremorine. There was no evidence from these results that the units which responded to tremorine had different firing rates from those which responded to intrastriatal oxotremorine. The overall average control discharge rate of the recorded nigral units was 273 ± 18 spikes/10s ($n = 25$) whereas the units which responded to oxotremorine had an average rate of 286 ± 34 spikes/10s (rising to 416 ± 64 ; $n = 8$) and the units which responded to tremorine had an average rate of 275 ± 39 spikes/10s (rising to 606 ± 64 ; $n = 10$).

From Table 10 it can be seen that the results obtained from pallidal units were more variable than those obtained from the SNR. Of the 18 pallidal units that were recorded during intrastriatal infusion of oxotremorine or carbachol, 10 (55%) showed no change, 5 (28%) showed a decrease, and 3 (17%) showed an increase in discharge rate. However, the response of pallidal units to systemically administered tremorine was somewhat different; of the 7 units recorded during tremorine administration, 5 (71%) showed an increase in firing rate while the remainder showed no change. In only 2

cases were the effects of intrastriatal oxotremorine or carbachol qualitatively similar to the effects of tremorine when evaluated on the same unit. In 3 cases where intrastriatal oxotremorine or carbachol produced no change in unit discharge rate, tremorine produced an increase. In a further 2 cases where intrastriatal oxotremorine or carbachol caused a change in unit firing rate, tremorine had no effect.

There is some evidence from these results to suggest that tremorine may only produce a change in the firing rate of units with below average discharge frequencies. The overall average control discharge frequency of the recorded pallidal units was 250 ± 19 spikes/10s ($n = 30$) whereas the average control firing rate of the units which responded to tremorine was 170 ± 14 spikes/10s (rising to 295 ± 55 ; $n = 5$). Although the variances of the group as a whole and the tremorine sensitive subset are different, the latter average firing rate is in the upper quartile range of the sample as a whole. Further experiments are required to determine if this is a significant observation.

The effects of intrastriatal apomorphine (dissolved in 0.9% sterile saline containing sodium metabisulphite) were also evaluated on pallidal unit activity. Of the 12 units studied, 6 (50%) showed no effect, while 4 (33%) showed a decrease, and 2 (17%) showed an increase in discharge rate. Haloperidol (2mg/Kg i.v.) successfully reversed the increase in discharge frequency produced by apomorphine. However, there was no effect (3 units)

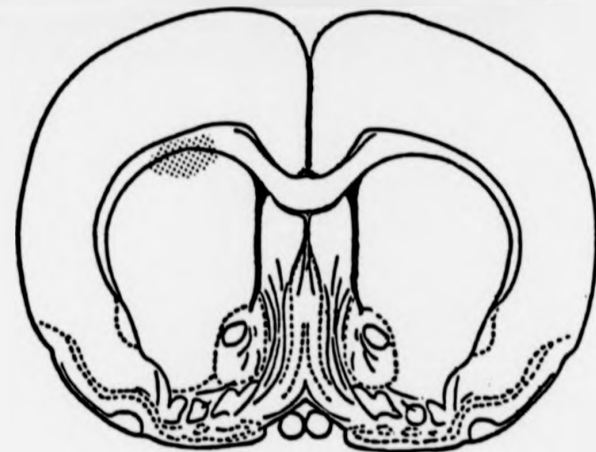
or a further reduction in firing frequency (1 unit) when haloperidol was administered following the apomorphine-induced reduction in unit discharge rate. These results, together with those obtained with oxotremorine and carbachol indicated that no consistent effect on pallidal unit activity could be produced by the intrastriatal administration of these agents.

At the completion of the experiment, the recording and infusion sites were confirmed by histological evaluation. A typical pontamine sky blue marked location of a recording electrode within the SNR is shown in Plate 2. All nigral unit recordings were made between 1.8 - 2.2A and 1.5 - 2.5L while all pallidal unit recordings were made between 6.3 - 6.6A and 2.0 - 2.6L. In neither nuclei could any correlation between recording site and discharge frequency be determined. The typical extent of diffusion of an intrastriatal drug infusion is shown in Fig.26. The drugs tended to diffuse from the micropipette tip back up the pipette track to the corpus collosum fibres. These presented a natural barrier to the diffusion of the drug and restricted it to the striatum. However, some of the drug solution did enter the deeper layers of the cortex in 3 experiments but this was always small compared to the amount within the striatum, and was restricted to the pipette track. There was no evidence in any of the experiments of the drug solution entering any other brain areas such as the lateral ventricles.

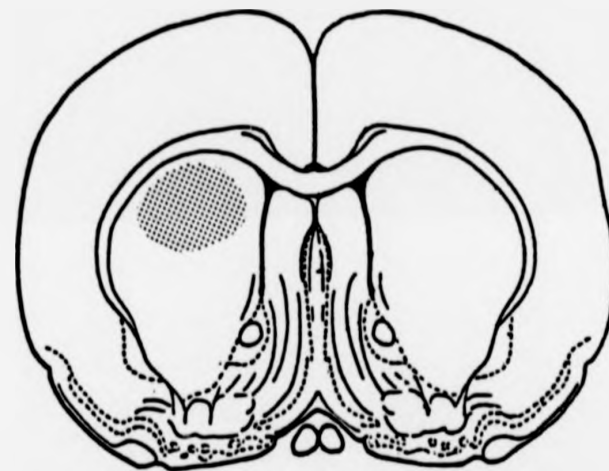
In summary, both the localised intrastriatal

Fig 26. Diagrammatic representation of the volume of the striatum infused in the electrophysiology experiments. The stippled area corresponds to the distribution of the infused pontamine sky blue observed at various anterior levels in a representative experimental animal.

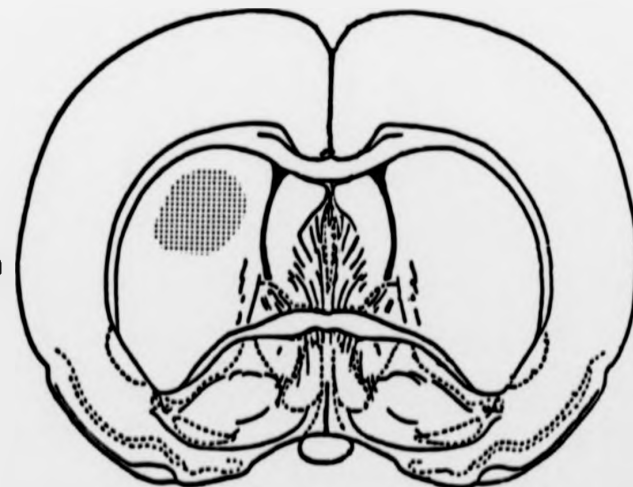
A 8.38mm



A 7.89mm



A 7.19mm



infusion of oxotremorine and the systemic administration of tremorine produced an increase in the single unit activity of the SNR. However, no such correlation could be determined from similar investigations of the globus pallidus.

3.5 BIOASSAY OF OXOTREMORINE IN BRAIN

A bioassay procedure was used to determine the amount of oxotremorine in rat brain after administration of the standard dose of tremorine (20mg/Kg i.p.) used in the limb tone experiments. A guinea pig isolated ileum preparation was used and the bioassay procedure is described in section 2.5.2. On two separate occasions brain extracts were prepared from 3 rats (see section 2.5.1) and assayed. One rat received no treatment before sacrifice, one received atropine MN (1mg/Kg i.p.) + tremorine, and the third received only tremorine. The rats were sacrificed 20min after tremorine administration. The guinea pig ileum preparations responded to as little as 10ng/ml bath of oxotremorine but they failed to contract in response to any brain extract with volumes of as great as 1ml. On one occasion a brain extract from an untreated rat brain produced a decrease in ileum tone of 5mm (equivalent to 0.25g). This lack of spasmogenic activity was not due to the presence of atropine MN antagonising the effects of the oxotremorine since no contraction of the ileum was produced by the brain extract of the rat which received only tremorine. Also no spasmogenic agents known to be present in brain (histamine, 5-hydroxytryptamine) were extracted. This

may have been due to the extract being performed in neutral buffered Krebs' solution.

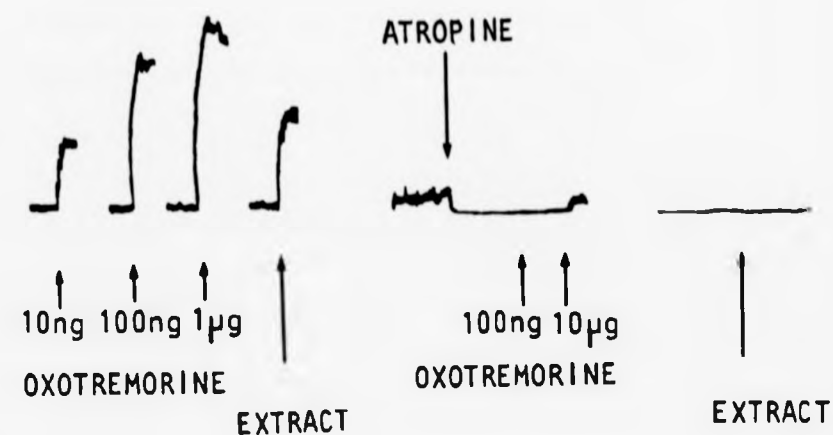
It was decided to test the extract procedure by producing an extract from a brain known to contain oxotremorine. In two separate experiments, a rat was anaesthetised with urethane and had 10 μ g of oxotremorine (in 1 μ l) microinjected into one striatum as described in 2.1.3. After 20min the rat was sacrificed and a brain extract was prepared. This extract caused a contraction of the guinea pig ileum and a 3-point bioassay was performed. Fig.27 shows typical responses produced. On both the test and concurrent control tissues oxotremorine and the brain extract produced distinctive short latency contractions. These were atropine sensitive and the atropine antagonism could be surmounted by the further addition of oxotremorine.

The results from the bioassay experiments were used to estimate the recovery rate of oxotremorine from rat brain by following the procedure of University of Edinburgh, Staff of Pharmacology (1968). One experimental study produced an estimate of 67-89% recovery, and a second gave an estimate of 65-92% recovery. Using the worst estimates, i.e. 65% recovery of brain oxotremorine, 1ml of extract being added to the tissue bath, and 10ng/ml bath being the least detectable amount of oxotremorine, the minimum detectable quantity of oxotremorine in the brain can be calculated. The resulting calculation becomes;

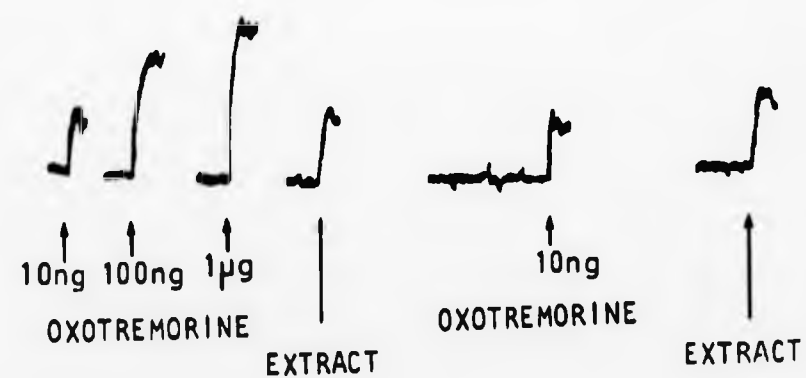
$$10 \times 30 \times 3 \times 100 / 65 = 1384.6\text{ng or } 1.4\mu\text{g}$$

Fig 27. Pen recorder trace showing the responses of isolated guinea pig ileum preparations to oxotremorine (g/ml bath) and the brain extract (0.3ml) prepared from a rat pre-treated with intrastriatal oxotremorine (10 μ g). Both the test and concurrent control tissues responded in a dose-dependent manner to oxotremorine. Atropine (250ng/ml bath) antagonised the oxotremorine and brain extract responses on the test tissue.

TEST TISSUE



CONCURRENT CONTROL TISSUE



1g
1min

Thus, the brain extract and bioassay procedures should be able to detect 1.4 μ g of oxotremorine in a rat brain. Therefore it can be concluded that the total amount of oxotremorine in the brain of a rat treated with tremorine (20mg/Kg i.p.) was less than 1.4 μ g.

SECTION FOUR : DISCUSSION

4.1 LIMB TONE MEASURING APPARATUS AND PRELIMINARY INVESTIGATIONS

Muscle rigidity is a phenomenon little studied in laboratory animals, mainly due to a lack of suitable methods for quantifying muscle tone. Most studies have used subjective (De Montis et al,1979) or semi-quantitative methods (Schallert et al,1979) to assess muscle tone. These have been useful in gross behavioural screening studies as the animals are not restrained, but they lack the objectivity of more quantitative methods such as those using electromyographic and mechanographic techniques. The electromyographic method requires the use of needle electrodes which are usually placed in the gastrocnemius-soleus muscle of the rat (Wand et al,1973; Ellenbroek, Schwartz, Sontag and Cools,1984). This is clearly not an ideal technique to use where multiple observations are required in conscious animals. The assumption behind this method is that the electromyographic activity recorded from one muscle accurately represents the rigidity of the limb as a whole. Since there is abundant evidence that proximal and distal muscle groups are controlled by different descending pathways (Kuypers,1981) this assumption may not be valid. One further drawback to this method, which is particularly relevant to the present studies, is that since it is an estimate of the overall electrical activity in the muscle concerned it is not possible to differentiate between the activity associated with rigidity from that causing tremor.

Several mechanographical methods of quantifying muscle tone have been reported (Dickinson and Slater, 1982; Johnels and Steg, 1982). These techniques require the animals to be restrained and muscle tone is measured as the resistance to flexion of the limb. The method of Johnels and Steg (1982) involved the movement of only the ankle joint, and as this joint is controlled by distal muscle groups this method has a similar disadvantage to the method using electromyography of the gastrocnemius-soleus muscle. The method of Dickinson and Slater (1982) had a movement which involved more than one limb joint but was found to be unsuitable to measure tremorine-induced rigidity as the movement caused by the concurrently induced tremor was also recorded and made the readings from the resulting pen trace inaccurate.

Due to the unsuitability of the existing mechanographical methods for quantifying tremorine-induced rigidity, a specifically designed apparatus (based on another apparatus in use in this laboratory; Dickinson, 1981) was constructed and rigorously evaluated. It was found that the two sides of the apparatus responded independently when the load differential on the two sides was less than 70g. This was considered to be satisfactory as it was expected (and subsequently found) that such a large differential would not arise during experiments on rat hindlimb tone. The apparatus was found to respond in a linear fashion over the range 0-90g which did not change significantly over

a 3h period, and was also shown to quantitatively record limb tone changes in the rat induced by a variety of drugs, including an increase in limb tone by morphine, oxotremorine and tremorine and a decrease in limb tone by methohexitone.

This apparatus overcame the disadvantages of other methods in several ways. First, all limb joints were flexed during the movement cycle. Secondly, both hindlimbs were evaluated simultaneously and finally, the tremorine-induced tremor did not interfere with the rigidity recordings. This apparatus gave different estimates of tremorine-induced rigidity than that described by Dickinson (1981) in that the response to tremorine per se was approximately 30g recorded with the present apparatus whereas with the same dose of tremorine it was about 15g on the older apparatus (Dickinson and Slater, 1982; Slater and Dickinson, 1983). The reason for this difference may be due to the fact that in the present apparatus simultaneous recordings were made from both hindlimbs whereas only one limb was tested at a time with the older apparatus. Taking these results together, it is clear that this newly constructed limb tone measuring apparatus constitutes a stable, reliable and objective method for quantifying limb tone changes in the conscious rat.

Preliminary studies were undertaken to select the most suitable dose of tremorine to produce a consistent limb rigidity. The well described, centrally mediated, motor effects of tremorine (rigidity and tremor) were

found to be dose-related, and overall the most appropriate dose of tremorine for the present study was found to be 20mg/Kg. The reproducibility of the limb rigidity induced by this dose of tremorine was evaluated. It was found that while the characteristic signs associated with tremorine administration were always produced (Everett et al, 1956; Korczyn and Eshel, 1979), the recorded values of hindlimb rigidity were not always the same: the magnitude of the hindlimb response to the first administration of tremorine was smaller than on subsequent occasions. However, the second and third administrations of tremorine produced closely similar results. As a result of this observation, it was decided to ignore the results obtained with the first dose of tremorine and to use the responses obtained from the second and subsequent administrations in this study. The reason why the initial response appeared to differ from subsequent responses is unclear and has not been reported before. Therefore, this observation has implications for other studies involving tremorine-induced limb tone changes where comparisons are made between initial and subsequent administrations of tremorine. The only studies in which a change of response to tremorine and oxotremorine has been reported suggests that chronic infusions of these compounds leads to the development of tolerance (Desci, Varszegi and Méhes, 1961; Marks, Artman and Collins, 1983). Whatever the reason for this apparent sensitisation to tremorine, its effect on the results of this study was

circumvented by not including data obtained after the first administration of tremorine.

In agreement with other studies, it was found that oxotremorine, the active metabolite of tremorine, produced qualitatively similar effects to tremorine on the behaviour of the rat (Ringdahl and Jenden, 1983). The one difference was that the changes in motor behaviour induced by oxotremorine were much shorter lasting. For example, oxotremorine caused immobility for 7-10min whereas tremorine produced immobility for more than 40min. This demonstrates the advantage of using pro-drugs, producing a prolonged and sustained pharmacological effect. This also provides justification for the use of tremorine rather than a directly acting, centrally active, muscarinic cholinomimetic agent to induce muscle rigidity.

4.2 THE STRIATUM AND TREMORINE-INDUCED RIGIDITY

The striatum is considered to be the major nucleus of the extrapyramidal motor system (Carpenter, 1981) and has one of the highest densities of muscarinic cholinergic receptors (Rotter, Birdsall, Burgen, Field, Hulme and Raisman, 1979a; Nonaka and Moroji, 1984) and richest cholinergic innervations of any brain region in the rat (Armstrong, Saper, Levey, Wainer and Terry, 1983; Wainer, Levey, Mufson and Mesulam, 1984). Also, this brain region has been previously implicated in the production of muscle rigidity induced by reserpine (Johnels, 1983), morphine (Havemann et al, 1981) and

tremorine in the rat (Dickinson and Slater, 1982). Consistent with these observations, in the present study the intrastriatal application of both oxotremorine and carbachol produced dose-related increases in rat hindlimb tone. The effective dose range for oxotremorine was between 1 and 10 μ g. There is evidence from the present findings that the dose-response relationship may have a bell-shaped curve as the 50 μ g dose produced a smaller response than both 30 and 10 μ g. It is not clear whether carbachol exhibited a similar dose-response relationship since this compound was less thoroughly investigated. However, the intrastriatal application of both oxotremorine and carbachol produced similar effects on motor behaviour (i.e. rigidity and tremor) to systemically administered tremorine. The finding that oxotremorine and carbachol both stimulated salivation and lacrimation when microinjected into the striatum suggests that activity in autonomic efferent pathways is influenced by striatal neuronal activity. This idea is supported by the observation that changes in blood pressure occurred in cats immediately following the intrastriatal administration of carbachol (Pazo and Medina, 1983) and that an increase in salivation has been reported following dopaminergic stimulation of the striatum in the rat (Pazo, Medina and Tumilasci, 1982).

In contrast to the effects of oxotremorine and carbachol on hindlimb tone, it was found that when the level of endogenous acetylcholine within the striatum was increased by the local administration of the

cholinesterase inhibitor neostigmine, not only was no rigidity recorded but also a significant reduction of hindlimb tone was induced after about 1h. The tremor induced by neostigmine also differed qualitatively from that produced by the stimulation of striatal muscarinic cholinceptors by oxotremorine and carbachol. There are several possible explanations why the intrastriatal administration of neostigmine and oxotremorine had different effects on limb tone and tremor. It may be that the dose of neostigmine used was insufficient to raise the levels of acetylcholine within the striatum sufficiently to activate the mechanisms which induce rigidity. As intrastriatal neostigmine caused tremor, this hypothesis implies that the threshold level of acetylcholine for producing limb rigidity is higher than for tremor. There is some supporting evidence for this from the preliminary dose-response experiments with tremorine. It was found that low doses of tremorine produced tremor without the concomitant induction of rigidity.

A second explanation may involve the acetylcholine receptor sub-types. Acetylcholine has an affinity for both muscarinic and nicotinic cholinceptor sub-types, whereas oxotremorine selectively stimulates the muscarinic sub-type (Ringdahl and Jenden, 1983). Although nicotinic receptors are not particularly dense within the striatum (Martin and Aceto, 1981; Rainbow, Schwartz, Parsons and Kellar, 1984), it is entirely possible that nicotinic receptor stimulation could counteract the

effect of muscarinic receptor stimulation. Against this argument is the fact that carbachol, which had a similar action to oxotremorine, is known to be an agonist at both cholinceptor sub-types, although it is more selective for the muscarinic receptors. To resolve the question of whether the discrepancies are due to the two sub-types of cholinceptor, the effect of intra-striatal administration of a selective nicotinic receptor agonist (such as nicotine) needs to be investigated.

The experiments discussed so far have been consistent with the hypothesis that tremorine produces limb rigidity and muscular tremor via the stimulation of muscarinic receptors within the striatum. It has been shown that the minimum amount of oxotremorine required within the two striata to produce these effects lies between 2 and 20 μ g. Therefore, experiments were carried out to estimate the amount of oxotremorine present in rat brain following the administration of tremorine using a bioassay procedure on an isolated guinea pig ileum preparation. It was found that no atropine-sensitive contractions were produced with brain extracts prepared from such tremorine intoxicated rats. The possibility existed that the extraction procedure did not recover the oxotremorine which was present in the brain. However, when oxotremorine was directly micro-injected into the brain of a rat and an extract prepared, an atropine-sensitive contractile substance was recovered. Using a 3-point bioassay procedure it was

estimated that the extraction procedure recovered a minimum of 65% of the oxotremorine administered. From these results it was calculated that the brain extraction-bioassay method should be able to detect as little as 1.4 μ g of oxotremorine in rat brain. This implies that there was less than this amount of oxotremorine present in a tremorine intoxicated rat brain. Since 2 μ g of oxotremorine microinjected into the striata was ineffective at producing an increase in hindlimb tone or tremor and the total brain content of oxotremorine following the systemic administration of tremorine (which does produce these effects) was less than 1.4 μ g this suggests that the striatum is not the major site of action of tremorine for the production of rigidity and tremor.

The possible role of the striatum in the control of resting limb tone and tremorine-induced rigidity was further evaluated with intrastriatal administration of pharmacological agents known to interfere with neurotransmitter function. It was found that modulation of striatal activity with pharmacological agents which increase dopamine or GABA activity or produce a temporary functional lesion (with a local anaesthetic) produced a statistically significant increase in resting tone. The neuronal interconnections within the striatum are not sufficiently understood to define precisely how these compounds produce their effects on limb tone. The complexity is illustrated by the observation that apomorphine and haloperidol did not have opposing actions on limb tone. These results may reflect the complicated pharma-

cology of the dopaminergic system. As many as four dopamine receptor sub-types have been described, based on ligand binding studies (Seeman, 1980), but a consensus on two receptor sub-types (D1 and D2) is beginning to emerge (Leff and Creese, 1983; Stoof and Kebabian, 1984). Studies using selective antagonists and agonists at these receptor sub-types have shown that unilateral stimulation of either D1 or D2 striatal dopamine receptors elicits turning behaviour in the rat, but the characteristics of the behaviours are qualitatively different (Herrera-Marschitz and Ungerstedt, 1984a). Further studies have led to the suggestion that D1 and D2 receptors may activate different striatal efferent pathways (Herrera-Marschitz and Ungerstedt, 1984b). Apomorphine is known to be able to stimulate both dopamine receptor sub-types (Kebabian and Calne, 1979) but it may have a degree of selectivity for the D1 receptor since apomorphine-induced turning behaviour has been shown to be insensitive to the D2 receptor antagonist sulpiride (Herrera-Marschitz and Ungerstedt, 1984a). In contrast, haloperidol may be a more effective antagonist at the D2 than at the D1 receptor. These differing pharmacological profiles may have some bearing on the results obtained concerning the effect of these compounds on resting limb tone. However, from the knowledge gained from the treatment of Parkinson's disease it would have been predicted that apomorphine would be more likely to reduce limb tone than to raise it.

Taking the results obtained with intrastriatal

administration of apomorphine, baclofen, and procaine together, these suggest that their effect on resting tone may be due to a membrane-stabilising effect, reducing the release of neurotransmitters within the striatum. The stimulation of pre-synaptic dopamine (Godukhin, Zharikova and Budantsev, 1984) and GABA receptors (Potashner, 1978) have been reported to reduce the release of neurotransmitters and it is likely that a local anaesthetic would have a similar (though non-specific) effect. Baclofen may also reduce neurotransmission within the striatum by a post-synaptic action at GABA_B receptors, producing inhibitory potentials by altering membrane calcium ion conductance (Bowery, Price, Hudson, Hill, Wilkin and Turnbull, 1984). Dopamine receptor stimulation may also have a similar inhibitory post-synaptic effect, although it might be mediated via an ionophore selective to some other ion (Stoof, De Boer, Sminia and Mulder, 1982) whereas procaine would be expected to non-specifically stabilise all striatal neuronal membranes.

In contrast to the effects of intrastriatal administration of drugs on resting tone, only one of the many compounds tested had any effect on tremorine-induced rigidity. The active compound was baclofen, which antagonised the effect of tremorine on limb tone. This is despite this same compound, together with apomorphine and procaine, being shown to increase resting tone, indicating that the striatum has a varied and complex influence over limb tone. Surprisingly, none

of the intrastriatal compounds tested was found to potentiate the effect of tremorine on hindlimb tone. One possible explanation is that the standard dose of tremorine used in this study (20mg/Kg) produced a maximal effect such that the hindlimbs could not be made more rigid. This is not likely since the hindlimb rigidity following intrastriatal apomorphine was significantly greater than in the control situation (although the tremorine response per se was unaltered) and also, the response to tremorine was significantly increased after lesions of the striatum - discussed below. Other suggestions are that the doses of the intrastriatal drugs used were not sufficient and that the injections were made into anatomically inappropriate parts of the striatum. These possibilities are unlikely because the doses of apomorphine and procaine employed were shown to affect resting limb tone. Furthermore, the stereotaxic placement of the cannulae were the same in all cases so if drugs administered to these areas can change resting tone it is likely that they are correctly placed to influence rigidity as well. The remaining conclusion is that tremorine was not acting primarily in the striatum to produce elevated limb tone. Strong support for this suggestion was provided by the studies with intrastriatal atropine, which was found to have no effect on tremorine-induced rigidity. It would have been expected that if tremorine was acting through a muscarinic mechanism within the striatum then intrastriatal atropine would antagonise the effects of

tremorine.

The possibility of striatal involvement in tremorine-induced rigidity was more directly investigated by the production of chronic unilateral lesions with the neurotoxins 6-hydroxydopamine, kainic acid, and ibotenic acid. All three lesions produced a slight, non-significant increase in resting tone of the contralateral hindlimb, with no effect on the ipsilateral hindlimb. It is possible that if bilateral lesions had been produced (as with procaine), these limb tone changes may have reached statistical significance. These results are not wholly in accord with the results obtained with the intrastriatal administration of pharmacologically active compounds. The difference concerns the 6-hydroxydopamine-induced lesion and the intrastriatal administration of haloperidol and apomorphine. The paradox is that the removal of a dopamine influence on the striatum with 6-hydroxydopamine produced effects more like those obtained with a compound which increased dopaminergic activity (apomorphine) than with those observed with a pharmacological antagonist of dopamine receptors (haloperidol). These results may again reflect the complex pharmacology of the dopamine system and/or the striatal circuitry.

The results obtained with the kainic and ibotenic acid lesions on resting tone are much more in agreement with previous experiments. These agents are known to produce axon-sparing, localised lesions of brain structures (Schwartz and Coyle, 1977; Coyle, 1983).

Although different striatal neurones have differing sensitivities to the action of these neurotoxins (e.g. somatostatin-containing neurones are more sensitive than acetylcholine-containing neurones), both acetylcholine-containing and GABA-containing neurones are equally sensitive (Araki, McGeer and McGeer, 1985). There is no evidence to suggest that other known striatal neurones, such as those containing substance P (Davies and Dray, 1976) or dynorphin (Vincent et al, 1982), are not susceptible to the neurotoxic effects of kainic and ibotenic acids. Therefore these lesions not only abolish synaptic activity in the affected parts of the striatum, but also reduce the efferent activity of this nucleus. Therefore baclofen, procaine and these neurotoxic agents may produce an increase in resting tone by a common action - decreased synaptic activity within the striatum.

The effect of morphine on hindlimb tone in animals with kainic acid-induced striatal lesions was evaluated. It has been previously reported that morphine-induced rigidity in the rat is mediated by opiate receptors within the striatum (Havemann et al, 1980, 1981). In the present study it was found that no attenuation of morphine-induced hindlimb rigidity resulted from a striatal lesion. There are two possible explanations for this. First, the lesion of the striatum was not sufficiently large and secondly, morphine may not have its major site of action within the striatum as intra-striatal morphine and its analogues failed to produce rigidity as measured by a mechanographical method

(Slater and Longman, unpublished observations). This may reflect the difference between electromyographic and mechanographic methods of measuring limb tone. Further experimentation is required to determine if either of these explanations are correct.

The lesions induced by 6-hydroxydopamine, kainic acid, and ibotenic acid all potentiated tremorine-induced rigidity of the contralateral hindlimb. The mechanism whereby at least two different lesions - one affecting afferent input and the other involving intrinsic neurones - produce similar effects, not only on resting tone but also on tremorine-induced rigidity, is not clear. However, the present results, involving kainic- and ibotenic acid-induced lesions, are at variance with some data reported earlier by Dickinson and Slater (1982). These authors reported that intrastriatal kainic acid prevented much of the tremorine-induced hindlimb rigidity in the rat. The dose of tremorine used in this latter study was the same as in the present. In contrast, the present results support the observations of Costall and Olley (1971b) in their studies of catalepsy. These authors reported that lesions of the striatum cause the catalepsy induced by muscarinic receptor stimulation by arecoline to be prolonged.

One possible explanation for the differing results obtained in the present study and those reported by Dickinson and Slater may relate to the size of the striatal lesions produced in the two groups of experi-

ments. In the present study there is histological and behavioural (apomorphine-induced turning) evidence suggesting a gross functional impairment of striatal activity in these lesioned animals. However, kainic acid is known to produce lesions not only at its site of application but also at more distant locations (Krammer, 1980). A benzodiazepine was used in the present study to reduce this distant damage (Ben-Ari et al, 1979) but such a treatment was not used in the experiments of Dickinson and Slater. This suggests that the differences between the two studies may be related to damage to structures outside the striatum. This is supported by the observation that ibotenic acid (a neurotoxin which may not produce such distant damage) had the same effect on tremorine-induced hindlimb rigidity as did kainic acid + benzodiazepine.

In summary, the present findings, involving different lesions and pharmacological manipulations, strongly suggest that the striatum is not the primary neural site of elicitation of the potent hindlimb rigidity induced by tremorine in the rat.

4.3 THE SUBSTANTIA NIGRA AND SUPERIOR COLLICULUS AND TREMORINE-INDUCED RIGIDITY

The substantia nigra pars reticulata (SNR) is a major output nucleus of the basal ganglia. Consequently, this nucleus has been implicated in several motor phenomena that may arise within the basal ganglia, including catalepsy (Costall and Olley, 1971a; Morelli, Porceddu, Imperato and Di Chiara, 1981), turning or

circling (Tulloch, Arbuthnott, Wright, Garcia-Munoz and Nicolaou, 1978; Dewar, Jenner and Marsden, 1983), and elevated muscle tone or rigidity (De Montis et al, 1979; Turski, Havemann and Kuschinsky, 1983). The role of the nigra in limb rigidity was investigated in the present study by bilateral intranigral administration of pharmacologically active compounds.

Several compounds were administered into the nigra, but only oxotremorine was found to have an effect on resting hindlimb tone. Oxotremorine produced rigidity similar to that produced by systemically administered tremorine. Other studies have reported that intranigral injection of muscarinic receptor agonists such as bethanechol (Turski, Havemann and Kuschinsky, 1984) and carbachol (De Montis et al, 1979) produced rigidity. Despite the fact that entirely different methods were used to quantify muscle tone, the results of the other studies are similar to the present findings. There is also some comparability between the effective doses of the three compounds - 2-15 μ g of oxotremorine, 5 μ g of bethanechol and 17.5nmols (approximately 3 μ g) of carbachol. These observations suggest that the SNR may be directly involved in generating rigidity induced by centrally acting cholinomimetics. This possibility is further supported by observations of De Montis et al (1979) in which bilateral, intranigral scopolamine prevented the rigid catalepsy induced by the systemic administration of arecoline. Taken together, these findings suggest that cholinomimetic-induced rigidity

may be caused by either a direct action in the nigra or is relayed by nigral cholinergic synapses.

However, in contrast, several other observations in the present study do not support the concept of the SNR being a primary structure involved in the production of rigidity by tremorine. Firstly, the intranigral injection of atropine not only failed to antagonise tremorine-induced rigidity but actually potentiated the response. Thus, the somewhat paradoxical situation arose in which a muscarinic agonist in the nigra caused limb rigidity, whereas a muscarinic antagonist exacerbated cholinomimetic induced rigidity. In passing, it is worth noting that the estimate of the oxotremorine levels in rat brain following the systemic administration of tremorine indicated that there was insufficient to produce rigidity solely by an action in the SNR. Finally, a temporary lesion of the SNR produced by procaine failed to alter significantly the rigidity induced by tremorine. However, it has been reported that bilateral electrocoagulation of the SNR greatly reduces the duration of arecoline-induced catalepsy (Costall and Olley, 1971a), but these authors made no mention of the concurrently induced rigidity. These same authors also reported that during the 4 days after the lesion, the effects of arecoline were potentiated, suggesting that the antagonism observed in subsequent trials may have been due to effects secondary to the lesion rather than to the damage to the SNR per se.

Evidence against a direct role for the nigra in

rigidity was reported by Slater and Dickinson (1983) who found that 14 days after a unilateral electrocoagulation of the SNR there was a reduction in the resting tone of the ipsilateral hindlimb but no effect on tremorine-induced rigidity. Some pharmacological evidence was obtained in the present study that the SNR can, to some extent, modulate the rigidity induced by tremorine. Intranigral baclofen was found to antagonise the effect of tremorine on hindlimb tone whereas other GABA-mimetic agents, muscimol and isoguvacine, were found to be ineffective. This suggests a nigral mechanism involving GABA_B receptors may be responsible for this antagonism (Bowery et al, 1984).

Electrophysiological recordings from the SNR showed that neuronal activity in the nucleus is affected by systemically administered tremorine. In the great majority of the single recordings there was an increase in spontaneous activity. Although this effect could be due to a direct action of oxotremorine (the active metabolite of tremorine) on SNR neurones, the stimulatory effect of intrastriatal infusions of muscarinic agonists on neuronal activity in the nigra suggests that the effects of tremorine may be mediated, at least in part, via the striatum. Changes in spontaneous multiunit activity of the SNR have been reported in the paralysed cat following intrastriatal infusion of carbachol (Pazo and Medina, 1982). These authors demonstrated that the changes in firing patterns depended upon the integrity of the striato-nigral pathway. They also noted that an

increase in activity was only found in dorsal SNR whereas a decrease in activity was observed in ventral SNR. No such regional variation was found in the present study. This discrepancy may be due to differences in either the recording techniques or the neuroanatomy of the two species. By monitoring multiunit activity, it is possible that Pazo and Medina (1982) may have recorded activity in the substantia nigra pars compacta as well as in the dorsal SNR. It is highly unlikely that pars compacta neurones were recorded in the present study because histological examination of the electrode tip placements indicated that they were invariably in the SNR. Furthermore, the spontaneous discharge rates of the units (200-300 spikes/10s) were closely similar to SNR units recorded previously (Guyenet and Aghajanian, 1978; Strahlendorf and Barnes, 1983) and differed considerably from those of identified pars compacta neurones (Guyenet and Aghajanian, 1978; Nakamura, Tsai and Iwama, 1981).

The increased firing of SNR units following intrastriatal administration of oxotremorine may be caused by several mechanisms. One possibility is direct excitation of the substance P-containing striato-nigral projection (Davies and Dray, 1976) since much of this projection arises in the antero-dorsal striatum (Ljungdahl, Hökfelt and Nilsson, 1978; Pycock and Phillipson, 1984), the site of the intrastriatal infusions. Other possibilities include disinhibition of the SNR by a reduction in the activity of the GABA-

containing striato-nigral projection (Fonnum et al,1978) or via indirect pathways such as the striato-pallido-nigral pathway.

Briefly, the present results are inconclusive concerning the involvement of the SNR in tremorine-induced rigidity. The electrophysiological studies demonstrated that the neuronal activity of this nucleus is increased following the administration of tremorine which may be mediated via a striatal mechanism. The earlier results indicated that the striatum was not a major neuronal site for the production of tremorine-induced rigidity which together with the results obtained with intranigral atropine and baclofen suggests that although the SNR may not be directly involved, it can modulate tremorine-induced rigidity.

One possible mechanism whereby the SNR may influence muscle tone involves the projection to the superior colliculus (Hopkins and Neissen,1976), a structure which has been suggested to be involved in orientating behaviour and posture (Imperato and Di Chiara,1981; Chevalier, Vacher and Deniau,1984; Geula and Asdourian,1984). The nigro-tectal pathway is believed to be GABA-ergic (Rinvik et al,1976; Vincent et al,1978; Kilpatrick et al,1982). It was reported that bilateral injections of muscimol into the intermediate and deep layers of the superior colliculus produced muscular rigidity (Ellenbroek et al,1984). These observations, together with the present results indicating that SNR activity increases following

tremorine administration suggests that an increased activity in the nigro-tectal pathway may contribute to the production of rigidity.

The results of Slater and Dickinson (1983) are in support of this hypothesis. They found that a unilateral lesion of the superior colliculus prevented tremorine-induced rigidity in the contralateral hindlimb and reduced the rigidity in the ipsilateral hindlimb. However, in the present study, a bilateral collicular lesion had relatively little effect on tremorine-induced rigidity. The explanation for these discrepancies might easily lie with differences in the site and size of the lesions produced in the two studies. In the experiments of Slater and Dickinson, unilateral lesions were produced by aspiration. The technique involved removal of the overlying cortex and probably involved damage to other brain regions surrounding the superior colliculus. In contrast, the lesions made in the present study were produced by electrocoagulation and were deliberately made more circumscribed. In addition, the lesions were carefully restricted to those areas of the colliculus that are known to receive the afferents from the SNR (Hopkins and Neissen, 1976; Graybiel, 1978; Edwards, Ginsburgh, Henkel and Stein, 1979). Naturally, any fibres passing through this region of the colliculus were also damaged but the fibre tracts of the underlying reticular formation remained unaffected. Although the most caudal and rostral parts of the colliculus were spared, the areas destroyed

showed a high degree of correspondence with areas thought to be involved in drug-induced postural changes (Imperato and Di Chiara, 1981; Geula and Asdourian, 1984).

Taken together, these reports suggest that the part of the superior colliculus that was destroyed in the present study was the area most likely to be involved in tremorine-induced rigidity, assuming that the colliculus is involved at all. However, very little effect was produced by the lesion and it is concluded that the superior colliculus plays little or no direct part in the expression of tremorine-induced rigidity. Equally therefore, the SNR-superior colliculus pathway is probably not involved in the motor behaviour.

4.4 THE GLOBUS PALLIDUS AND TREMORINE-INDUCED RIGIDITY

In man, the globus pallidus is intimately associated with the expression of the rigidity of Parkinson's disease since stereotactically placed lesions which are successful in alleviating this symptom involve either the globus pallidus or pallidal efferent fibres (Hassler, 1984). In animals it has been shown that the administration of pharmacological compounds into the pallidum not only induced limb tone changes (Campbell and Dill, 1974) but also modulated other basal ganglia mediated motor effects such as catalepsy (Costall and Olley, 1971b; Ossowska, Wedzony and Wolfarth, 1984) and turning behaviour (Pycock, 1980).

The electrophysiological experiments performed in the present study indicated that, in the rat, the spon-

taneous activity of pallidal neurones is altered by tremorine administration. The spontaneous frequency of the pallidal units recorded in this study (approximately 250 spikes/10s) is closely similar to the frequencies reported in other studies (Napier, Pirch and Peterson, 1983; Stone, 1983). When tremorine was administered this activity increased in the majority of units tested. Comparisons of the spontaneous firing rates of the units which responded to tremorine with those of the total sample of 30 unit recordings suggest that pallidal neurones which respond to tremorine initially had below average discharge rates. In contrast to the effects of tremorine on nigral neurones, there was no indication that any of the effects of tremorine on pallidal units were mediated via the striatum because, of the eight pallidal neurones which had their discharge frequency modulated by intrastriatal oxotremorine or carbachol only 3 responded with an increase in activity. In many cases, intrastriatal infusions of oxotremorine or carbachol had no effect on pallidal unit activity.

There are several possible reasons why intrastriatal drug infusions produced inconsistent effects on pallidal unit activity. Firstly, the infusions were made within 2mm of the recording electrode so that some of the observed effects on pallidal unit activity may have been caused by the infusion. Saline infusion into the striatum would confirm this. Secondly, the striatum is a large nucleus with a highly topographical projection to the globus pallidus (Carpenter, 1981) so the striatal

infusions may have been made to regions of the striatum which did not project to the pallidal units from which recordings were made. Further experiments are required to examine this possibility. Thirdly, the quantity of drug infused may have been insufficient, although this seems unlikely since the same doses infused into the striatum caused selective increases in the spontaneous activity of some nigral units. Finally, the results obtained may reflect the complex effects produced by the administration of pharmacologically active compounds into the striatum.

Compounds which interfere with GABA, 5-hydroxytryptamine, acetylcholine and enkephalin neurotransmission were locally applied to the globus pallidus. Only baclofen was found to modify hindlimb tone. When this drug was administered unilaterally, the animal became sedated and the resting tone of the contralateral hindlimb was significantly reduced. In contrast, following bilateral injections of baclofen, the decrease in resting tone was not statistically significant. It is unlikely that the changes in resting tone associated with unilateral administration of baclofen were due to the production of postural asymmetry because asymmetry was more pronounced with the GABA antagonists picrotoxin and bicuculline yet neither compound altered resting limb tone.

The observation that muscimol, picrotoxin and bicuculline had no effect on resting limb tone whereas baclofen did may be related to the existence of two

pharmacologically distinct sub-types of GABA receptor (Hill and Bowery, 1981). Muscimol, isoguvacine, bicuculline and picrotoxin interact preferentially with the GABA_A receptor and baclofen with GABA_B (Bowery et al, 1984). Both GABA_A and GABA_B sites can be detected in many regions of the brain but a clear separation can only be demonstrated in the cerebellum, thalamus and olfactory bulb (Bowery et al, 1984). This suggests that both GABA_A and GABA_B receptors are present in the globus pallidus. Therefore, the variations in the effects of intrapallidal administration of GABA agonists and antagonists on hindlimb tone may reflect the differing function of the GABA receptor sub-types in this region.

Baclofen administered into the globus pallidus was the only compound which modulated tremorine-induced rigidity. Baclofen antagonised the rigidity whereas compounds that modify 5-hydroxytryptamine, enkephalin or acetylcholine function had no effect. Involvement of the pallidum in tremorine-induced rigidity has been suggested by Dickinson and Slater (1982) who reported that unilateral lesions of the globus pallidus increased the rigidity of the contralateral hindlimb whilst reducing the rigidity in the ipsilateral hindlimb. Also, using similar doses to those used in the present study, these same authors found that unilateral intrapallidal administration of bicuculline and picrotoxin increased tremorine-induced rigidity in the contralateral hindlimb whereas baclofen antagonised the rigidity. There is no obvious reason for the differing results concerning the

GABA antagonists in these two groups of studies. In support of the present results, studies on catalepsy have found that chronic bilateral pallidal lesions had no effect on arecoline-induced catalepsy (Costall and Olley, 1971b) but neuroleptic-induced catalepsy was abolished (Costall and Olley, 1971b; Ossowska, Smialowska and Wolfarth, 1983).

In summary, the present results suggest that the globus pallidus may not be of primary importance for the production of rigidity but can modulate the effects of tremorine on limb tone.

4.5 THE EFFECT OF 3-ACETILPYRIDINE LESIONS ON TREMORINE-INDUCED RIGIDITY

Although some previous studies concerning the sites of the lesions in the central nervous system produced by 3-acetylpyridine (3-AP) showed that these were predominantly within the medulla oblongata (Desclin and Escubi, 1974; Llinás et al, 1975) a more recent report provided evidence of damage to other areas, including the substantia nigra and entopeduncular nucleus (Balaban, 1985). In the present study, no neuronal degeneration was detected in structures above the hindbrain including the superior colliculus, substantia nigra, entopeduncular nucleus, globus pallidus, striatum and thalamus. In the brain stem the greatest damage occurred in the inferior olive, the nucleus ambiguus, and the nuclei of the X and XI cranial nerves, together with some degeneration in the vestibular nuclei. The apparent absence of degenera-

ting neurones in higher brain areas suggests that these areas do escape damage, or, less likely, that the method of Fink and Heimer (1967) which was used to visualise degenerating neurones in the present study is less sensitive than the cupric-silver method used by Balaban (1985).

The lesion produced by 3-AP resulted in an increase in the resting tone of the hindlimbs and the development of a resting tremor. Other workers have reported an increase in limb tone following brain stem lesions. Extensor hypertonus together with abnormal positioning of the hindlimbs has been observed in the cat following electrocoagulation or aspiration of the inferior olive (Wilson and Magoun, 1945; Murphy and O'Leary, 1971). One possible mechanism for the increase in resting tone observed in the present study involved changes in the output of the lateral vestibulo-spinal tracts, as 3-AP-induced lesions have been reported to reduce the inhibitory action of cerebellar Purkinje cells on Deiters' nucleus (Ito et al, 1978). The lateral vestibulo-spinal tract fibres have an excitatory action on extensor motor-neurones innervating forelimb, axial, and hindlimb muscles (Wilson and Yoshida, 1969; Grillner, Hongo and Lund, 1971). Therefore, the loss of the inhibitory action of Purkinje cells on the neurones of Deiters' nucleus may increase the excitatory drive on the extensor motor-neurones of the hindlimbs producing the observed increase in resting tone. However, with 3-AP lesions, the loss of Purkinje cell inhibition on Deiters' nucleus observed by

Ito et al (1978) could be due to the effects of 3-AP on the olive resulting in a loss of climbing fibre input, or to a direct action on the Purkinje cell itself. Therefore, the Purkinje cell inhibition of neurones of Deiters' nucleus needs to be evaluated following more selective lesions of the inferior olive to determine if the observed increase in hindlimb tone is associated with the loss of climbing fibre input to the cerebellum.

The study of Modianos and Pfaff (1976) found that electrolytic lesions of the lateral, medial and superior vestibular nuclei as well as the inferior olive produced resting tremor. This suggests that the damage observed to the inferior olive and/or the vestibular complex may have been responsible for the production of the resting tremor exhibited by rats lesioned with 3-AP. Other motor deficits observed in rats with 3-AP, such as a decrease in body elevation and an increase in tremor during movement, were also found with electrolytic lesions of the inferior olive and vestibular complex (Modianos and Pfaff, 1976). Therefore, although many of the effects produced by the administration of 3-AP are similar to those produced by electrocoagulation of the inferior olive, the participation of other hindbrain areas cannot be ruled out.

The lesion produced by 3-AP not only increased resting tone but also antagonised the rigidity induced by tremorine. However, the rigidity produced by morphine was unaffected by the lesion. This suggests that hindbrain mechanisms damaged by 3-AP are required for the

expression of tremorine-induced rigidity, but not for that induced by morphine. This is supported by the suggestion that morphine-induced rigidity may be mediated by an action in the striatum and substantia nigra (Havemann et al, 1980; Turski et al, 1982). Neither of these areas were affected by 3-AP treatment.

The observation that the antagonism of tremorine-induced rigidity by 3-AP was greater on day 10 than on day 4 after the administration of 3-AP suggests that the lesions may develop over a period of days. This idea is supported by the demonstration of both immediate and delayed effects of 3-AP on Purkinje cells. Although changes in the discharge patterns of Purkinje cells, such as a loss of complex spikes and an increase in the frequency of simple spikes, have been shown to occur within 3-10h of 3-AP administration (Batini and Billard, 1985), further morphological changes occur up to 6 weeks later (Desclin and Colin, 1980). Therefore, the progressive antagonism of tremorine-induced rigidity may be due to the progressive changes in the cerebellum.

In contrast to rigidity, tremorine-induced tremor was exacerbated by the lesions produced by 3-AP administration. This suggests that different brain mechanisms are involved in the production of tremor and rigidity. There is circumstantial evidence to suggest that the increase in tremorine-induced tremor that was observed in this study may have been due to cerebellar dysfunction. In human subjects, lesions of the cerebellar cortex have been reported to produce tremor in muscles which are

generating tone (Silferskiöld, 1977). Also, tremor has been observed in the chronically hemi-cerebellate baboon following the administration of drugs which increase muscle tone (Walter, Guiot, Basso, Oviedo, Jedynak and Gautron, 1974). Therefore, the potentiation of tremorine tremor by 3-AP lesions found in the present study could be the result of two factors. There may have been a secondary tremor stimulated by an increase in muscle tone via the lesioned cerebellum which was superimposed on the tremorine-induced tremor. Alternatively, there may have been a real potentiation of the tremorine tremor mechanism.

The results obtained with the lesions induced by 3-AP do not allow a precise definition of the site of action of tremorine as several areas of the hindbrain were damaged. However, the findings suggest that the vestibulo- and reticulo-spinal pathways are important for the expression of tremorine-induced rigidity. As both of these descending systems receive a large efferent innervation from the cerebellum (Eccles, Nicoll, Schwarz, Taborikova and Willey, 1974; Batton, Jayaraman, Ruggiero and Carpenter, 1974; Armstrong, 1978), tremorine may produce rigidity by an action in the cerebellum. This may be the result of a direct action on the climbing fibres, because muscarinic receptors are present in the inferior olive in many species including the rat (Rotter, Birdsall, Field and Raisman, 1979b) and man (Cortes, Probst and Palacios, 1984). Alternatively, tremorine may act at one or more of the brain areas which project to

those nuclei from which the vestibulo- and/or reticulospinal pathways originate. Therefore, more selective lesion experiments of the inferior olive, superior, lateral and medial vestibular nuclei, nucleus reticularis pontis caudalis and nucleus reticularis gigantocellularis are required to further identify the brain mechanisms involved in tremorine-induced rigidity.

In summary, the central lesions produced by 3-AP suggest that tremorine-induced hindlimb rigidity in the rat may be mediated via the cerebellum and the reticulo- and vestibulo-spinal tracts although other areas influencing these descending pathways cannot be ruled out.

4.6 SUMMARY AND CONCLUSIONS

In this study, the local application of muscarinic receptor agonists to the striatum and substantia nigra produced similar effects to those observed following systemic administration of tremorine, namely tremor, rigidity and akinesia. Paradoxically, lesions of the striatum or the administration of atropine into the striatum and substantia nigra either exacerbated or had no effect on tremorine-induced rigidity. Further studies using local application of pharmacologically active compounds to basal ganglia nuclei showed that only baclofen antagonised the effect of tremorine on limb tone. As no statistically significant effects were produced with GABA_A receptor agonists or antagonists, the mechanism activated by GABA_B receptors appears to be particularly important for the observed antagonism of tremorine-induced rigidity. Unfortunately, there are no

other available agonists or antagonists which selectively interact with GABA_B receptors which could have been used to ensure that the effects found with baclofen were indeed associated with an action at GABA_B receptors.

There are problems associated with techniques involving pharmacological manipulations of neurotransmitter function in localised brain areas, such as whether a sufficient dose of drug is used, how selective the drug is and the actual volume of brain affected by the microinjection. These must be borne in mind when interpreting the data obtained with such methods. However, despite the similarities in the effects elicited by tremorine and the signs and symptoms observed in patients suffering from Parkinson's disease, the balance of the evidence obtained in this study indicates that the basal ganglia may contribute to, but have only a minor role in, the expression of tremorine-induced rigidity. This is supported to some extent by studies on cholinomimetic-induced rigid catalepsy.

This hypothesis is not as unexpected as would have been first thought. Muscarinic receptors are not only the most plentiful of the acetylcholine receptor subtypes in the brain, they are also widely distributed throughout the neuro-axis, from the spinal cord (Gillberg, Nordberg and Aquilonius, 1984) to the cerebral cortex (Rotter et al, 1979a). Also, muscarinic receptors are found in many areas of the central nervous system (CNS) known to be associated with motor control, such as the ventral horn of the spinal cord, the cerebellum, the

basal ganglia and the sensorimotor cortex (Rotter et al, 1979a and b; Gillberg et al, 1984). In comparison, the central lesions associated with Parkinson's disease, although not fully and accurately characterised, are relatively well localised. The observations that intra-striatal oxotremorine can produce an increase in limb tone suggests that if sufficient oxotremorine were present in the brain it would stimulate the striatal mechanism to produce rigidity. However, as tremorine is administered systemically, all the muscarinic receptors in the CNS are equally likely to be stimulated. This indicates that the rigidity observed after tremorine administration may be the product of muscarinic receptor stimulation in a number of areas in the CNS.

The pharmacological manipulation of neurotransmitter function and lesions of the individual nuclei of the basal ganglia, produced only small effects on tremorine-induced rigidity, whereas lesions of the hind-brain (with 3-acetylpyridine), where influences from several motor areas, e.g. cerebellum and cerebral cortex (Peterson, Anderson and Filion, 1974; Armstrong, 1978) converge, were found to have a profound effect. These observations can be viewed as supporting the concept of multiple sites of action within the CNS of the active metabolite of tremorine, with lesions more caudal in the neuro-axis having greater effects than lesions at more rostral sites. Further support for this suggestion is from the observation that very low levels of oxotremorine are present in rat brain following tremorine administra-

tion in comparison to the levels of oxotremorine required to stimulate rigidity via the striatum and substantia nigra. Alternatively, these findings may suggest that the cerebellum has a major role in the expression of tremorine-induced rigidity as 3-acetylpyridine is known to produce a major dysfunction of the cerebellum (Llinás et al, 1975) and also the nuclei which give rise to the vestibulo- and reticulo-spinal tracts have large innervations from the cerebellum (Eccles et al, 1974). The suggestion that tremorine may produce rigidity via the stimulation of a localised brain area is not unreasonable as it has been suggested that systemically administered morphine produces rigidity by an action within a part of the basal ganglia (striatum and substantia nigra) rather than via an action throughout the neuro-axis (Havemann et al, 1980; Turski et al, 1982).

However, the most likely mechanism of rigidity induced by tremorine is via an action at several sites within the CNS. This makes the sites of action very difficult to investigate as many possible mechanisms have to be evaluated. For example, tremorine may act directly on the α -motoneurons causing them to be more easily depolarised by excitatory inputs from descending pathways or direct sensory stimuli. Alternatively, rigidity may be mediated by descending pathways which represent the algebraic summation of mild stimulation (due to the low levels of oxotremorine in brain) of several brain areas which may include the basal ganglia

and cerebellum. However, the possible role of the cerebellum and its associated hindbrain nuclei are more easily investigated. Therefore, although the findings of the present study do not preclude the concept of a multiple site of action of tremorine, perhaps the most logical progression in experimental studies of the mechanism of action of tremorine is to define more precisely which hindbrain structures are involved.

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