The Effects of Age and L-DOPA Replacement On Locomotor Deficits in the AS/AGU Mutant Rat

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Payne, A.P., Campbell, J.M., Russell, D. et al. (2000) The AS/AGU rat: a spontaneous model of disruption and degeneration in the nigrostriatal dopaminergic system . *J. Anat.* 196: 629-633.

Campbell, J.M., Gilmore, D.G., Russell, D. et al. (2000) Pharmacological analysis of extracellular dopamine and metabolites in the striatum of concious AS/AGU rats, mutants with locomotor disorder. *Neurosci.*, 100(1): 45-52.

Campbell, J.M., Favor, G., Russell, D. et al. (1999) A microdialysis study of evoked dopamine release and re-uptake blockade in the AS/AGU rat. *Congress of the Turkish physiological society abstracts*. P87.

Russell, D., Campbell, J.M., Favor, G. et al (1998) Locomotion in the AS/AGU mutant rat: progressive deficits and the effects of L-DOPA replacement. *Society for Neuroscience Abstracts* 24: 1718.

General Publications

Al-Fayez, M.A.S., Russell, D., Campbell, J.M. et al. (2001) Deficiencies in the raphe-striatal serotonergic system of the AS/AGU rat. *XIV International Congress on Parkinson's Disease abstracts.*

Payne, A.P., Sutcliffe, R.G., Campbell, J.M. et al. (1998) Disordered locomotion in the AS/AGU mutant rat and the effects of L-Dopa or fetal midbrain grafts. *Movement Disorders* 13: 832-834.

Campbell, J.M., Gilmore, D.P., Russell, D. et al. (1998) Microdialysis studies of dopamine and it's metabolites in the corpus striatum of conscious AS/AGU mutant rats. *Neurosci.*, 85: 323-325.

Campbell, J.M., Payne, A.P., Gilmore, D.P. et al. (1997) Age changes in dopamine levels in the corpus striatum of albino Swiss (AS) and AS/AGU mutant rats. *Neurosci. Lett.* 239: 54-56.

Campbell, J.M., Payne, A.P., Gilmore, D.P. et al.(1996) Neostriatal dopamine depletion and locomotor abnormalities due to the albino Swiss rat agu mutation. *Neurosci. Lett.* 213: 173-176.

LIST OF ABBREVIATIONS

6-OH-DA	6-Hydroxydopamine
ACSF	Artificial Cerebrospinal Fluid
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
AS	Albino Swiss
AS/AGU	Albino Swiss/Anatomy Glasgow University
CM/PF	Centromedian-Parafasicular
CNS	Central Nervous System
COMT	Catechol-o-Methyl Transferase
DHBA	Dihydroxybenzylamine
DOPAC	3,4-Dihydroxyphenylacetic Acid
EDTA	Diaminoethanetetra-acetic Acid
EPN	Entopenduncular Nucleus
GABA	Gamma-Aminobutyric Acid
GP	Globus Pallidus
Gpe	External Globus Pallidus
Gpi	Internal Globus Pallidus
HBN	Lateral Habenular Nucleus
HD	Huntington's Disease
HPLC-ECD	High Performance Liquid Chromatography with Electrochemical Detection
HVA	Homovanillic Acid
L-AAAD	L-Aromatic-amino Acid Decarboxylase
L-DOPA	L-3,4-Dihydroxyphenylalanine
MAO	Monoamine Oxidse
MPTP	1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine
MSA	Multiple System Atrophy
NMDA	N-methyl-D-aspartate
PD	Parkinson's Disease
PKCγ	Protein Kinase C gamma
PET	Positron Emission Tomography

PPN	Pendunculopontine Tegmental Nucleus
PSP	Progressive Supranuclear Palsy
RF	Response Factor
SC	Superior Colliculus
SN	Substantia Nigra
SNpc	Substantia Nigra Pars Compacta
SNpr	Substantia Nigra Pars Reticulata
STN	Subthalamic Nucleus
Thal	Thalamus
VM-VL	Ventromedial/Ventrolateral Complex
VTA	Ventral Tegmental Area

SUMMARY

The AS/AGU mutant rat was derived from Albino Swiss (AS) parent stock and is characterised by an ungainly staggering gait, hindlimb rigidity, whole body tremor and difficulty in initiating movement. It is highly deficient in extracellular dopamine in the dorsal caudate-putamen. It is important to know which aspects of locomotor performance show progressive deficits and which ones are responsive to L-DOPA replacement. Initially, locomotor tests were video-recorded and used to assess an age series of both AS/AGU (mutant) and AS (parent strain) rats between 20 days and 1 year. Progressive deficits with age (with initial success c. 80-90% declining to nil at one year) were found in the performance of simple tasks such as mid-air righting (being dropped upside down from 50cm and successfully turning) and walking down a series of inclined ramps of increasing width and not falling off. In infra-red monitoring, mutant rats actually showed more movement than controls a) in an acute test of 12 minutes and b) in extended tests of 22 hours. Secondly, images taken from a footfall chamber were studied using a Kontron image analysis system to determine paw contact patterns. In AS rats, whilst contact progresses forwards it is centred around the midline of the foot throughout. Conversely, mutant rats exhibited frequent footfalls in which contact was eccentric (either medial or lateral). There appeared to be most contact on the medial side of the foot than on the lateral side, confirming the staggering gait nature of the AS/AGU rat. This is indicative of the animals inability to balance as well as its failure to sustain purposeful movement. The several elements of the movement disorder are brought back into the wild-type range by treatment

with L-DOPA administration (25 mg/kg/day ip + benserazide 2.5mg/kg/day ip) for 4 weeks. This drug is the standard treatment for human Parkinson's Disease. L-DOPA ameliorated mid-air righting, and performance on certain inclined ramps in AS/AGU rats. Most striking was the improvement in locomotor performance on a 5 cm ramp after week 3 of treament. The width of this ramp corresponded to the AS/AGU rat's natural gait width. The improvement of AS/AGU rats in both of these simple locomotor tasks, continued to improve over the course of the study.

These results indicate that the progressive nature of the AS/AGU movement disorder and its amelioration after L-DOPA treament, would suggest that this rat could prove to be invaluable to the study of basal ganglia related disorders. It is therefore suggested that this naturally occurring mutant model could have many advantages over existing animal models, such as those with 6-OHDA and MPTP lesions.

INTRODUCTION

INTRODUCTION

1.1 The Basal Ganglia

As early as 1664, a number of subcortical structures were identified and described by the British anatomist Thomas Willis. What is now known as the basal ganglia were then referred to as the corpus striatum, a structure thought to both receive all sensory modalities and initiate all motor acts. This idea seemed to be reinforced by its central position and visible ascending and descending fibre systems.

As the histological organisation of the cortex became clearer, it became obvious that many of the functions originally assigned to the corpus striatum were in fact properties of neighbouring corticospinal paths (Wilson, 1914). At the beginning of the twentieth century, the corpus striatum was studied in some detail and gained particular importance when it was discovered that lesions of its component parts often resulted in disorders of motor functions in humans.

The term "extrapyramidal motor system" loosely grouped the striatum with an array of brain stem nuclei and reflected the growing assumption that this grouping constituted a complete and independent motor unit (Carpenter, 1981). The term basal ganglia has been generally used to refer to these anatomical telencephalic subcortical nuclei at the base of the forebrain.

1.1.1 Components of the Basal Ganglia

The term basal ganglia refers to the large nuclear masses i.e., the caudate, putamen and globus pallidus (GP), which are found in the basal part of the telencephalon and are best known for their motor functions. The substantia nigra (SN) and subthalamic nucleus (STN), both closely related to the preceding structures, are also usually considered part of

the basal ganglia. These structures are linked together by a complex series of intrinsic loops, most of which are reciprocal, and to other brain structures (cortex and thalamus) by a parallel circuit. Other regions that are important for basal ganglia function are cortical motor, somatosensory and premotor areas; ventral anterior (VA), ventrolateral (VL), and centromedian-parafascicular (CM-PF) nuclei of the thalamus; monoamine containing cell groups such as ventral tegmental area, retrorubral field, dorsal raphe nucleus and the tegmental pedunculopontine nucleus. Together these nuclei enable not only controlled motor execution but also more complex aspects of behaviour such as motor planning and sequencing, motor learning, and cognitive and motivational drives.

1.1.2. Striatum

The term striatum refers to the functional unit of caudate nucleus and putamen, together called the dorsal striatum or neostriatum. In man the caudate nucleus is a large C-shaped structure located chiefly medial to the internal capsule and related throughout its length to the lateral ventricle. It has a large rostral component (head) that bulges into the lateral wall of the anterior horn of the lateral ventricle. The head tapers to form the body which in turn further attenuates to form the tail terminating at the amygdaloid body. The putamen is separated, though incompletely, from the caudate nucleus by the internal capsule. The ventral striatum is the region at the base of the caudate-putamen complex that lies mainly anterior to the anterior commisure. The ventral continuations of the caudate nucleus and putamen, the nucleus accumbens septi and parts of olfactory tubercle are all contained within the ventral striatum.

Striatal neurons are of two different types, projection neurons and interneurons. About 90 percent are medium sized (12-20μm diameter) with spiny dendrites (Wilson, & Groves, 1980), and have a receptive/outflow (projection) function. They use gamma-

aminobutyric acid (GABA) as their main neurotransmitter (Oertel & Mugnaini, 1984; Ribak et al., 1979), but also co-express neuroactive peptides, such as substance P, enkephalin, dynorphin and neurotensin (Fallon & Leslie, 1986; Hökfelt et al., 1977). The remaining striatal neurons are of large elongated (50x15μm) and medium (14-15μm) sizes, lack dendritic spines, and function as intrinsic interneurons, using the excitatory transmitter acetylcholine (Ach).

1.1.3. Globus Pallidus

The globus pallidus (GP) or palaeostriatum, represents the efferent side of the basal ganglia. It is situated medial to the putamen and is separated from it by the external medullary lamina. The GP is divided into external (GPe) and internal (GPi) segments by the medial medullary lamina, and is traversed by numerous myelinated fibres giving it a characteristically pale appearance in stained sections. Both segments contain a relatively small number of large neurons which typically have long dendrites whose branching tends to be discoidal in nature. These dendritic formations lie parallel to the lateral segmental boundaries.

1.1.4. Substantia Nigra.

The substantia nigra (black substance) is an elongated nucleus located medial to the basis pedunculi throughout the rostrocaudal extent of the midbrain. Although a component of the midbrain it is considered part of the basal ganglia due to its functional significance. The SN is divided into two distinct parts: the ventrally situated pars reticulata (SNpr), and the more dorsal pars compacta (SNpc), which have different connections and distinct neurotransmitters.

1.1.5. Subthalamic nucleus

The subthalamic nucleus is a biconvex structure located within the caudal diencephalon ventral to the thalamus and just medial to the internal capsule.

1.1.6. Compartmental Organisation of the Striatum.

Regular Nissl staining of the striatum shows a largely homogeneous mass. Discontinuous areas were observed, however, when techniques such as retrograde tracing were combined with histochemistry, immunohistochemistry and histopharmacology. Two levels of compartmental organisation have been described in the striatum: patch-matrix compartments, and the organisation of separate striatopallidal and striatonigral systems. Striatal patch-matrix systems are demonstrated and determined by specific neurochemical markers and also by underlying neuronal connections. The patch compartment is defined by μ-opiate binding (Herkenham & Pert, 1981), and by low expression of acetylcholinesterase (Butcher & Hodge, 1976). The term striosome ("striatal body") was first used by Graybiel and Ragsdale (1978), to describe these areas of low cholinesterase activity. Five-nucleotidase (Schoen & Graybiel, 1992) was also found to be an excellent marker for striosomes in the rat.

The matrix compartment can be defined by staining for the calcium binding protein calbindin, and by the immunoreactivity of somatostatin positive fibres (Gerfen et al., 1985). Patch-matrix organisation is predominantly found in the caudate- putamen, extending into the dorsolateral and ventromedial areas.

In the rat, it was observed that compartmental organisation of cortico-striatal afferents was related to their laminar origin rather than in their cortical areas of origin (Gerfen, 1989), with different cortical laminae innervating patch and matrix compartments. Thus, corticostriatal neurons in infragranular layers project principally to patches, with the

matrix being the main recipient of axons from supragranular layers. A similar pattern exists in primates but is complicated by the fact that allocortical areas have a higher concentration of corticostriatal neurons arising from infragranular layers, whereas in neocortical areas, supragranular corticostriatal neurons are more numerous (Arikuni & Kubota, 1986). These corticostriatal projections terminate principally on the dendrites of medium spiny projection neurons where they have an excitatory effect due to glutamate. These findings are consistent with studies that show the developmental relationship between medium spiny neurons of the patches and matrix, and neurons of deep and superficial cortical laminae respectively (Fishell & van der Kooy, 1987).

If striatal medium spiny neurons can be divided into two populations (patch and matrix) that have a relationship with the laminar (Gerfen, 1989) and regional (Donoghue & Herkenham, 1986) organisation of the cortex, they can also be characterised by their respective projections to globus pallidus, entopenduncular nucleus (EPN) and substantia nigra, with striatopallidal neurons arborizing within the GP, and striatonigral neurons extending to the EPN and SN (Parent et al., 1984). Gerfen (1992) hypothesised that striatopallidal projections to mainly cholinergic and substance P expressing areas of the ventral pallidum originate largely in the patch striatal area, whereas matrix neurons tend to project to the predominantly GABAergic enkephalin-containing neurons.

Tracing techniques in rats have shown that although both types of neurons project to the substantia nigra, patch neurons project to the dopaminergic cell groups in the SNpc, and cell islands in the SNpr, whereas matrix neurons project to areas in the SNpr containing GABAergic neurons (Gerfen, 1984;1985).

1.1.7. Parallel organisation of cortico-basal ganglia loops.

Early data on the function of the basal ganglia suggested its role in the assimilation of information coming in from all areas of the cerebral cortex, funnelling through the ventrolateral thalamus specifically to the motor cortex to complete a circuit that would facilitate, amongst other things, the initiation and control of movement (Kemp & Powel, 1971; Alexander et al., 1986). A revised view was formed when subsequent studies showed the segregation of information from the sensorimotor and associative cortices through the basal ganglia-thalamocortical pathways (DeLong & Georgeopoulus, 1981). It was suggested at that time that there might be two separate circuits through the basal ganglia i.e. the "motor" loop, passing mainly through the putamen, receiving inputs from the sensorimotor cortex transmitting ultimately to premotor areas, and the "association" or "complex" loop which took information from the association areas, passed through the caudate nucleus, ultimately returning to areas of the prefrontal cortex (DeLong & Georgeopoulus, 1981; DeLong et al., 1983).

There is good neuroanatomical and neurophysiological evidence that primate supplementary motor area I and the frontal eye field give rise to pathways in which the basal ganglia-thalamocortical outputs remain segregated and terminate in the cortical areas from which they originated, thus forming largely closed "motor"and "oculomotor" circuits (Alexander & Crutcher, 1990; Hoover & Strick, 1993).

The organisation of corticostriatal systems has been described by Alexander et. al.(1986). Segregated parallel circuits connect limbic, prefrontal, oculomotor and motor cortical areas through specific subregions of the basal ganglia, which in turn project back to the same cortical areas via ventral tier thalamic nuclei. There seems, therefore, to be a progressive "funnelling" of multiple corticostriate inputs at striatal, pallidal/nigral and

thalamic levels. It also seems that each basal ganglia-thalamocortical circuit uses multiple inputs only from cortical areas that are functionally related and mostly interconnected.

The five proposed basal ganglia-thalamocortical circuits (motor, oculomotor, dorsolateral prefrontal, lateral orbitofrontal and anterior cingulate) each involve specific regions of the cerebral cortex, striatum, pallidum, substantia nigra and thalamus. The best understood is the motor circuit.

1.1.8. The Motor Circuit

The motor circuit begins with major somatotopically organised excitatory projections, which use glutamate as a neurotransmitter from the motor (Kunzle, 1975), and somatosensory (Kunzle, 1977) cortices. In primates, both motor and somatosensory cortical "leg" areas project to a dorsolateral area, the "face" areas to a ventromedial area and the "arm" areas to an region in between (Kunzle, 1975; 1977). In addition to motor and somatosensory projections, the putamen also receives inputs from area 5, lateral area 6 including the arcuate premotor area, and from the supplementary motor area (Alexander & Crutcher, 1990). Although outputs from each area venture slightly into neighbouring areas of the caudate nucleus, the putamen controls all of the terminal arborizations.

The putamen sends topographically organised efferents to the ventrolateral two thirds of both the internal and external segments of the globus pallidus (Devito et al., 1980; Parent et al, 1990), and to caudolateral portions of the substantia nigra (Parent et al, 1990). The portion of the GPi segment that receives putamen efferents projects in turn to the oral part of the ventrolateral nucleus of the thalamus (Vlo) (Devito & Anderson, 1982). Schell & Strick (1984) showed that the Vlo projects to the supplementary motor area. It is thus demonstrated that somatotopically organised projections from the supplementary motor area, the arcuate premotor area, motor cortex and somatosensory cortex exert their

influence through the motor circuit projecting ultimately back to a single cortical region, the supplementary motor area. Each basal ganglia-thalamocortical circuit contains separate parallel "direct" and "indirect" pathways to the output nuclei (GPi, SNr, and ventral pallidum) (Figure 1).

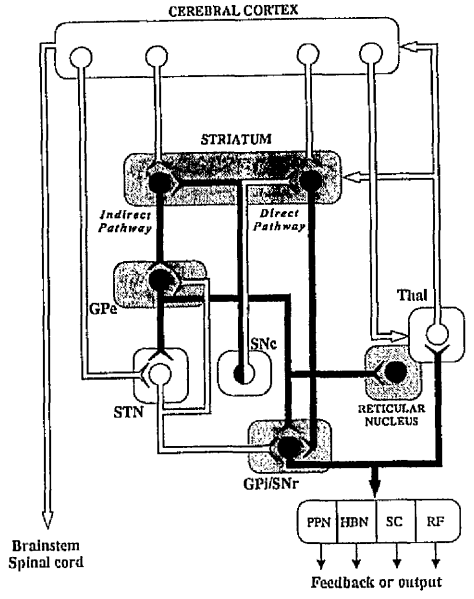


Figure1. An updated version of the basal ganglia circuitry originally represented by Alexander and Crutcher (1986), showing the connectivity of the direct and indirect pathways of the basal ganglia. Inhibitory projections are shown as filled arrows, exitatory projections as open arrows. GPe = external segment of the globus pallidus; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; STN = subthalamic nucleus; Thal = thalamus; PPN = pedunculopontine tegmental nucleus; HBN = lateral habenular nucleus; SC = superior colliculus; and RF = reticular formation.

The "direct" pathway arises from inhibitory striatal efferents, projecting from spiny neurons directly to the output stations that contain both GABA and the neuropeptides Substance P (Albin et al., 1989), and dynorphin (Vincent et al., 1982). These neurons preferentially express the D_1 subtype of dopamine receptor. Activation of this pathway disinhibits the thalamic stage by double inhibition, as the subsequent thalamic afferents are also inhibitory projections (Chevalier et al., 1985). In addition to the cerebral cortex, the intralaminar thalamic nuclei are a major source of glutamatergic inputs to the striatum.

In the "indirect" pathway, striatal efferents project from spiny neurons and contain GABA and the neuropeptide enkephalin (Alexander & Crutcher, 1990). These neurons

preferentially express the D_2 subtype of dopamine receptor. These projections exert an inhibitory influence on the GPe which in turn projects to the subthalamic nucleus via a purely GABAergic pathway, and finally to the GPi/SNr via an excitatory glutaminergic projection from the subthalamic nucleus. The GABA/enkephalin inhibiting influence on the GPe from the striatum tends to suppress the activity of striatal neurons which, along with the similar inhibitory influence of GPe neurons on the subthalamic nucleus, has the net effect of disinhibition on the subthalamic nucleus. This increases the excitatory drive on the output nuclei (GPi, SNpr and EPN), subsequently increasing inhibition of their efferent targets within the thalamus. One of the most important new findings is the demonstration of multiple indirect pathways of information flow through the basal ganglia. In addition to the classic indirect route via GPe and STN, the GPe has been shown to project also to the output nuclei (SNpc/Gpi) as well as to the reticular nucleus of the thalamus. These projections are GABA-ergic in nature. The cortico-subthalamic projection is an additional route by which cortical information flows in order to reach the basal ganglia output structures (Smith et al., 1998).

Thus the "direct" and "indirect" pathways have opposite effects on the output nuclei (inhibition and activation respectively). This in turn has differing effects on thalamic targets, with the "direct" pathway disinhibiting, and the "indirect" pathway inhibiting the target nuclei.

The thalamic targets include the ventromedial/ventrolateral complex (VM-VL), the centromedian-parafasicular complex, and the mediodorsal nucleus and adjacent intralaminar nuclei. Other targets of the entopenduncular nucleus and SNr are the superior colliculus and the pendunculopontine tegmental nucleus (Parent, 1990).

The concept of two opposing pathways has been a major factor in the advancement of research on the anatomical and functional aspects of the basal ganglia. It has also led to

the development of new therapies and surgical strategies in the treatment of basal ganglia disorders such as Parkinson's disease and Huntington's chorea.

1.1.9. The Dopaminergic System

Neurons in the SNpc are typically densely packed and are 11-20μm in size. They have dendrites which spread ventrolaterally into the SNpr. Dopamine released from these dendrites interacts with dopamine autoreceptors, and with dopamine receptors on adjacent dopamine neurons (Cheramy et al., 1981). A second type of neuron is medium sized radiating medio-laterally, but without apical dendrites that run into the SNpr. This separation of dendritic projections forms the basis for the functional segregation of input and output areas in the SNpc (Gerfen, 1992). A third type of neuron, a non-dopaminergic interneuron, makes up about 10% of the SNpc total.

80% of the neurons in the VTA are dopaminergic. The rat VTA has more in common with human than with non-human primates which gives added validity to the use of the rat brain as a model for human study (Halliday & Tork, 1986).

Projections from the SNpc and VTA to the cortex, striatum and to a lesser degree pallidum arise topographically and are largely ipsilateral. These projections have a significant influence on post synaptic neurons and play an important part in the role of dopamine in facilitating loops and outputs in the extrapyramidal motor system.

It is not only the substantia nigra, however, that provides dopaminergic inputs to the striatum. Other midbrain dopaminergic cell groups which do so include: the retrorubral region (Cell group A8 which is contiguous with the caudal portion of the SNpc); and the medially situated ventral tegmental area (A10) which is the main source of dopamine for the ventral striatum, prefrontal cortex and limbic targets although these regions also receive some connections from the medial SNpc (Fallon & Loughlin, 1995).

There are four main inputs to the SNpc, (which is also referred to as group A9 in the mesencephalon): striatal input from striosomes and ventral striatum; limbic inputs from amygdala and lateral preoptic area of the hypothalamus; cortical inputs from prefrontal cortex including premotor cortex; and some fibres from pallidum and subthalamic nucleus. Thus, a number of nuclei, many related to the limbic system, are able to have an influence on striatal activity via the dopaminergic connections from the SNpc (Fallon & Loughlin, 1995).

Dopaminergic neurons are strongly influenced by striatopallidal inputs. The presence in differing degrees of both dopaminergic and GABA-ergic neurons in the SNpc and VTA allow similar striatal projections to influence both systems simultaneously. For example, axons arising from the striatal matrix may terminate on both SNpr GABA-ergic neurons and SNpc dendrites that extend from the SNpc to the SNpr.

Dopaminergic inputs to the striatum follow an ordered pattern. Although some nigral dopaminergic cells project to both striatal compartments, the ventral part of the SNpc and the SNpr (in rats) exclusively innervate striosomes whilst other dopaminergic cells (cell groups A8 and A10) project only to matrix (Fallon & Loughlin, 1995).

Neurons projecting to these different areas could be under different presynaptic control. An indication of differences between these neurons is the fact that dopaminergic cells which innervate striosomes develop to maturity more quickly (Graybiel, 1990), while those which innervate matrix areas exhibit a faster dopamine turnover, contain the calcium binding protein calmodulin, and are more sensitive to MPTP toxicity.

Dopamine projections from SNpc/VTA extend to various other structures including the caudate-putamen, nucleus accumbens, olfactory tubercule, amygdala, and also to prefrontal, cingulate, perirhinal and entorhinal cortices. Non-dopaminergic projections

include thalamus, superior and inferior colliculi, mesopontine tegmental/PPN and visual cortex (Fallon & Loughlin, 1995).

1.1.10. Dopamine receptors in the striatum

The dopamine receptor can be divided into five subtypes, D_1 to D_5.This section will focus mainly on D_1 and D_2 subtypes. Several studies have shown that these receptors are localized postsynaptically on different striatal neuronal systems. D_1 is expressed on striato-nigral (direct) neurons, D_2 on striato-pallido-subthalamo-nigral neurons (Gerfen, 1990). Other studies have also concluded that about 20-40% of D_2 receptors are localized on cortico-striatal terminals (Schwarcz et al., 1978). This, however, has been debated (Filloux et al., 1988).

Dopaminergic inputs to the striatum have differing effects on the 'direct' and 'indirect' pathways. Dopamine has an excitatory influence on the 'direct' pathway via D_1 receptors and the neuropeptides substance P and dynorphin. In the 'indirect' pathway, dopamine has an inhibitory effect via D_2 receptors, that is influenced by enkephalin. When dopamine is substantially depleted, for example in Parkinson's disease or in rats with 6-OHDA lesions, the resulting effect on the 'direct' pathway is to reduce the inhibitory influence of striatal GABA-ergic neurons on SNpr/GPi, thus increasing inhibition of the thalamus and reducing locomotor activity. In the 'indirect' pathway, a reduction in dopamine has the effect of increasing enkephalin and there is an increased firing of striatal GABA-ergic neurons due to reduced inhibition via dopamine, which results in down regulation of the GPe and subsequent over-activity of the STN. This has the effect of over inhibiting the thalamus.

In a review article, Levy and colleagues (1997) consider studies that suggest that the GPe is not hypoactive in Parkinson's disease or in MPTP monkeys, and that the activity of

GPe neurons is not dependent only upon striatal influence, but perhaps also upon excitatory projections such as those from the STN via a sub-loop (glutamate), cortex (glutamate), parafasicular nucleus (glutamate), and SNpc (dopamine).

Cortico-striatal glutamatergic neurons innervate either striosomes or matrix depending on the layer of origin. They project onto medium spiny GABA-ergic neurons that activate both direct and indirect pathways. In vitro studies have shown glutamate also stimulates tonic and phasic dopamine release presynaptically via AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) and NMDA (N-methyl-D-aspartate) receptors, some of which are located on dopamine nerve terminals (Krebs et al., 1991; Gauchy et al., 1991). Dopamine itself inhibits its own release by acting on DA autoreceptors (D_2 and D_3) on dopamine nerve terminals. Acetylcholine, located in striatal interneurons, can also facilitate or reduce the release of dopamine (Gauchy et al., 1991).

1.1.11. The 6-Hydroxydopamine (6OHDA) rat model

Experiments relating to the role of the substantia nigra and the striatum in motor control have given an insight into the site of dopamine action in the nigrostriatal circuit. Ungerstedt (1968) developed the 6OHDA rat model for the dopaminergic loss in Parkinson's disease. This destruction of the nigrostriatal pathway, and unilateral loss of dopamine release, was achieved by a unilateral injection of the toxin into the ascending forebrain bundle. Dopaminergic cell loss in the SNpc results in reduced tonic dopamine release in the striatum due to a reduction in the number of terminals. Compensatory mechanisms such as the sprouting of remaining terminals, increased production and release from these terminals, and an increase in the of postsynaptic dopamine receptor numbers were found to be a feature of these models.

1.2. Movement Disorders

Dysfunction of the basal ganglia is the root cause of many movement disorders. These diseases can be classified into two categories; hypokinetic and hyperkinetic. Hyperkinetic diseases such as Huntington's disease are generally associated with decreased output from the basal ganglia, and are characterised by an excess of movement that tends to be uncontrolled and relatively rapid.

Hypokinetic diseases, such as Parkinson's disease, are associated with increased basal ganglia output which typically results in symptoms such as akinesia, bradykinesia and rigidity (Albin et al., 1989). Pharmacologically, they tend to show the inverse of hyperkinetic diseases in that they are exacerbated by D_2 antagonists and cholinergic agonists, and are improved with dopamine agonists and anti-cholinergic treatment (Lang & Lozano, 1998).

1.2.1. Multiple System Atrophy

The term multiple system atrophy (MSA) refers to a group of sporadic hypokinetic disorders that tend to occur in middle age, presenting with extrapyramidal, cerebellar or autonomic symptoms (Wenning et al., 1997). Symptoms include ataxia, rigidity and general autonomic failure. Degeneration occurs in a variety of subcortical systems clinically presenting as striato-nigral degeneration, in which Parkinsonism predominates; olivopontocerebellar atrophy, which affects balance, coordination and speech, and Shy-Drager syndrome in which such autonomic functions as blood pressure and urinary control are also affected. Levodopa is an effective treatment in striato-nigral degeneration.

Pathological studies have shown that up to 10% of patients thought to have Parkinson's disease actually have MSA.

1.2.2. Progressive Supranuclear Palsy

Progressive Supranuclear Palsy (PSP), also known as Steele-Richardson-Olszewski syndrome, is a sporadic, late-onset hypokinetic disease that is rarely familial. It is clinically characterised by rigidity, akinesia, postural instability, vertical gaze palsy and frontal lobe dementia, and is L-DOPA resistant. One to eight percent of patients with Parkinsonism suffer from this syndrome (Collins et al., 1995). Nigrostriatal dysfunction is the main cause of PSP with an 80-90% decrease in dopamine, tyrosine hydroxylase and D_2 receptors in the striatum. Damage to the SNpr, STN and pallidum causes severe disruption of the pathways to the motor thalamus.

1.2.3. Corticobasal Degeneration

This rare, late onset disorder may clinically resemble Idiopathic Parkinson's disease and PSP (Bergen et al., 1998). It is characterised by an asymmetrical akinetic-rigid syndrome associated with cognitive (apraxia and aphasia) and extrapyramidal motor dysfunction (rigidity and dystonia). Moderate dementia is occasionally observed late in the course of the disease.

Neuropathological examination reveals lobar frontal or parietal atrophy, severe neuronal loss and gliosis in the cortex with the presence of swollen, achromatic, tau-positive staining Pick cells. The basal ganglia and substantia nigra show severe degeneration. The disease is poorly L-DOPA responsive.

1.2.4. Parkinson's Disease

Described over 180 years ago by James Parkinson (1817), Parkinson's Disease (PD), the classic hypokinetic disorder, is characterised by its main symptoms, namely,

resting tremor, rigidity, bradykinesia, and akinesia. There is also disruption of normal gait patterns and of posture. Dementia is an important part of PD in elderly patients with the frequency being 6.6% up compared with normal ageing (Mayeux et al., 1990). Pathological examination in PD remains the only means of diagnosis and there is still no biological marker. Pathology normally reveals significant cell losses in dopaminergic neuromelanin containing cells in the SNpc, serotonergic cells in the raphe nucleus, noradrenergic cells in the locus ceruleus, the cholinergic nucleus basalis of Meynert, hypothalamus, small cortical neurons particularly in cingulate gyrus and entorhinal cortex, olfactory bulb, sympathetic ganglia, and parasympathetic neurons in the gut (Lang & Lozano, 1998). The presence of Lewy bodies is also a pathological feature.

SNpc loss is greatest in the ventrolateral tier (60-70% at onset), followed by the medial ventral tier and dorsal tier. This pattern is relatively specific to PD (Fearnley & Lees, 1991). These dopaminergic deficits result in regional loss of striatal dopamine, mainly in the dorsal and intermediate subdivisions of the putamen (Kisch et al., 1988). This is believed to account for akinesia and rigidity.

Among the factors thought to cause neurodegeneration in PD (Olanow & Tatton, 1999) are:

i.) Oxidative stress, where damaging levels of hydrogen peroxide and other reactive oxygen species are increased as a result of a) increased dopamine turnover, b) a deficiency in glutathione, and c) a build up of reactive iron which can lead to hydroxyl radical formation.

ii) Mitochondrial dysfunction, in which a selective decrease in complex1 activity of the mitochondrial respiratory chain affects the SNpc.

iii) The toxic effects of increased glutamate formation.

The resurgence of surgical procedures as treatment for PD has come with the increased understanding of the pathophysiology of the disease. Medial pallidotomy has been shown to be effective in reducing or even abolishing L-DOPA induced dyskinesias (Dogali et al., 1995). High frequency electrical pallidal stimulation is reported to give similar results (Arcusa et al., 1996). Given that striatal dopamine depletion in PD is associated with reduced inhibition of the GPi (Figure 2), the improvements due to pallidal surgery seem to have the effect of removing this excessive inhibition, reducing it's inhibitory effect on the thalamus, and thereby facilitating movement. Other surgical approaches involve the stimulation of STN, which reduces it's excitatory input to, and stimulation of the thalamus. The latter effectively abolishes tremor (Benabid et al., 1991).

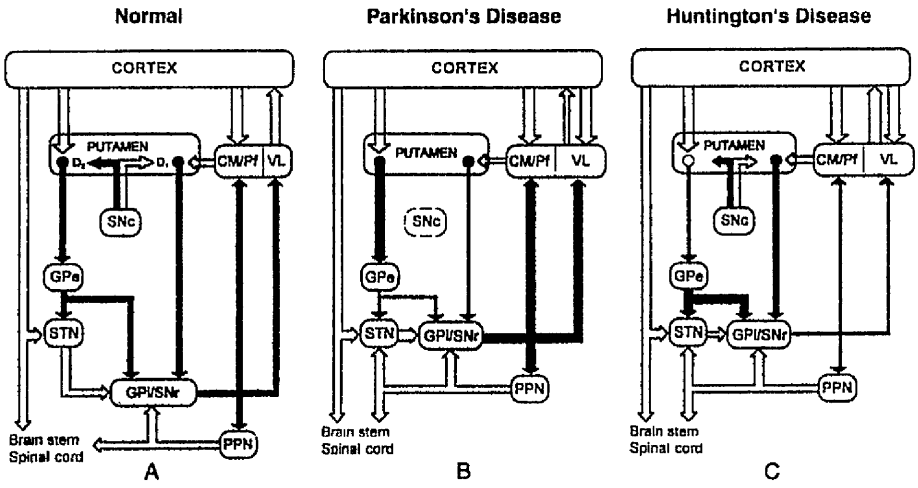


Figure 2. Basal ganglia thalamocortical circuitry A) under normal conditions. B) with Parkinson's disease C) with Huntington's disease. Inhibitory projections are shown as filled arrows, exitatory projections as open arrows. Changes in arrow thickness reflect differential changes between disease states.
GPi = internal segment of the globus pallidus; GPe = external segment of the globus pallidus; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; STN = subthalamic nucleus; VL=ventrolateral thalamus; PPN = pendunculopontine tegmental nucleus; CM/PF = centromedian/farafasicular nucleus of the thalamus. From Wichmann and DeLong (1998).

A range of drugs has been used to treat PD with various levels of success. The dopamine precursor L-DOPA has for many years been the most effective. Used along with the decarboxylase inhibitor carbidopa, it is converted to dopamine thus improving striatal

deficits. The prolonged use of L-DOPA can, however, result in hyperkinetic type abnormal involuntary movements (Marconi et al., 1994).

Other drug types include acetylcholine blockers (trihexy phenidyl, benztropine), dopamine metabolism enzyme (MAO/COMT) blockers (selegiline, tolcapone), and dopamine agonists which mimic the action of dopamine on dopamine receptors (bromocryptine, apomorphine).

1.2.5. Huntington's Disease

A hyperkinetic disorder, Huntington's Disease (HD) was first described as a specific disease in 1872 by the American Physician George Huntington, who believed that the distinguishing features of the disorder, namely the late onset and its hereditary nature, made it quite distinct from the chorea described prior to its discovery. The mutation which is responsible for HD is an expanded polyglutamine repeat, (CAG), within exon 1 of the gene that codes for the protein Huntingtin (Reddy et al., 1999; Hedreen & Folstein, 1995).

HD is the classic hyperkinetic disorder characterised by progressive chorea, rigidity, dementia, dystonia, cognitive deficits and psychological disturbance. It is an autosomal dominant hereditary neurodegenerative disease. Pathologically, there is an apparent non-random degeneration of medium sized striatal projection neurons early in the course of the disease. Striatal GABA-ergic output neurons projecting to the GPe appear to be affected initially. The dying off of these neurons leads to reduced inhibition of neurons in the GPe, and the subsequent increased inhibition of STN neurons. This results in a decrease in the inhibitory output from the GPi, which effectively over activates the thalamus causing increased glutamatergic stimulation of the cortex, resulting in chorea (Figure 2).

Damage also extends to the nucleus accumbens, pallidum, ventrolateral thalamus, STN and SNpc and includes loss of cortico-striatal neurons in layer V of the cortex. When

the disease becomes more advanced, striatal output neurons to the GPi (direct pathway) are affected, resulting in disinhibition of GPi neurons. The increased activity of these neurons has the effect of reducing chorea sometimes to the extent where hypokinetic symptoms such as decreased motor activity and rigidity occur (Albin et al., 1990). PET studies have shown reductions in both D_1 and D_2 receptor binding in the striatum of HD patients with both rigid and choreic predominance. Decreases of at least 40% have been found in early stages of the disease (Brooks, 1998). This opens the possibility of detecting pre-clinical features of the disease in patients carrying the HD gene.

1.3. The AS/AGU Rat

The AS/AGU rat arose as a spontaneous mutation within a closed inbred colony of Albino Swiss (AS) rats. The mutation is recessive (Campbell et al., 1996) and the rats have been isolated as a true breeding substrain. Phenotypic differences are the result of a single mutation. The mutant is characterised by serious movement impairments including rigidity, a wide staggering gait with the tendency to fall over every few steps, a slight whole body tremor and difficulty in initiating movement, particularly in older animals. (Clarke & Payne, 1994; Payne et al., 1998).

There are no obvious morphological differences between normal AS and AS/AGU mutants with neocortical and cerebellar areas appearing normal. Immunocytochemical studies, however, revealed a deficit of up to 60% in tyrosine hydroxylase immunoreactive cells in the SNpc of 12-14 month mutants compared with AS controls (Clarke & Payne, 1994).

Studies by Lam and colleagues (1998), revealed significant depletion of 2-deoxy-glucose uptake in SNpc, STN and ventrolateral thalamus in mutants. These findings

pointed to a wider basal ganglia involvement, possibly indicating that the indirect pathway might be more affected than the direct.

When whole tissue dopamine levels were measured in striatal micropunches (Campbell et al., 1996) using High Performance Liquid Chromatography with electrochemical detection (HPLC-ECD), AS/AGU mutants showed a reduction of 20-30% compared with the parent AS strain. This reduction does not occur until the animals are 6 months old, which is well after the time of onset of locomotor abnormalities. These symptoms can, in fact, be observed when the animals are as young as 21 days old. In-vivo microdialysis studies on freely moving rats have found a massive reduction of extracellular dopamine, as measured with HPLC-ECD, in the dorsal caudate putamen of AS/AGU rats aged 3 months and older, compared with control rats. Three months is the earliest point at which stereotaxic surgery can be confidently carried out using a standard stereotaxic atlas. It is possible that rats at even younger age points may have similar (if not greater) striatal dopamine deficits. Abercrombie and colleagues (1990) reported little change in extracellular dopamine until tissue levels had fallen to around 20% of control values. The opposite seems to be the case with the AS/AGU rat. With large dopamine reductions having been found in mutants younger than 6 months with extracellular studies, but not in whole tissue levels, it is reasonable to assume that there may be a problem with dopamine release in the AS/AGU rat. Indeed, pharmacological studies with dopamine release evoking agents such as amphetamine, as well as monoamine oxidase inhibition with clorgyline, and dopamine re-uptake blockade using nomifensine, seem to strengthen this hypothesis (Campbell et al., 2000).

1.3.1. The AS/AGU Mutation

The *agu* gene encodes the brain-restricted isoform of Protein Kinase C gamma (PKC-γ). Genetic mapping demonstrates very tight linkage between the *agu* mutation and a marker in the 3' UTR encoding region of the PKC-γ gene (Craig et al., 2001). A premature stop codon is found in the PKC-γ gene of mutant rats, which would prevent synthesis of the catalytic domain of the protein. The result is that the AS/AGU rat has a truncated form of this protein with the active component missing. Therefore PKC-γ activity is completely abolished in this animal model.

PKC-γ is a 78.4 kDa protein thought to be specifically present in high concentration in neuronal tissues and has been implicated in a broad spectrum of neuronal functions. PKC activation within nerve cells is frequently associated with the modulation of ion channels (Shearman et al., 1989), the desensitisation of receptors (Huganir and Greenyard, 1990), and the enhancement of neurotransmitter release. PKC-γ is one of 14 isoforms identified in mammalian tissues. It is known to be highly expressed in purkinje cells (PCs), medium sized neurons of the striatum and globus pallidus, which project to the substantia nigra pars reticulata, and the spinal cord (Chen et al., 1995). However, the overall distribution of PKC-γ in the CNS remains unclear.

1.3. Aims of the Study

The purpose of this study was to look closely at the effect that these deficits might have on locomotor performance, how this might alter with age, and whether the pharmacological approach of L-DOPA replacement would restore extracellular dopamine levels to normal AS control values.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study consisted of two sets of experiments. In experiment 1, a study of locomotion in AS and AS/AGU animals was made at various ages using four tests (a) Inclined ramp test, (b) Mid-air righting, (c) Gait analysis and (d) Infrared movement analysis. Experiment 2 was an investigation into the effects of L-DOPA on the movement disorder and included locomotor assessment, and the measurement of striatal dopamine and its metabolites using in-vivo microdialysis and high performance liquid chromatography. Details of all chemicals and equipment used during this study are listed in appendix 2.

2.1. Locomotor Assessment

A number of studies were carried out to assess locomotion in the mutant AS/AGU rats and to compare them with the parent AS strain.

Initially, a total of 187 AS (control) and 176 AS/AGU male mutant rats were observed and scored in two simple tests of movement: a) the inclined ramp test, and b) mid-air righting. The rats ranged in age from 20 days to 1 year. The numbers in each age group are shown below.

AGE (days)	AS	AS/AGU
20-30	17	17
40-50	35	27
60-70	37	24
80-90	23	39
100-130	34	27
140-150	22	20
365	19	20

2.1.1. Inclined ramp test

The rats were observed whilst walking down a series of ramps of various widths (12, 9, 7, 5, 3.5 and 1cm). Each ramp was 80cm in length and was placed at an angle of 40° into a plastic container (150×70×20cm) that contained a deep layer of bedding medium that served to protect the rats that fell from the ramps during unsuccessful attempts.

Figure 3 shows a rat walking down the 90mm ramp. The upper surface of each ramp was roughened with saw-cuts to provide a good grip.

Figure 3. Inclined Ramp Test showing an AS rat negotiating the 9cm Ramp.

Animals were placed at the top of each ramp (starting with the broadest) facing downward, and scored on their ability to walk to the bottom of the ramp without falling off. Three attempts were allowed on each ramp. All tests were video recorded and assessed on playback.

2.1.2. Mid-air righting

The same animals used in the previous test were held upside down, horizontally above a thick layer of bedding contained in the plastic container previously described, and dropped.

The ability of the animal to right itself in mid-fall and land on its feet was recorded as a success. Animals landing on their back, side or head were considered to have failed the test. Adult animals were dropped from a height of 50cm, but rats aged less than 50 days were dropped from 30cm to account for their smaller size. Heights were confirmed against a vertical calibrated scale that formed a backdrop for video recording.

2.1.3. Gait analysis

Secondly, in order to make a detailed study of the effects of the mutation on normal locomotion in the AS/AGU rat, a computerised gait analysis system was built to determine the pattern of foot-floor contact and to compare it with that of normal AS animals. This process was undertaken in 4 steps:

i) Recording of footfall patterns using a footfall chamber and video camera.

ii) Digitizing of footfall images using a silicon graphics computer and software.

iii) Analysis of images on Kontron KS400 system.

iv) Handling of data in Microsoft excel.

i) Footfall chamber

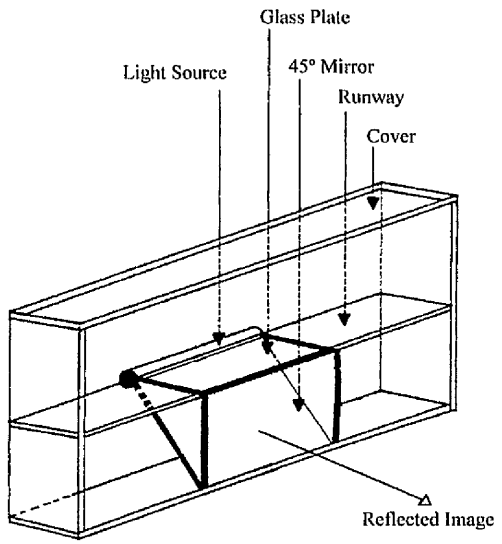


Figure 4. Footfall chamber based on a design by Clarke (1992).

This apparatus, shown in figure 4, was constructed in-house and based on an original design by Clarke (1992). The 100×20×10cm plexiglass box contains an enclosed platform which has as it's central area a 6mm thick glass plate measuring 30×10cm. The relative

narrowness of this passage is designed to encourage the rat to walk in a straight line across the glass, which is the recordable part of the platform. Light from an 8-watt fluorescent tube is shone horizontally through the edge of the glass plate, and is totally internally reflected unless broken and scattered downward by the contact of a foot on the glass. Pad to glass contact is thus represented by a white pattern which is recorded onto a videotape via a 45° mirror. The resulting monochrome image - white areas against black (unlit) glass - is vital in achieving separation of individual pads during subsequent analysis. All footfall recordings were carried out in a quiet, dark room. Each rat was placed onto one end of the platform, and at least 3 spontaneous, complete crossings of the glass area were recorded. Rats aged 9 months were assessed (AS n=9, AS/AGU n=9).

ii) Digitization of video images

The hind foot of the rat contains 11 pads: 5 digital tips (1,2,3,4 and 5), 4 distal pads (A, B, C and D) and 2 proximal pads (E and F- see figure 5a)

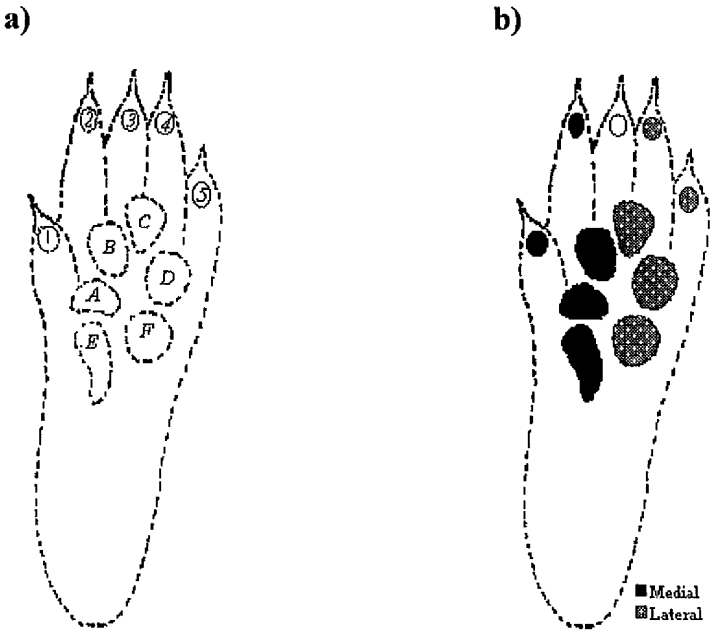


Figure 5. Diagrams showing the pad arrangement on the hind left foot of a rat. **a)** The 11 pads are labelled: 5 digital tips (1,2,3,4 and 5), 4 distal pads (A, B, C and D), and 2 proximal pads (E and F). **b)** The foot is divided along the mid-anterior/posterior line in order to determine lateral and medial groupings.

The videotape was viewed frame by frame, and the frames were digitized using a silicon graphics workstation to obtain individual images throughout each step taken by both the left and right hind feet. This resulted in a set of images per step ranging between 6 and 20 in number depending on speed of movement (see figure 6). Only steps falling within this range were considered, in order to ensure that the speed was neither too slow nor too fast. This helped to ensure consistency and accuracy.

The images were then saved in a form (.bmp) that could subsequently be handled by the imaging software.

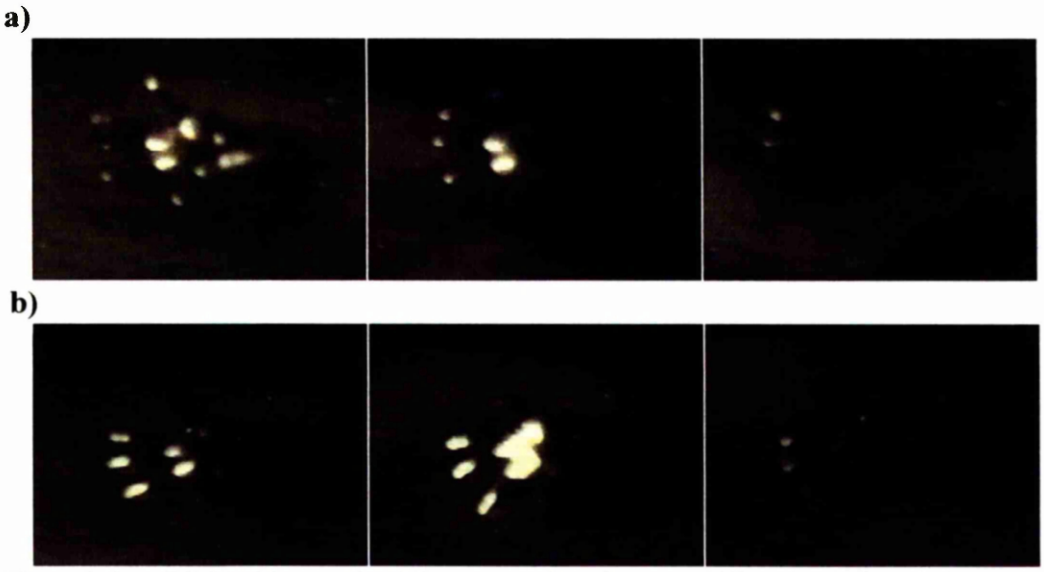


Figure 6. Sets of digitised images of pad-floor contact from the start, middle and end of a typical step.
a) The AS rat starts the step with all pads in contact with the glass and is linear throughout the step.
b) TheAS/AGU begins with incomplete contact and continues through the step with abnormal contact, and with the foot at an angle.

iii). Image analysis

A Kontron KS400 image analysis system was used to a) process footfall images into a binary form which was necessary for automatic measurements, b) present the images to be interactively separated into individual pads, and c) measure the area of contact of each pad, and display these areas.

iv). Data handling

Data was transferred directly into Microsoft Excel. Pad areas from the whole foot were divided into 2 groups, ie., those pads falling in the lateral half of the foot (1, 2, A, B and E), and those of the medial half (4, 5, C, D and F). The foot was divided along the mid-anterior/posterior line in order to determine the groupings (figure 5b).

Areas within both segments were added together to give total values for lateral and medial sides that were then expressed as percentages of the whole foot contact area.

2.1.4. Infrared movement analysis

A third study was undertaken to evaluate normal spontaneous locomotor activity within the cage environment, using the computerised infrared sensor system which is shown in figure 7.

This equipment, a Coulburn Instruments infrared motion activity system, is capable of detecting movement within the cage in three dimensions, and recording information about the number and duration of movements based on pre-programmed criteria.

Figure 7. Cages with sensors which detect spontaneous locomotor activity as part of the infrared movement monitoring system.

An infrared sensor is attached to the top of a cage of dimensions 50×32×18 (cm) facing into the cage at an angle of 45° to ensure that activity in all areas of the cage is detectable. The sensors are connected to a computer software package from which data can be collected

AS control and AS/AGU male rats aged 9 and 12 months (n=9 for each strain in each age group) were assessed. Activity was monitored over two different time periods: a) a 12-minute run, with data being reported every minute, and b) A 22-hour run, which reported every 15 minutes. Movements of two different durations were recorded ie: **small** movements (1-3 seconds duration), and large movements (more than 3 seconds). This ensured that movements lasting less than 1 second would not be reported. In this way natural phenomena such as breathing, or tremor in the case of mutants, were ignored.

2.2. L-DOPA treatment

This consisted of two studies. One to examine the effects of L-DOPA on locomotion, the second to evaluate the effect of L-DOPA administration on dopamine release in the striatum.

2.2.1. Locomotor assessment

Twenty AS/AGU animals aged three months were used to assess the effects on the locomotor disorder of the classic Parkinson's drug L-DOPA which can cross the blood-brain barrier and is therefore given instead of dopamine. Rats were divided into 2 treatment groups with 10 in each group. One group was treated with L-DOPA and the other with saline as a control injection. Animals in the drug treatment group were given intraperitoneal injections of L-DOPA (25mg/kg in 1ml of saline) daily for 4 weeks. Each injection was preceded by an injection of the peripheral DOPA decarboxylase inhibitor benserazide (2.5mg/kg in saline) 30 minutes prior to L-DOPA treatment. This ensured that the administered L-DOPA was being metabolised in the brain only. All treatments were given at the same time point each day.

Mid-air righting and inclined ramp tests as described above were carried out at weekly intervals on all animals, two hours after the L-DOPA or saline injections. Testing was restricted to one session per week in order to minimise learning effects.

2.2.2. Measurement of extracellular levels of Dopamine, DOPAC and HVA in the striatum.

Microdialysis, followed by high performance liquid chromatography with electrochemical detection (HPLC-ECD), was used according to the method adopted by Campbell et al. (1998), in order to determine extracellular levels of dopamine and its

metabolites in the striatum in freely moving rats. This technique allows the monitoring of normal basal neurotransmitter levels in conscious animals, and also has the benefit of being able to show the effects of drugs administered either before or during the microdialysis process. Samples can be collected at chosen flow rates thus determining sample volume, and neurotransmitters can be detected at very low concentrations (picomoles) in protein free samples. This eliminates the need to pretreat samples before HPLC analysis.

There are three main stages to the extracellular sampling study; i) implantation of a guide cannula, ii) collection of microdialysis samples via a probe, and iii) HPLC-ECD analysis.

After the four week L-DOPA treatment described above, 4 AS and 4 AS/AGU rats were studied using these techniques, under Home Office project licence 60/2167.

i) Implantation of cannula.

This procedure was carried out by Dr Jackie Campbell under the above licence. Animals were anaesthetised using Vetalar (ketamine hydrochloride; 100mg/ml) and Rompun (Xylazine hydrochloride 2%) in a ratio of 2:1. Intraperitoneal Injections were given at 1.1ml/kg of body weight.

Deep anaesthesia was achieved after 5-10 minutes. The 'toe pinching' technique was used to determine this, with no reflex withdrawal of the foot being observed when full anaesthesia was established. The animal was then placed into ear bars on a Kopf stereotaxic frame, and the head held in place using tooth and nose bars. This stabilised the head to allow a midline incision to be made in a pre-shaved area of the scalp using a scalpel blade, the skin being held back from the skull using skin retractors.

Scraping of the skull using a scalpel blade revealed the point of intersection of the coronal and sagittal cranial sutures (bregma), which was marked using a pencil.

Coordinates derived from a standard atlas of the rat brain (Paxinos and Watson, 1982), were used to locate the dorsal caudate-putamen. These coordinates were; 1mm (anterior/posterior), 3.5mm (lateral) and 4.5mm (ventral), using bregma as a starting point. This is shown in

figure 8.

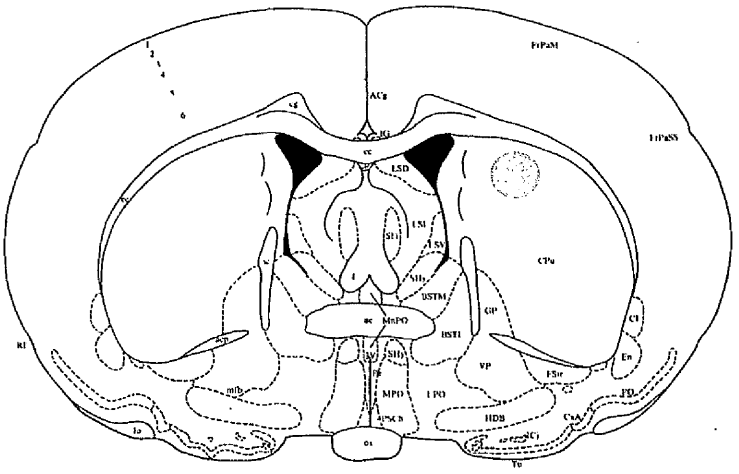


Figure 8. Diagram a brain section showing the level of the striatum from which microdialysis samples were taken. The grey circle indicates the dorsal sampling point which is represented by the coordinates: 1mm (anterior/posterior), 3.5mm (lateral) and 4.5mm (ventral). From 'The rat brain in stereotaxic coordinates' (Paxinos and Watson, 1982).

Firstly, the anterior/posterior and lateral coordinates were used to determine the point on the skull into which a hole was drilled using a 1mm drill. The cannula was then placed over and lowered into the hole slowly until the ventral coordinate was reached.

In order to ensure that the cannula would remain in place until the end of the experiment, it was secured using *Redifast* dental cement. A further two holes were drilled into the skull in an area close to the probe, into which two screws were placed securely before the cement was poured over the whole area which was contained within a plastic 'hat' which was made by cutting a 5ml plastic syringe to obtain a 1cm deep cylinder. Being

embedded in the cement, the screws had the function of ensuring that the cannula assembly was properly secured onto the rat's head.

Once the cement hardened, a guide cannula was placed inside the previously inserted cannula in order to seal and protect it from damage. The rat was then removed from the stereotaxic frame, a subcutaneous injection of 0.1ml Antisedan (atipamezole hydrochloride, 5mg/ml) was given to reverse the effect of the anaesthetic, and the animal was allowed to recover in an incubator before being placed back into its cage.

This whole procedure had to be performed within a 40 minute period in order to ensure that the animal did not recover before the operation was complete. The position of the cannula was confirmed at a later date by sectioning the brain on a Jeol cryoststat.

ii) Microdialysis

After a period of two days recovery in their normal cage environment, animals were linked up to a microdialysis system. This consisted of a CMA automatic microinjector, a series of lengths of narrow bore tubing, a microdialysis probe and a Perspex box (42×23×41cm). The Perspex box contained rat bedding material, food and water, and had a metal lever (reflex arm) attached which allowed the rat to move around the box freely without disrupting the tubing or probe.

The rat was held securely with its head immobilised, and the probe was then inserted carefully into the cannula, and secured with superglue. The microsyringe was attached to the probe's inflow tubing via a connection at the collection port that was situated at the end of the reflex arm. The outflow probe tubing was connected to a plastic collecting vial at this port.

The microdialysis probe

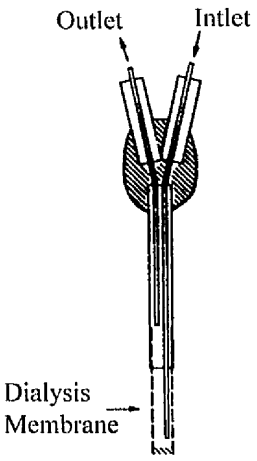


Figure 9. Single cannula type probe
used to collect microdialysis samples.
Based on a method by Carswell et al., (1997).

Shown in diagram 9, the microdialysis probe is a single cannula type and is a variation on that used by Carswell and colleagues (1997). It consists of a series of tubing types of different diameters ranging from a 1mm diameter plastic tube down to a silica glass tubing which is situated innermost in a concentric arrangement, and has an outer diameter of 25μm.

The probe's active area, which forms the tip, consists of a 200μm ID semi permeable tubular dialysis membrane with a 40,000 molecular weight cut-off. The pore size is 60Å. Microdialysis occurs over the exposed surface of this membrane, and its length can be adjusted to suit the size of the brain area under investigation. Artificial cerebrospinal fluid (ACSF) flows into the region where the microdialysis takes place and the dialysed liquid then flows back out of the probe, via the silica glass tubing that is situated inside the microdialysis tubing, the tip of which is plugged with *RS* epoxy resin.

The dialysis membrane allows the two-way passage of fluid with the exclusion of any high molecular weight protein matter that would contaminate the sample. Neurotransmitters are thus passed through a concentration gradient into the transmitter- free ACSF. This allows us to monitor basal extracellular concentrations under standardised conditions as well as following pharmacological interventions. This fluid is passed through the probe at a flow rate of 2µl per minute via a length of narrow bore perspex tubing (0.28mm internal diameter) and is collected after microdialysis in the plastic collecting vial. Samples are collected every 20 minutes giving a sample volume of 40µl. No pre-HPLC treatment is required for these pure samples, hence they can be either injected onto the HPLC system immediately or frozen in liquid nitrogen for injection at a later time.

Animals were allowed to move around freely in the Perspex box for a period of between 1 and 2 hours before samples were collected, and after a series of 3 consistent samples showing steady neurotransmitter levels, basal sample collection began. Basal samples (resting levels after 4 weeks of L-DOPA treatment) were collected for a **period of** 100 minutes before a bolus injection of Benserazide (10mg/kg in 0.1% sodium bisulphite) and L-DOPA (100mg/kg in sodium bisulphite) were given half way during the collection of the last basal sample. Samples were then collected for a further 5-hour period.

i) HPLC-ECD analysis

Sample analysis was carried out according to the method used by Campbell et al (1998). The HPLC system consisted of a Gilson 305 pump with 805 manometric module, a 7125i Rheodyne sample injector, a microbore column and an ANTEC *intro* electrochemical detector. The column (stationary phase) was a Hichrom microbore reverse phase column with a C18 ODS2 5µm packing gel. This allowed effective and consistent separation of catecholamines when used with a buffered mobile phase consisting of: Citric

acid (83 mM), EDTA (1mM), Disodium Hydrogen Phosphate (43 mM), Octane Sulphonic Acid (0.2 mM) and Methanol (10%). PH was 3.5.

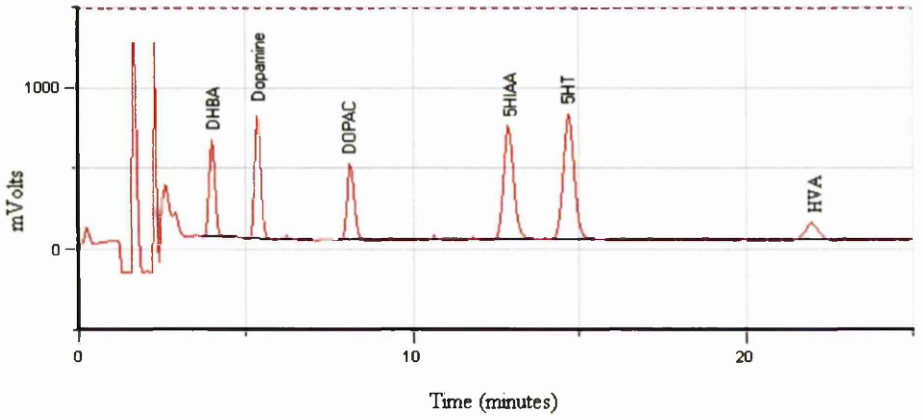
A 100µl sample loop was used on the injector to ensure that the whole sample (45µl) was injected onto the column. This maximised the amount of detectable neurotransmitter.

A composite standard containing all of the detectable metabolites (Dopamine, DOPAC, 5HT, 5HIAA and HVA) was prepared at a concentration of 10 nanograms per ml. for each. An internal standard, 3,4,dihydroxybenzylamine (DHBA), was added to the composite mixture at the same concentration. This composite mixture has two functions. Firstly, it is used to determine the time that it takes each substance to be eluted from the column, and detected by the ECD (retention time). Secondly, the peak areas from the resulting composite chromatogram are used to calculate the response factor (RF) **for each** substance. This factor (DHBA peak area divided by area of transmitter peak), is later used when calculating final concentrations.

A typical 45µl microdialysis sample contains 40µl of dialysate and 5µl of DHBA at the same concentration as in the composite standard. With some experiments, a sample volume of slightly less than 45µl was injected due to a smaller return per 20 minutes via the microdialysis probe. The 5µl of DHBA (internal standard) was added in order to obtain a peak from each sample that represented a known concentration. This internal standard was chosen because it is stable, is not found in the brain, and it is not known to react with any of the catecholamines present in the sample. This peak area for DHBA was used to calculate the unknown concentrations of catecholamines within the sample. These were expressed in picograms per sample.

Typical composite standard and sample chromatograms are shown in Figures 10a) and b).

a)



b)

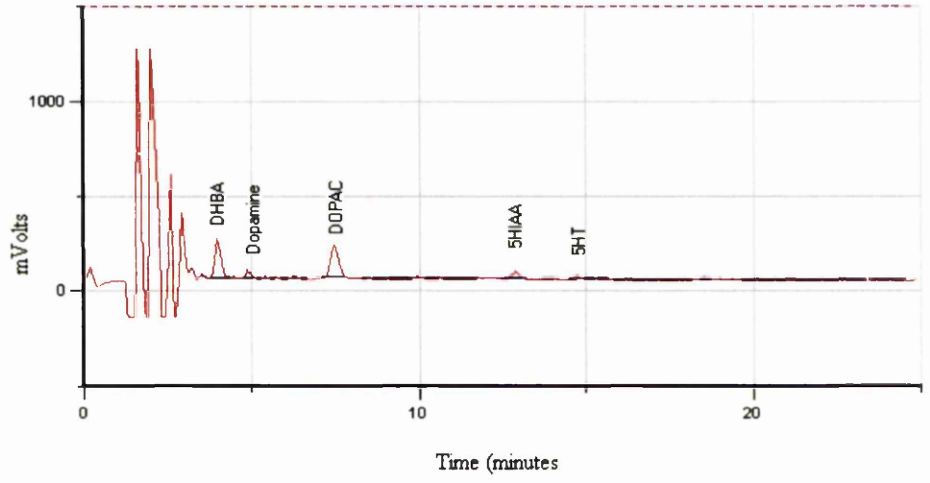


Figure 10. Typical HPLC chromatograms from **a)** Composite standard, and **b)** Microdialysis sample. Peaks in order of elution are DHBA (internal standard), Dopamine, DOPAC, 5HT, 5HIAA and HVA (not in this sample).

These chromatograms show the order of elution of the internal standard and neurochemicals under investigation. The catecholamine peaks are identifiable by their retention times, a line drawn underneath the peaks, and the areas calculated by the computer software. The calculation by which the peak concentrations are determined from these areas is shown in appendix 1.

2.3 Statistical Analysis

In both the age series study and the L-DOPA investigation, performance on the series of ramps of decreasing width was analysed statistically using analyses of variance (F). Ramp width constituted a non-continuous variable in the analysis. With mid-air righting tests, comparisons were carried out using chi square (χ^2) tests. All of the remaining statistical analyses were performed using Student's t-tests.

RESULTS

RESULTS

3.1. Locomotor Assessment

3.1.1. Inclined ramp test

The results of the inclined ramp test are shown in Figure 11.

AS animals

As shown in figure 11a, AS control animals in all of the age groups tested performed well across the range of ramp widths except for the narrowest (1cm), where the percentage of rats completing was found to be reduced as the animals got older and bigger. This resulted in only 60% success from age 100 days. This figure is reduced to a 10% success rate by 1 year of age. Performance on the second narrowest ramp (3.5cm) was also affected, but to a much smaller degree. Here, 80% of rats at 1 year still managed to walk down the ramp without falling off.

AS/AGU animals

The general pattern seen in figure 11b) is of a decline in performance on all ramp widths with age. No AS/AGU rat tested, regardless of age, was able to successfully walk down the 1cm ramp. None of the mutant rats aged 50 days or older were able to negotiate the 3.5cm ramp with any success. There was also a very poor success rate (maximum 10%) on the 5cm ramp after 50 days.

Performance according to age was analysed for both the AS and AS/AGU groups by analyses of variance (F); see Appendix 3a. Because ramp width constituted a non-continuous variable, each ramp was assigned a score from 1 (the narrowest) to 6 (the widest). Each animal at each age received a score in this range which was therefore the narrowest ramp it was capable of negotiating successfully; an animal which could not walk down any ramp received a score of 7. Both groups of animals showed a highly significant variance in performance with age. For AS males ($F = 33.23$, df 5 x 158, $p < 0.001$) and AS/AGU males ($F = 35.17$, df 5 x 129, $p < 0.001$) in which there was an increase in the narrowest ramp they were capable of walking down. However, in the case of AS rats, this simply meant that performance declined from all rats being capable of negotiating the narrowest ramp (1cm) to most rats only being able to negotiate the second narrowest ramp (3.5cm). Performance at the earliest three ages did not differ significantly, but animals aged 100-130 days had a significantly poorer performance ($p < 0.05$), whilst animals aged 140-150 days and 365 days exhibited a poorer performance than all younger animals ($p < 0.05$). In the case of AS/AGU rats, performance declined much more drastically (from most animals being able to negotiate the third narrowest ramp at 20-30 days to very few animals being able to walk down even the widest ramp at 365 days). Thus, performance at 40-50 days was significantly worse than at 20-30 days ($p < 0.05$); performance over the age range of 60-150 days was worse still ($p < 0.05$) and performance at 365 days was worse again ($p < 0.05$). For each age, comparisons between AS and AS/AGU rats were made by t-tests (see Appendix 3b); in each case these revealed a highly significant difference between the two strains with locomotor performance being worse in the AS/AGU group (**$p < 0.001$**). Comparisons could not be made for the youngest group (20-30 days) because all AS rats could negotiate all ramps and therefore there was no variability in the data.

The general decline for the remaining 3 ramp widths (7, 9 and 12cm) resulted in the 1 year animals having no success on any ramp except for the small number (20%) that managed to complete the task on the broadest ramp.

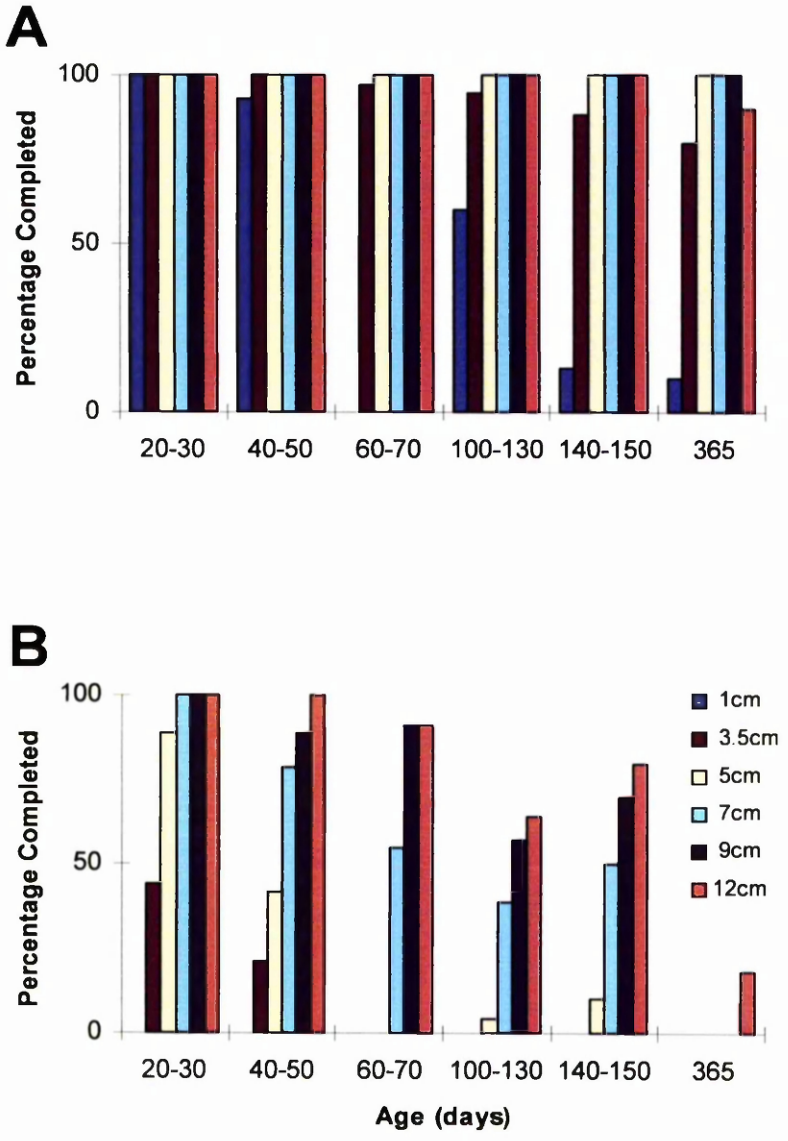


Figure 11. Inclined Ramp Test
The percentage of AS and AS/AGU rats capable of walking down inclined ramps of various widths is shown as a function of age up to one year.

3.1.2. Mid-Air Righting

AS animals

Figure 12 shows that the majority of AS control rats throughout the age groups tested were able to turn in mid-air and land on their feet. Excepting the one-year age group, all animals showed at least a 90% success rate. One year old AS control rats righted in mid-air less consistently, although over 70% still managed to land in an upright position.

AS/AGU animals

AS/AGU rats, shown in figure 12, proved to be much less capable than AS controls with this task. The youngest animals tested showed only a 60% pass rate. This tailed off gradually as older animals were tested, with 80% of mutants at 140 days showing an inability to land on their feet. By the age of 1 year, no AS/AGU rat was able to right in mid-air.

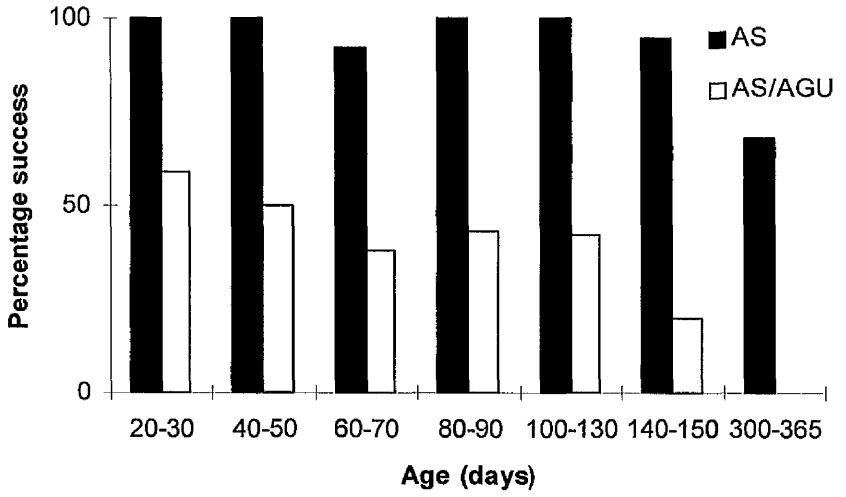


Figure 12. Mid-air Righting
The percentage of AS and AS/AGU rats capable of righting in mid-air is shown as a function of age up to one year.

Comparisons between the ability of AS and AS/AGU rats to right in mid-air were carried out using chi-square (χ^2) tests. The results of these are shown in Appendix 4, from which it can be seen that the two strains differ at all the ages tested. In each case, performance of AS/AGU animals was worse than AS controls.

3.1.3. Gait analysis

Footfall patterns were recorded from the left and right hind feet of 9 month AS (n=8) and AS/AGU (n=7) rats. Up to 4 recordings were digitized from each animal, depending on their willingness to cross the recordable area of the apparatus. Initially, the approach taken by Clarke (1992) was used whereby footfalls were analysed on an anterior/posterior basis, with the footfalls being divided into 3 zones.

Zones were arranged thus (see figure 6a):

A - Pads 2, 3 and 4

B - Pads 1, 5, B and C

C - Pads A, D, E and F

This arrangement was designed by Clarke (1992) to assess weight distribution in the anterior-posterior plane as the animal walked freely. In the present study, however, it became clear from observing movement in the rats, that this method of assessment of weight distribution would not be the best approach as thy tend to stagger from side to side. As an alternative, foot contact was analysed across the foot in a medial-lateral plane. This established a difference between AS control and AS/AGU mutant footfalls.

Computed gaits were compared between AS and AS/AGU rats in several ways.

- First of all, the "best" footfall of each rat was compared in the two strains. In this case, the notion of "best" is that closest to a "perfect" footfall in which weight distribution (averaged over the period of contact) is 50% on either side of the mid-line of the foot. From Appendix 5 (a) it can be seen that the two groups did not differ in this measure.

- Secondly, the "worst" footfall of each rat was compared in the two strains. In this case, the notion of "worst" is that furthest from a "perfect" footfall as defined above. From Appendix 5(b) it can be seen that the two groups differed significantly in this measure ($p < 0.05$) with the AS/AGU rats diverging considerably from the perfect footfall.

- Thirdly, the difference for each animal between "best" and "worst" footfalls (as defined above) in analysed in Appendix 5(c). From this it will be seen that the two groups differed significantly in this measure ($p < 0.05$) which was much greater in the AS/AGU rats than in AS ones.

Shown in figure 13, is the percentage lateral contact (area of foot/floor contact made by the lateral side, expressed as an average of total contact) for all footfalls analysed in the study. Thus, 100% laterality would be found if an animal walked on the lateral edge of it's paws, 0% laterality if it walked on the medial edge. Taking paw contact overall, AS control animals did not show extremes of contact in either a lateral or a medial direction and most footfalls were grouped around the central (50%) point. Indeed the mean for control animals was 53.19 and the standard deviation was 8.58.

The central coloured bands in the chart represent one and two standard deviations either side of the mean for the normal control animals (ie 34% and 96% respectively, of control values). It can be seen from this chart that a large number of mutant footfalls fall outwith this band, and many show excessive contact of either lateral or medial sides of the foot. This was in excess of 80-90% in the worst cases.

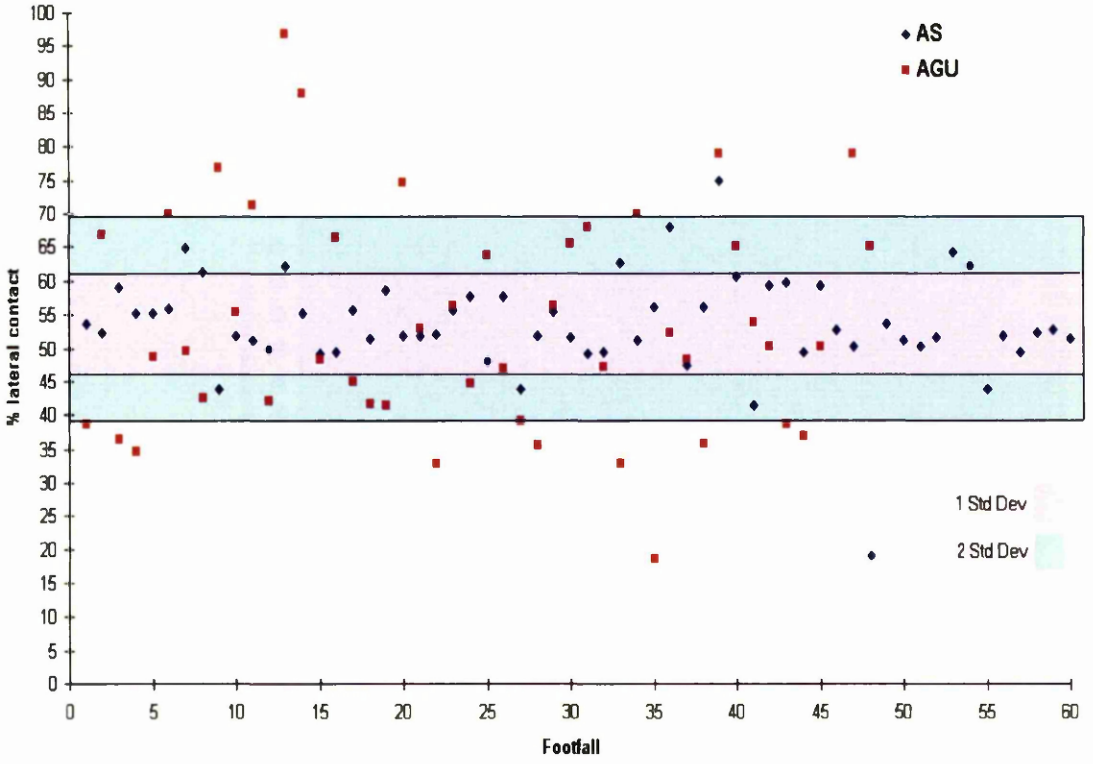


Figure 13. Gait analysis
Footfall contact shown as percentage contact made by the lateral side of the foot for both left and right feet.

In Figure 14, the same set of footfalls is organised into 3 footfall types:

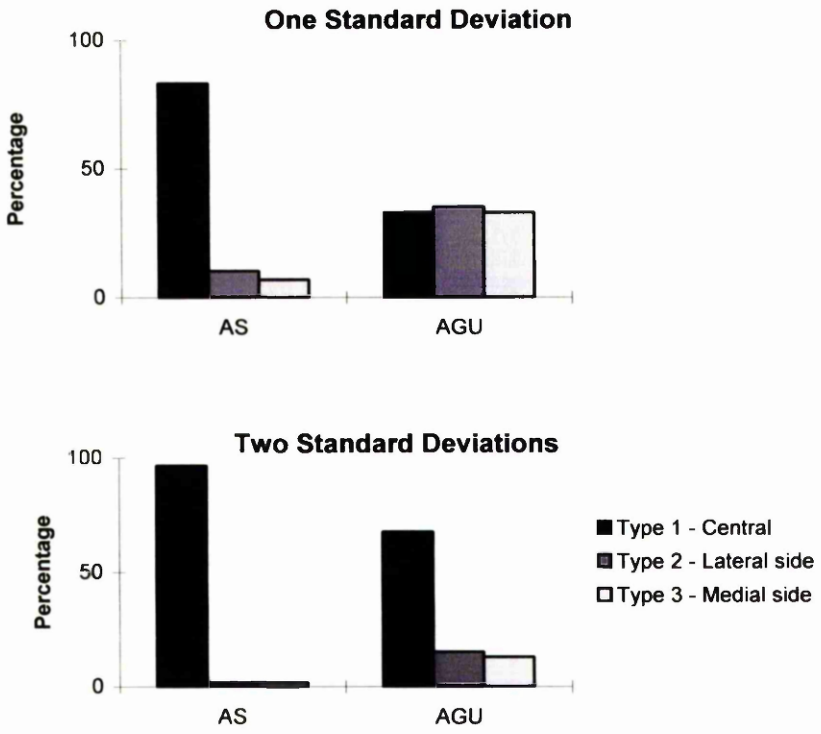


Figure 14. Gait analysis
Footfall contact in shown as percentage of each of three footfall types after one(a) and two(b) standard deviations.

Type 1 - Footfalls falling within the first and second standard deviation bands from Figure 13.

Type 2 - Outwith these bands and weighing on the lateral side.

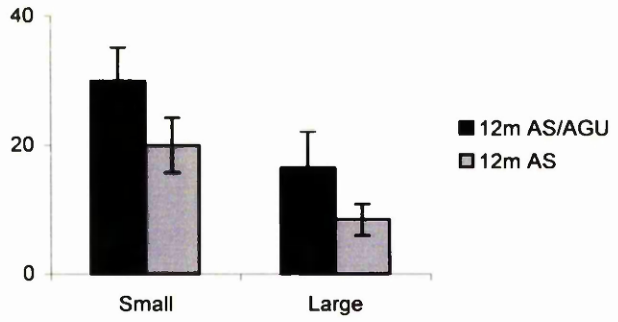
Type 3 - Outwith these bands and weighing on the medial side.

32% of control footfalls lie outside the 1x standard deviation band (and 4% outside the 2x standard deviation band). Using these same control means and standard deviations, it can be seen that 67% of mutant footfalls lie outside the control 1x standard deviation band (and 40% outside the 2x standard deviation).

3.1.4. Infrared Movement Analysis

In general, AS/AGU mutants were more active under the testing conditions than normal AS controls. When spontaneous movement was measured over the shorter time span of 12 minutes (Figure 15), AS/AGU rats aged both 9 and 12 months made more movements in both small and large categories. Comparisons were made between both 9 month and 12 month male AS and AS/AGU rats for both small and large movements, in both 12-minute and 22-hour sessions. The results of these are shown in appendix 6. There was no significant difference between the groups tested in the 12-minute sessions. Over 22 hours the result is different, with significant differences being found between the two strains at both age points, for both small and large movements (P=<0.05 in all cases).

a)



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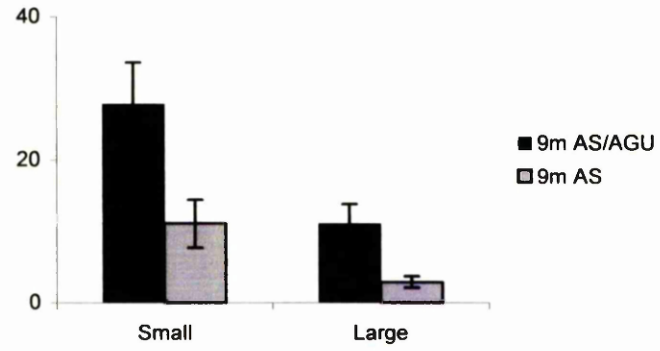


Figure 15. Infra-red movement analysis
Number of spontaneous small and large movements made by 12(a) and 9(b) month AS and AS/AGU rats during a 12-minute test period. Bars are standard errors.

The same pattern was observed in both age groups during the 22-hour sessions (Figure 16).

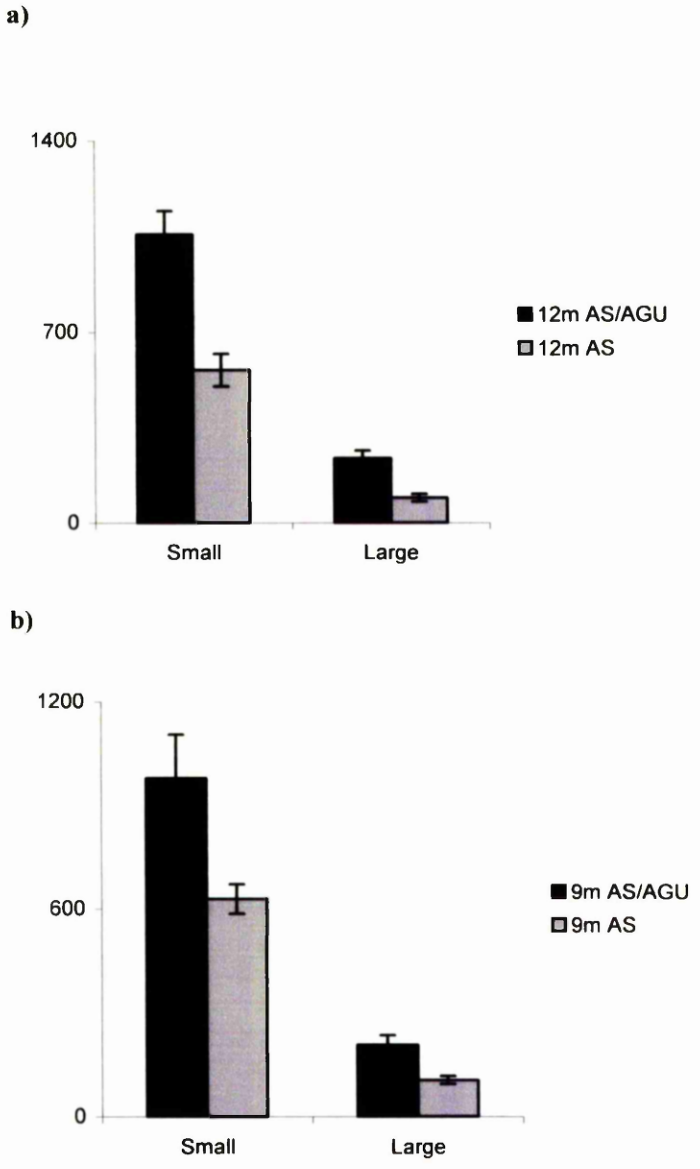


Figure 16. Infra-red movement analysis
Number of spontaneous small and large movements made by 12(a) and 9(b) month AS and AS/AGU rats during a 22-hour test period. Bars are standard errors.

3.2. L-DOPA treatment

3.2.1. Inclined ramp test

Figure 17 shows that in AS controls, no difference was seen between saline and L-DOPA administration, ie., all rats showed maximum performance with both treatments.

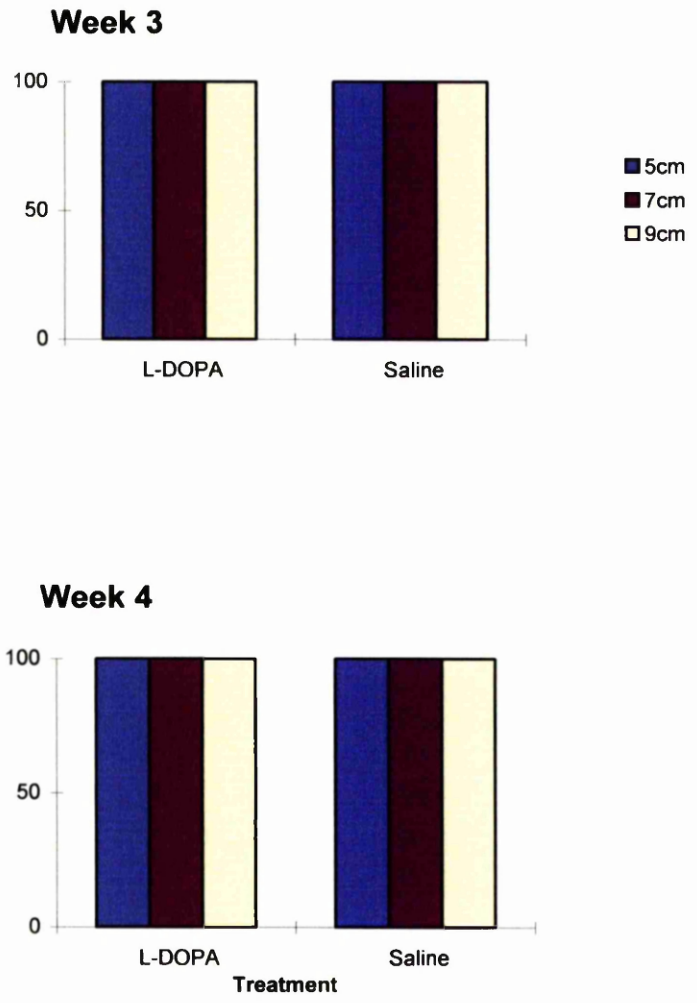


Figure 17. L-Dopa treatment: Inclined ramp test.
The effects of saline or L-Dopa on the ability of AS rats aged 3 months to walk down inclined ramps of 5, 7 and 9cm widths. Weeks 3 and 4 are shown.

Figure 18 shows the effects of dopamine replacement by L-DOPA administration in AS/AGU animals after weeks 3 and 4 of the experiment. Although the animals were tested on their ability to negotiate all 6 ramp widths as in the previous experiment, only 5, 7 and 9cm ramps are shown here, for simplicity, this being the range within which improvement due to the action of the drug was observed. In the third week of treatment the narrowest ramp successfully negotiated by a saline treated AS/AGU rat 9cm wide whereas L-DOPA treated mutants were successful on both 9cm (60%) and 7cm (50%).

By week 4, performance on the aforementioned ramps had increased to 90% and 80% respectively for L-DOPA treated animals. Moreover, 60% of this group were able to complete the 5cm ramp by this stage.

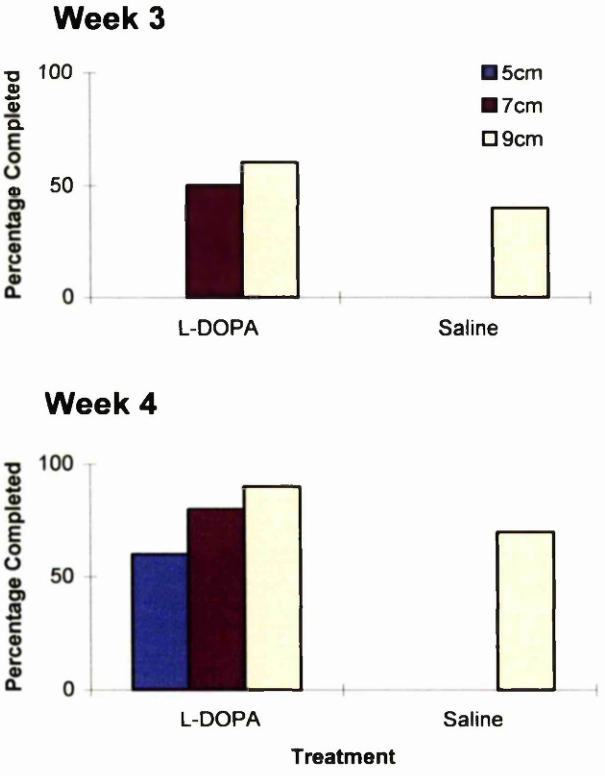


Figure 18. L-Dopa treatment: Inclined ramp test.
The effects of saline or L-Dopa on the ability of AS/AGU rats aged 3 months to walk down inclined ramps of 5, 7 and 9cm widths. Weeks 3 and 4 are shown.

Performance in the Inclined Ramp test was analysed for the AS/AGU males treated with saline or L-DOPA groups by a two-way analysis of variance (F); see Appendix 7a. As has been discussed in the age series results section, because ramp width constitutes a non-continuous variable, each ramp was assigned a score from 1 (the narrowest) to 6 (the widest). Each animal received a score in this range which was therefore the narrowest ramp it was capable of negotiating successfully; an animal which could not walk down any ramp received a score of 7.

However, it rapidly became clear

- That drug effects could not be seen prior to the third and fourth weeks of testing
- That the greatest changes were in the ability of animals to negotiate the planks of middling width, viz. 5, 7 and 9 cm wide.

A two-way analysis of variance (Appendix 7a) was therefore carried out on AS/AGU rats to compare a) the performance on weeks 3 and 4, and b) the treatment received (saline or L-DOPA). Appendix 7a reveals that there was a highly significant variance for both treatment and week of treatment.

Student's t-tests were carried out between the performance of saline and L-DOPA treated males on the two weeks concerned. The differences did not reach significance on Week 3, but did on Week 4 ($p < 0.01$) with L-DOPA treated males having a better performance than saline-treated ones (see Appendix 7b).

3.2.2. Mid-Air Righting

Figure 19 shows that in AS controls, no difference was seen between saline and L-DOPA administration, ie., all rats showed maximum performance with both.

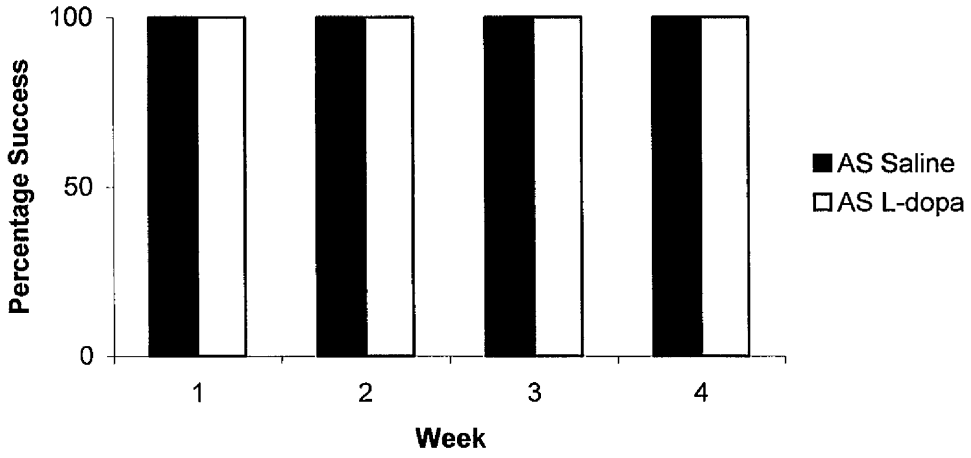


Figure 19. L-Dopa treatment: Mid-air righting.
The effects of saline or L-Dopa on the ability of AS rats aged 3 months to right in mid-air.

The percentage of saline and L-DOPA treated AS/AGU rats capable of mid-air righting throughout the course of the treatment is shown in Figure 20. On the first week of treatment, both saline and L-DOPA treated animals had similar rates of success (around 25%). Improvement in performance was subsequently observed only in the L-DOPA group, which showed a steady increase as the trial progressed, reaching a high of 90% by the fourth week.

The effects of L-DOPA on mid-air righting performance in AS/AGU rats compared to AS/AGU rats treated with saline were analysed by chi-square (χ^2) tests. These can be seen in Appendix 8. Whereas there are no differences after either one or two weeks of treatment, there are significant differences in the numbers of animals carrying out mid-air righting successfully at three and four weeks of treatment ($p < 0.01$)

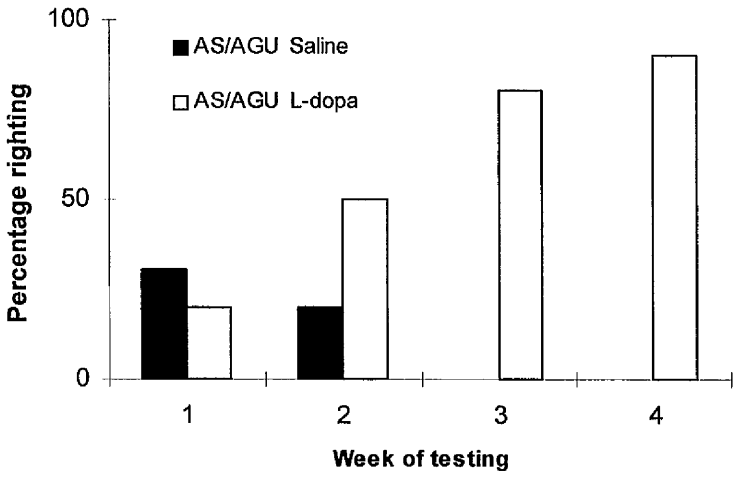


Figure 20. L-Dopa treatment: Mid-air righting.
The effects of saline or L-Dopa on the ability of AS/AGU rats aged 3 months to right in mid-air.

3.2.3. Measurement of extracellular levels of Dopamine, DOPAC and HVA in the striatum.

Previous work on the AS/AGU rat has shown vastly reduced levels of extracellular dopamine in the dorsal caudate putamen as measured in dialysates by HPLC/ECD (Campbell et al. 1998). These dopamine levels are shown as the first part of Figure 21a).

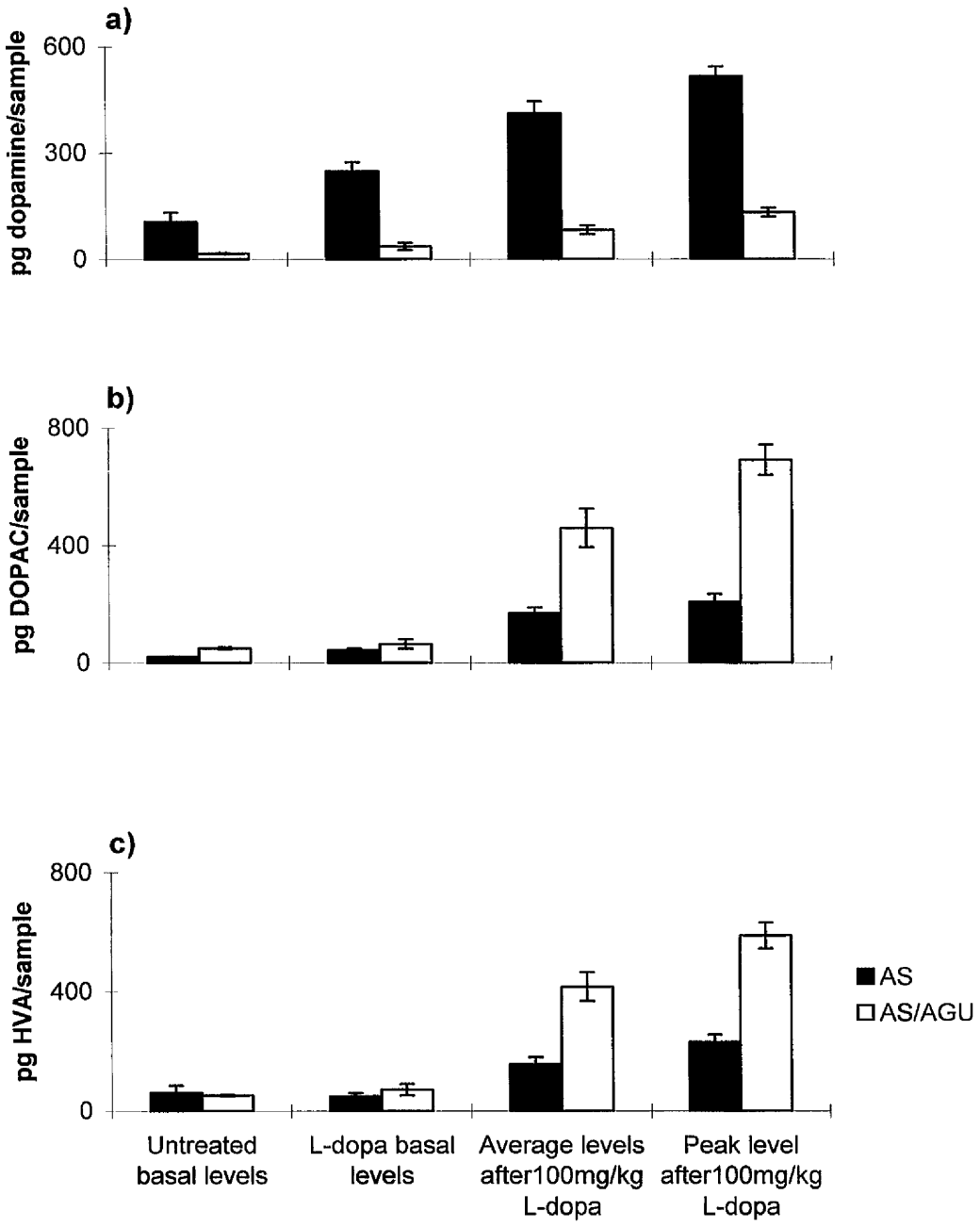


Figure 21. L-Dopa treatment: Comparison of levels of extracellular dopamine(a), DOPAC(b) and HVA(c) obtained by microdialysis and HPLC/ED, before and after L-Dopa treatment. Bars are standard errors.

From figure 21 it can also be seen that in both AS and AS/AGU strains, "resting" dopamine levels obtained 24 hours after the last L-DOPA injection, are almost twice those found in saline treated rats. Dopamine levels taken after the administration of a 100mg/kg bolus injection are also shown. Average dopamine levels found during the period between the injection and the termination of the experiment, and peak levels during this period, are represented in bands c) and d) respectively.

It can be noted from these average and peak levels that there is a significant increase for both AS and AS/AGU animals compared with their "resting" levels, and that dopamine levels in the mutant almost reach those of untreated AS controls animals. Analysis of variance over the three L-DOPA treatment groups was highly significant ($F=496$, df 5×18, $p<0.001$), and inter group comparisons using Confidence Intervals showed that both peak and mean dopamine values after bolus injection were significantly higher than pre-administration 'resting' levels in both AS and AS/AGU rats ($p<0.05$ for all).

Figures 21b) and 21c) show the same treatment groups as in figure 21a). In previous experiments, low levels of extracellular dopamine have been combined with greatly increased levels of the dopamine metabolite DOPAC, and relatively unchanged levels of HVA (Campbell et al. 1998). Twenty-four hours after the last injection of L-DOPA (25mg/kg), the level of DOPAC was found to be about double that of untreated basals, with HVA being largely unchanged. After the 100mg/kg bolus injection, however, striatal concentrations of both metabolites had significantly increased in average (c) and peak (d) levels. Analysis of variance over the three L=Dopa treatment groups was highly significant (for DOPAC $F=428$, df 5×18, $p<0.001$; for HVA $F=568$, df 5×18, $p<0.001$), and inter group comparisons show that both peak and mean dopamine values after bolus injection were significantly higher than pre-administration 'resting' levels in both AS and

AS/AGU rats (p=0.05 for all). Figures 23a), b) and c), are time course graphs that follow sample collection from initial L-DOPA resting levels, through the bolus injection to the end of the experiment. This covers a time span of over 6 hours.

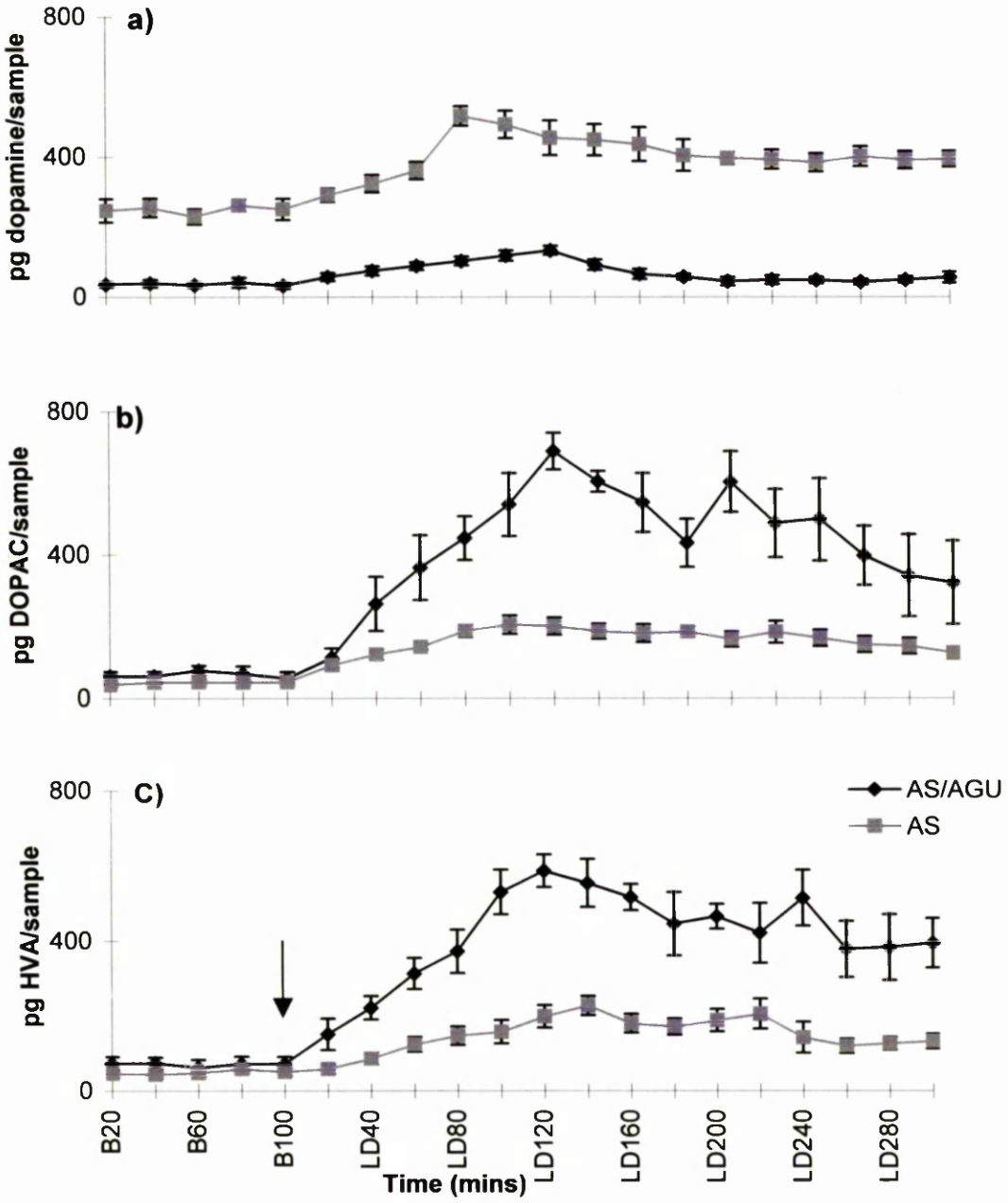


Figure 22. L-Dopa treatment: Time course of extracellular levels of dopamine(a), DOPAC(b) and HVA(c) obtained by microdialysis and HPLC/ED, before and after L-DOPA treatment, over a four hour period. The arrow marks the time of bolus injection. Bars are standard deviations.

The time of bolus injection administration (B100), is shown by an arrow. AS control dopamine values reach a peak level 40 minutes earlier than those of mutant animals, and whilst AS values do not return to resting levels by the end of the experiment, AS/AGU resting levels are restored after about 100 minutes. Levels of both DOPAC and HVA remain elevated throughout the post-bolus period in both Control and mutant animals. It should be noted that figure 22 illustrates the *time course* of extracellular release of dopamine, DOPAC and HVA in the L-DOPA experiment and does not include any statistical analysis. This analysis is to be found in appendix 8 and figure 21.

DISCUSSION

DISCUSSION

The discovery of a naturally occurring, spontaneous mutation in the Albino Swiss colony at Glasgow University created a whole series of research possibilities. It was soon established that physical characteristics of this mutant, such as difficulty in initiating movement, whole body tremor, a wide staggering gait and the inability to walk even a few steps without falling over, along with the finding of a reduced number of dopaminergic neurons in the SNpc (Clarke and Payne, 1994), made it a potentially valuable model for a Parkinson's like movement disorder. Its advantages over existing toxin induced models such as those using 6-Hydroxydopamine (Ungerstedt, 1968; Sakai and Gash, 1994) or MPTP (Moratalla et al., 1992; Herrero et al., 1993; Gnanalingham et al., 1995) were clear in that the progressive nature of the disorder could be monitored closely from the earliest onset of locomotor symptoms through to the advanced stages where locomotor and neurochemical deficits would be most pronounced. The possibility of gaining insight into the genetic aspects of a condition that displayed the symptoms of a hypokinetic movement disorder was also an exciting prospect.

The need to devise a series of simple, robust locomotor tests was an early priority, in order to assess and characterise the physical nature of the mutation. This would form a basis for future therapeutic investigations.

4.1. Locomotor Assessment Techniques

Locomotor and other behavioural testing have been used in the assessment of laboratory animals such as rodents and non-human primates for many years. Various methods have been used in the attempt to assess and quantify a variety of abilities in a) normal control animals b) animals that are being screened for the physical effects of

administered toxic substances such as 3-Acetyl pyridine and acrylamide (Jolicoeur et al., 1979) and ββ-iminodipropionitrile (Ivens, 1990) and c) mutant animals with locomotor dysfunction such as the shaker rat, which has deficits in the purkinje cells of the cerebellum (Wolf et al., 1996), or the weaver mouse which has both cerebellar and dopaminergic deficits (Eisenman et al., 1998). There has also been much use of locomotor testing in models of Parkinson's disease such the rat 60HDA model (Sakai and Gash, 1994; Borlogan et al., 1996; Mukhida et al., 2001), and in non-human primates (Herrero et al., 1993; Gnalalingham et al., 1995; Moratalla et al., 1992) and in mice (Rosas et al., 1998; Fredriksson et al., 1990; Nishi et al., 1991) with MPTP lesions.

Spontaneous locomotor activity, usually in a cage environment, has been monitored in most of the aforementioned studies and interpreted as a good indicator of general motor activity. The effects of drugs, toxins and genetically-induced locomotor deficits can then be quantified over time, and depending on how sophisticated the monitoring system is, various parameters such as number, duration speed and direction of movement can be measured (Borlogan et al., 1996; Doan et al., 1999; De Leonibus et al., 2001; Mead et al., 1994)

Apart from non-specific spontaneous locomotion, a wide range of other simple tests have been devised. These include hand gripping tests, which have been used on monkeys (Rouiller et al., 1998; Lemon et al., 1996) and also on rodents (Colotta et al., 1990; Ivens, 1990; Gad, 1982) to assess motor control of fingers. Forelimb and hindlimb placement (Wolf et al., 1996); the ability to walk along a rod, right in mid-air and on a surface (Wolf et al., 1996; Gad, 1982).

Another common method in the study of locomotor deficits is the measurement of gait parameters in normal walking patterns. Apart from the use of the footfall chamber in determining pad-floor contact areas (as used in this study), other parameters such as stride

length, gait width, and gait symmetry have been used to quantify abnormal gait patterns. (Wolf et al., 1996; Dorner et al., 1996; Jolicouer et al., 1979; Parker and Clarke, 1989).

The use of the Morris water maze whereby an animal is tested on its ability to swim to and find an underwater platform (Setlow and McGauch, 1999) involves movement and spatial memory. In the case of the AS/AGU rat, cognitive tests such as the water maze have yet to be carried out.

The rotorod, a revolving cylinder with adjustable speed, has proven to be a useful test of hind and forelimb dexterity, motor function and co-ordination. Rodents are tested on their ability to remain on the rod for set time periods as the speed of rotation is increased (Ivens, 1990; Colotta et al., 1990; Rozas et al., 1998). This useful but relatively expensive equipment has not yet been acquired for use with the AS/AGU mutant.

As mentioned before, models of movement disorders which involve the selective destruction of dopaminergic neurons and systems have proven to be useful in gaining understanding of the mechanisms of neurodegeneration in diseases such as Parkinson's disease. The development of pharmalogical approaches to these diseases, and an improved understanding of the functional anatomy and physiology of the basal ganglia are also major benefits derived from these models. Locomotor and behavioural testing has played an important part in reaching these findings.

Bilateral injection of 6OHDA into the striatum causes akinesia, aphagia and adipsia with increased mortality (Ungerstedt, 1968; Sakai and Gash, 1994). Unilateral lesions, however, have allowed the use of behavioural testing in the form of the assessment of rotational behaviour. After lesioning, these rats show normal behaviour, and circling occurs only when drugs affecting the dopaminergic system are given. Rotations contralateral to the lesioned side occur when dopamine agonists such as apomorphine are administered, whereas circling which is ipsilateral to the lesioned side is caused by dopamine releasing

drugs such as amphetamine (Borlongan et al., 1996; Mukhida et al., 2001; Hefti et al., 1980). This so called 'Ungerstedt' model is potentially useful in testing novel anti-parkinsonian drugs. Behavioural improvements after unilateral 6OHDA lesions have been reported with the use of L-DOPA (Sakai and Gash, 1994) and dopaminergic transplants (Mukhida et al., 2001). In the AS/AGU rat, the assessment of rotational behaviour is not a relevant testing method as the dopaminergic deficits in these animals are usually bilateral in nature. There may be some scope, however, in the use of 6OHDA in future AS/AGU studies in order to investigate the effects of apomorphine and amphetamine on behaviour in rats with natural bilateral lesions.

MPTP is used mainly in non-human primates and in mice, and does not appear to be effective in rats. Its action causes parkinsonian-like motor symptoms. Rozas and colleagues (1998), suggest the rotorod as being an excellent method for testing basal locomotor capacity in MPTP treated mice, as well as the effects of treatments such as L-DOPA and apomorphine. L-DOPA has also been used in reversing MPTP-induced hypoactivity in mice as measured by spontaneous locomotor activity (Frieriksson et al., 1990; Nishi et al., 1991).

4.2. Locomotor Characterisation of the AS/AGU Rat

The initial characterisation of the AS/AGU rat involved the use of a number of locomotor tests base on those in Marshall & Teitelbaum (1974), in order to find out which would provide the simplest and most effective means by which to quantitate the differences between mutants and the parent AS strain. Tests which required the animal to grip and hang onto metal bars were not of any value as the forelimbs seem to be relatively unaffected in the mutant. An open field test whereby the rat is placed in the centre of a flat Perspex sheet with concentric, evenly spaced rings to measure distance travelled, was

intended as a test of initiation of movement and time taken to travel short distances. Although some interesting observations were made, with AS/AGU rats taking longer to initiate movement, this was not consistent. The conditions under which the test had to be carried out, i.e., a novel environment, were such that stress and mood probably had an influence on behaviour in this task. A stepping test, whereby the rat's forelimbs were placed on a polystyrene step with animals having to lift their hind limbs onto the step, also proved inconsistent despite the obvious effects of the mutation on hind limbs. Inclined ramp, mid-air righting, spontaneous locomotor activity and gait analysis were chosen as being the most consistent, robust and reliable means by which to characterise and measure the locomotor deficits in the AS/AGU rat.

4.3. The Effects of Age on Locomotor Behaviour in the AS/AGU Rat

Inclined ramp and mid-air righting tests quickly established differences in abilities between AS/AGU and control animals. Payne and colleagues (1998) reported data for these tests with 6 month old rats which showed significant differences between mutants and controls in both tests.

4.3.1. Inclined Ramp Test

The testing conditions for mid-air righting in previous AS/AGU work were the same as with the present study. The inclined ramp test, however, differed in that previously only one ramp (13cm) was used (Payne et al., 1998). This single ramp established a clear difference in performance between the two groups. The animals earlier investigations did not vary much in size, and the chosen ramp width was appropriate for the size of the animal. As the purpose of the present work was to study the progressive nature of the disorder at various age points at which both body size and locomotor ability might change,

a series of ramps of different widths was felt necessary. Thus, it should be possible to test a rat of any size, within the age range, on a ramp that most suited its size and gait width, and to test also on ramps that would present a range of increasingly difficult challenges. Rats could thus be assessed also on their ability to adjust their gait width in a controlled manner in order to negotiate ramps which were narrower than this.

As can be expected, normal control rats are generally successful on a range of ramp widths. In the vast majority of cases, AS rats can comfortably walk down ramps of any width used in this study. Bigger, heavier control animals in the oldest age groups (5 months and above) tended to struggle, however, with the narrowest (1cm) ramp used. These rats seemed able to adjust their gait width to the width of the ramp, but were inhibited by their size and weight.

The choice of ramp widths used in this study proved successful in demonstrating the progressive nature of the mutant's movement disorder. Thus, poor general performance rates by AS/AGU rats was manifested by (for example) a) no mutant of any age being able to negotiate the narrowest (1cm) ramp, and b) none of the oldest animals being able to negotiate any ramp except the broadest. The physical features of the locomotor impairment itself may serve as the main reasons for the general lack of success:

- A wide gait makes it difficult for a mutant to walk down a ramp unless that ramp is of a width that is much broader than the gait width, as observed when the animal is first placed on the ramp. AS/AGU animals seem unable to adjust gait width in a controlled manner in order to negotiate ramps that are narrower than the natural splay of their hind limbs.
- Having difficulty in initiating movement, particularly with older animals, mutants often eventually set off in an uncontrolled manner, resulting in their

falling off near the top of the ramp. Occasionally this latency can result in the rat falling off as soon as it attempts to move.

- The staggering nature of movement in the mutant renders the rat unable to recover balance on a ramp unless that ramp is much broader than the width of the animal's body. This results in the animal falling off after a few strides down the ramp.
- As the worst affected animals fall over every few steps when walking on a flat surface, animals are generally unable to remain on ramps of any width after a small number of steps.

4.3.2. Mid-Air Righting

The study of the righting reflex in mid-air provided a robust test which demonstrated a clear difference in ability between control and mutant groups (Payne et al., 1998). The air-righting reflex consists of a chain of events that occur after a rat is released in mid-air. Initial labyrinthine stimulation triggers rotation of the shoulders, which carry the head and neck around also. Cervical righting reflex then moves the lower end of the body round in tandem with the forelimbs, resulting in a horizontal position which is achieved very quickly, allowing the rat to land on all four limbs comfortably (Pellis et al., 1991). Rats are able to right in mid-air successfully at about age 16 days postnatal (Hard and Larsson, 1975).

A minority of the oldest AS control animals in this study (1 year old rats, which are also the largest and heaviest) failed to right in mid-air, but all other age groups showed complete or almost complete success. This provided an ideal basis for comparison with mutant animals. AS/AGU rats showed an obvious inability in this test from the earliest age tested. Whether or not the rat lands on its feet after mid-air righting is normally a clean cut

observation. When a control animal is held upside down until relaxed, and dropped, it turns 180 degrees instantly and lands comfortably on all fours. AS/AGU animals do not display such slick movement, and show varying patterns when dropped, such as a) completely failing to turn and landing flatly on their backs, b) turning only either hind or forelimbs and landing on their sides or c) turning too far and landing heavily on the bedding layer. These awkward patterns normally result in animals landing either on their back, side, head or tail. It is possible that different patterns may emerge as the animals get older. It was observed as the animals were dropped that the one year old mutants failed to turn to any degree, and landed directly upside down more frequently than younger age groups.

When a rat is held upside down and dropped, its first reaction is to turn immediately through 180 degrees in order to land on its feet. It is likely that, although this inclination is present in AS/AGU rats (a reasonable number up to the age of 3 months land on their feet), it is the varying degree of disability which renders the mutants unable to move their body in line with this 'desire' to turn and land safely on all fours. This may make mutants less able to initiate the air righting sequence than normal control animals. There is no evidence to suggest that mutants lack the motivation to turn.

4.3.3. Gait Analysis

The use of a footfall chamber and an image analysis macro program, for the close study of footfall patterns, was successful in achieving its aim. It was possible to quantitate the pattern of weight distribution in the lateral-medial plane in the AS/AGU rat and to compare it with that of AS controls. In AS animals, the distribution of pressure on the footpads is evenly distributed along a line passing down the centre of the foot, whereas in AS/AGU rats pressure is often concentrated on either the medial or lateral side. When all of the

footfalls from all of the animals tested are shown together in a scatter diagram (Figure 3), mutant weight distribution is much more widely scattered than that of controls and about 40% of AS/AGU footfalls lie outwith two standard deviations of the mean for AS control values. Moreover, AS/AGU footfalls that lie outwith the normal AS range are evenly distributed between those falling heavily on the lateral side of the foot, and those which show excessive medial contact. This pattern of distribution is what you might expect with a rat that walks in an ungainly manner with a staggering gait. When a mutant rat leans excessively on one side, lateral foot-floor contact is generally excessive on that side with percentage lateral pad contact being increased (and the medial contact decreased) as the rat leans further. As the animal's weight swings across to the other side, the same pattern is repeated for that side. This, however, was not always the case due to the fact that occasionally the weight bearing foot would be placed so awkwardly on the walking surface during motion that it would have uniform, or even excessive, medial, pad contact. On those occasions, the rat was more likely to slow down and stop walking, or to fall over by tripping on the awkward foot.

4.3.4. Infrared Movement Analysis

Spontaneous locomotor activity in a cage environment was found to be increased in both short (12-minute) and long (22-hour) sessions, and significantly so during the longer sessions.

AS/AGU rats eat, drink and groom normally (Clarke and Payne, 1994), activities that would normally cause the animal to register small and large movements with this apparatus. During the 12-minute sessions, it is possible that mutant rats do not settle as quickly as AS controls in an unfamiliar cage, which is larger and deeper than their normal

cage environment. Normal sized cages could not be used during this experiment because the optimised use of the infrared sensors required a larger cage.

With the 22-hour sessions, however, this is less likely to be the case. It was not practical to observe the rats during these sessions and so the exact nature of movements is unknown over a day-long session part of which covers a dark period during which rats are naturally more active. It is possible that grooming, for example, occurs more regularly in the AS/AGU rats or lasts longer during this period. Limitations of the infrared activity apparatus such as the inability to record the speed, direction or type of movements, or the relatively crude time parameter options, make it difficult to determine whether tremor in the mutant rat is detected by the sensors. With the mutant rat being significantly more active than controls during the longer sessions, there is the possibility that tremor is being detected.

These data confirm that the AS/AGU rat, although showing signs of locomotor disability and having neurochemical deficits, is still active enough to be used in locomotor testing.

4.4. L-DOPA Replacement

Locomotor deficits in the AS/AGU rat seem likely to arise mainly from deficits in the dopaminergic system, in particular the heavy loss of available neurotransmitter in the striatum, with reductions of up to 80% in extracellular fluid at 3 months of age and beyond (Campbell et al., 1998), and 30% reductions in striatal whole tissue levels from 6 months. In light of this, the efficacy of L-DOPA, a dopamine precursor, in ameliorating the resulting locomotor symptoms is not an unexpected occurrence.

Improvements in performance when L-DOPA treated AS/AGU animals were tested on inclined ramp and mid-air righting tests could be attributed to a general overall

improvement in motor ability. They seemed more willing to initiate and more able to execute movement. In the mid-air righting test, it is thus possible that the mutant rat was more able to rotate its body in accordance with vestibular stimulus than untreated animals. This ability improved as the chronic treatment progressed. Similarly, in the inclined ramp test, mutants would have been able to walk down ramps, and to adjust gait width, in a more controlled manner in order to negotiate narrower ramps than their untreated counterparts.

4.4.1. Pharmacokinetics

It is supposed that, although L-DOPA is itself proposed to be a neurotransmitter, having pre- and post-synaptic responses (Misu et al., 1996), in order to be effective in improving locomotor responses, L-DOPA must firstly be converted to dopamine in the striatum by the enzyme L-aromatic amino acid decarboxylase (L-AAAD). Newly synthesized dopamine must then be released and interact with striatal dopamine receptors to produce an effect. The amount of LAAAD influences the rate of this process by controlling the amount of dopamine produced by exogenous L-DOPA. This whole process, from L-DOPA administration through to it producing chemical and physical effects, is thought to take between 45 and 90 minutes to complete (Shoulson et al., 1975).

The time course from L-DOPA administration to dopamine release is slower in the AS/AGU rat as compared to AS control (see Figure 12a); it takes 120 minutes for a peak level of dopamine to be achieved compared with 80 minutes in the control animal. Although we have no information on this, it could be a reflection of the amount of L-AAAD available to the administered L-DOPA in mutants, with conversion to dopamine being delayed. In this connection, reductions in L-AAAD have been found in both Parkinson's disease and 6-hydroxy dopamine lesioned rats (Wooten, 1987).

After 4 week administration of 25mg/kg L-DOPA, resting levels of extracellular dopamine were approximately doubled in both AS controls and AS/AGU mutant rats compared to untreated controls. However, after the administration of 100mg/kg of L-DOPA by a bolus I.P. injection, mutants showed a higher percentage rise in extracellular DA levels, compared with resting levels, than the controls. This was the case both with average and peak post-bolus values. Absolute levels, however, remained lower in the AS/AGU than in the mutant.

In a study on the effects of L-DOPA on normal and 6-hydroxy dopamine lesioned rats, Abercrombie and colleagues (1990) suggested that i) low doses of L-DOPA enhance the storage pool of dopamine in dopaminergic neurons making it susceptible mainly to normal impulse evoked release whereas ii) higher doses of the drug cause leakage of dopamine from synaptic vesicles directly into the extracellular fluid. Hence, dopamine formed from exogenous L-DOPA (100mg/kg) in 6-OHDA treated rats is released in an impulse independent manner rather than being stored in vesicles for subsequent release, and this mechanism along with lesion induced loss of re-uptake sites is responsible for the sharp increase in extracellular dopamine levels.

It is possible that this hypothesis may apply in the present study and that the 25mg/kg dose is increasing dopamine stores, and therefore effecting a lower release rate in AS/AGU animals compared with controls. In order to elucidate this further, it would be worthwhile studying the same L-DOPA doses as in this study, combined with a re-uptake inhibitor such as nomifensine. This may show whether a possible reduction in dopamine re-uptake is also a factor in the pharmacokinetics of the 100mg/kg dose. Campbell and colleagues (2000) reported that after nomifensine administration, AS/AGU rats showed a much smaller drug induced increase in extracellular dopamine levels (due to re-uptake inhibition) than AS controls. This confirms that under normal conditions, synaptic release

in the mutant is low, and demonstrates that re-uptake differences do not underlie the release problem.

Higher extracellular dopamine rises relative to basal and L-DOPA resting levels in AS/AGU compared with AS controls may also reflect quicker penetration of dopamine into neurons in the mutant. These neurons may have a greater capacity for dopamine influx due to a natural deficit. This may explain the higher percentage rise in post-bolus DOPAC and HVA levels in the mutant extracellular fluid, as compared with control levels. Moreover, if normal AS cells and terminals are packed with a large influx of dopamine, which would not normally exist, they may struggle to cope with this due to increases over normal capacity.

4.5. The AS/AGU Rat as a Model for a Movement Disorder

The inclined ramp and mid-air righting tests employed in this study were successful as simple, consistent and reproducible methods. They were thus important and useful in the characterisation and measurement of the locomotor disorder due to the AS/AGU mutation, and in confirming its progressive nature . The use of a footfall chamber in studying footfall patterns was both an interesting and dynamic technique which showed a dramatic difference between mutant and parent strains. This was, however, a complex and time consuming method which gave a relatively small amount of information. The infrared movement system, a widely used method in the field of locomotor study, was confirmed as a valuable tool in this study.

The use of L-DOPA, as a method of replacing dopamine in deficient mutants, was successful in both enabling the mutants to perform more effectively under testing conditions, and also in increasing extracellular dopamine levels in the striatum.

In terms of the genetic component of this animal model, a loss of PKC-γ may explain some of the neurological dysfunction present. Although PKC-γ is not involved exclusively in the packaging and release of neurotransmitters from vesicles, it may still play an integral role in these processes (Chen et al., 1995). This is because other types of PKC (PKC alpha and beta) may be up-regulated as a result of PKC-γ deficiency using cellular compensatory mechanisms. Therefore loss of PKC-γ in the AS/AGU rat may partly explain why the neurotransmitter release mechanism seems to be affected in these animals.

In summary, the AS/AGU rat model is indeed an excellent model for a movement disorder. Both the known characteristics, and those yet to be discovered, make this a valuable testing ground for potential drug treatments for Parkinson-like diseases.

CONCLUSIONS

CONCLUSIONS

Although the use of toxin- and lesion-induced models of basal ganglia based movement disorders has long been of significant benefit to the understanding of the aetiology of diseases such as Parkinson's Disease and Huntington's Disease, and has also advanced the knowledge and understanding of basal ganglia circuitry and function, these models have intrinsic limitations. Such models, although producing relevant disease characteristics, are generally transient in nature with significant recovery being a feature of certain methods. Lesions can be non-specific, affecting also neurotransmitters not directly related to the area of study. Furthermore, with induced models, progression of disease cannot be measured from a young age through to an advanced state at older ages.

Models that show a movement disorder as the result of a mutation have potential advantages over toxic models whilst retaining many of the useful features of these animals. In the AS/AGU mutant, which seems to be unique in having no obvious cerebellar dysfunction, symptoms are detectable at a very young age. There is the advantage with this mutant of studying the natural progression of both the chemical and physical deficits present in this innate condition. Its genetic nature may offer insight into the genetic aspects of basal ganglia disease. There is, for example, the opportunity to study the possible connection between PKC-γ and Parkinson-like symptoms, in terms of cell function and synaptic release.

Improvements, both physical and chemical, as a result of L-DOPA replacement in the AS/AGU rat mirror those in patients with Parkinson's disease. It would be interesting to study the effects of L-DOPA over a longer time period with the mutant rat, in order to assess whether the detrimental effects due to prolonged use in humans occur in this rat. This would also present the possibility of testing other drugs alongside L-DOPA which

may reduce these side effects. This potentially important model could, in future, be used as a testing ground for drugs such as dopamine agonists, MAO inhibitors or specific PKC drugs.

Future work might include closer study of enzymes such as MAO and L-AAAD; investigations into other components of the basal ganglia such as STN or GP; gaining more knowledge of other dopaminergic areas including the hypothalamus and the olfactory system; or the study of dopamine receptors, in the AS/AGU rat.

REFERENCES

REFERENCES

Abercrombie, E.D., Bonatz, A.E. & Zigmond, M.J. (1990) Effects of L-DOPA on extracellular dopamine in striatum of normal and 6-hydroxydopamine-treated rats. *Brain Res.*, 525: 36-44.

Albin, R.L., Reiner, A., Anderson, K.D., Penney, J.B. & Young A.B. (1990) Striatal and nigral neuron subpopulations in rigid Huntington's disease: Implications for the functional anatomy of chorea and rigidity-akinesia. *Ann. Neurol.*, 27: 357-365.

Albin, R.L., Young, A.B. & Penney, J.B. (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci.*, 12: 366-375.

Alexander, E.G., DeLong, M.R. & Strick, P.L. (1986) Parallel organisation of functionally segregated circuits linking basal ganglia and cortex. *Ann. Rev. Neurosci.*, 9: 357-381.

Alexander, G.E. & Crutcher, M.D. (1990) Functional architecture of basal ganglia circuits: neural substrates and parallel processing. *Trends Neurosci.*, 13(7): 266-271.

Arcusa, M.J., Barcia-Salorio, J.L., Burguera, A. et al. (1996) Chronic high frequency stimulation of the globus pallidus internus in Parkinson's disease. *Neurology*, 46(2): 490-495.

Arikuni, T. & Kubota, K. (1986) The organisation of prefrontocaudate projections and their laminar origins in the macaque monkey. A retrograde study using HRP gel. *J. Comp. Neurol.*, 244: 492-510.

Benabid, A.L., Pollak, P., Gervason, C., et al. (1991) Long term suppression of tremor by chronic stimulation of the ventral intermediate thalamic nucleus. *Lancet*, 337: 403-406.

Bergen, C., Pollaner, M.S., Weyer, L. et al. (1998) Unusual clinical presentations of cortical-basal ganglionic degeneration. *Ann. Neurol.*, 40: 893-900.

Borlogan, C. V., Freeman, T.B. & Hauser, R.A. (1996) Cyclosporine-A increases locomotor activity in rats with 6-hydroxydopamine-induced hemiparkinsonism: relevance to neural transplantation. *Surg. Neurol.*, 46:384-388.

Brooks, D.J. (1998) Positron emission topography studies in movement disorders. *Neurosci. Cli. N. Amer*, 9(2): 263-281.

Butcher, L.L. & Hodge, G.K. (1976) Postnatal development of acetylcholinesterase in the caudate-putamen nucleus and substantia nigra of rats. *Brain Res.*, 106: 223-240.

Campbell, J.M., Gilmore, D.G., Russell, D. et al. (2000) Pharmacological analysis of extracellular dopamine and metabolites in the striatum of concious AS/AGU rats, mutants with locomotor disorder. *Neurosci.*, 100(1): 45-52.

Campbell, J.M., Gilmore, D.P., Russell, D. et al. (1998) Microdialysis studies of dopamine and its metabolites in the corpus striatum of conscious AS/AGU mutant rats. *Neurosci.*, 85: 323-325.

Campbell, J.M., Payne, A.P., Gilmore, D.P. et al.(1996) Neostriatal dopamine depletion and locomotor abnormalities due to the albino Swiss rat agu mutation. *Neurosci. Lett.* 213: 173-176.

Campbell, J.M., Payne, A.P., Gilmore, D.P. et al. (1997) Age changes in dopamine levels in the corpus striatum of albino Swiss (AS) and AS/AGU mutant rats.
Neurosci. Lett. 239: 54-56.

Carpenter, M.B. (1981) Anatomy of the corpus striatum and brain stem integrating systems. In *Handbook of physiology* Vol II.v; Brooks (ed) pp749-955 Amer. Physiol. Soc., Bethesda.

Carswell H.V.O., Graham D.I. & Stone T.W. (1997) Kainate-evoked release of adenosine from the hippocampus of the anaesthetised rat: possible involvement of free radicals. *J. Neurochem.*, 68: 240-247.

Chen, C., Kano, M., Abeliovich, A. et al., (1995) Impaired motor co-ordinattion correlates with Persistent multiple climbing fibre innervation in PKC-γ mutant mice. *Cell* 83:1233-1242.

Cheramy, A., Leviel, V. & Glowinsky, J. (1981) Dendritic release of dopamine in the substantia nigra. *Nature*, 289: 537-542.

Chevalier, G., Vacher, S. Deniau, J.M. & Desban, M. (1985) Disinhibition as a basic process in the expression of striatal functions. I. The striato-nigral influence on tecto-spinal/tecto-diencephalic neurons. *Brain Res.*, 334(2): 215-226.

Clarke, D.J., & Payne, A.P. (1994) Neuroanatomical characterisation of a new mutant rat with dopamine depletion in the substantia nigra. *Eur. J. Neurosci.* 6: 885-888.

Clarke, K.A. (1992) Disturbances of spatiotemporal footfall contact patterns in the rat by TRH analogue CG3703. *Neuropeptides*, 23: 33-38.

Collins, S.J., Ahlskog, J.E., Parisi, J.E. & Maraganore, D.M. (1995) Progressive supranuclear palsy: Neuropathologically based diagnostic clinical criteria. *J. Neurol. Neuosurg. Psychia.*, 58: 167-173.

Colotta, V.A., Flores, E., Oscos, A., Meneses, A. & Tapia, R. (1990) Effects of MPTP on locomotor activity in mice. *Neurotoxicol. Teratol.*, 12(4): 405-407.

Craig, N., Duran Alonso, M., Hawker et al. (2001) A new candidate gene for neurodegenerative disorders: mutation in the PKC-γ gene causes a Parkinsonian syndrome in rats. Submitted for publication.

De Leonibus, E., Mele, A., Oliverio, A. & Pert, A (2001) Locomotor activity induced by the non-competitive N-methyl-D-aspartate antagonist, Mk-801: role of nucleus accumbens efferent pathways. *Neurosci.,* 104: 105-116.

DeLong, M. & Georgeopoulus, A. (1981) Motor functions of the basal ganglia. In *Handb. Physiol.* Sect. 1, The nervous system, vol 2, motor control, Part 2. Ed. J. M. Brookmart, V.B Mountcastle, V.B Brooks. p.p. 1017-1061.

DeLong, M., Georgeopoulus, A. & Crutcher, M.D. (1983) Cortico-basal ganglia relations and coding of motor performance. *Exp. Brain Res.*, Suppl. 7: 30-40.

Devito, J.L. & Anderson, M.E. (1982) An autoradiographic study of efferent conections of the globus pallidus in macaca mulatta. *Exp. Brain Res.*, 46: 107-117.

Devito, J.L., Anderson, M.E. & Walsh, K.E. (1980) A horse radish peroxidase study of afferent connections of the globus pallidus in macaca mulatta. *Exp. Brain Res.*, 38: 65-73.

Doan, V.D., Grondin, R., Tahar, A.H., Gregoire, L. & Bedard, P.J. (1999) Effect of the selective D_1 antagonists SCH 23390 and NNC 01-0112 on the delay, duration and improvement of behavioural responses to dopaminergic agents in MPTP-treated monkeys. *Clin. Neuropharmacol,*. 22(5): 281-287.

Dogali, M., Fazzini, E., Kolodny E. et al. (1995) Stereotaxic ventral pallidotomy for Parkinson's disease. *Neurology*, 45: 753-761.

Donoghue, J.P. & Herkenham, M. (1986) Neostriatal projections from individual cortical fields conform to histochemically distinct striatal compartments in the rat. *Brain Res.*, 365: 397-403.

Dorner, H., Otte, P., Platt, D. (1996) Training influence on age-dependant changes in the gait of rats. *Gerontol.*, 42: 7-13.

Eisenman, L.M., Gallacher, E. & Hawkes, R. (1998) Regionalization defects in the weaver mouse cerebellum. *J. Comp. Neurol.*, 394(4): 431-444.

Fallon J.H.& Leslie F.M. (1986) Distribution of dynorphin and enkephalin peptides in the rat brain. *J Comp. Neurol.*, 249(3): 293-336.

Fallon, J.H. & Loughlin, E.S. (1995) Substantia nigra. From *The rat nervous system* **Ed. G.** Paxinos, Academic Press.

Fearnley, J.M. & Lees, A.J. (1991) Ageing and Parkinson's Disease: substantia nigra regional selectivity. *Brain*, 114: 2283-301.

Filloux, F., Liu,T.H., Hsu, C.Y., Hunt, M.A. & Wamsley, J.K. (1988) Selective cortical infarction reduces [H-3]sulpiride binding in rat caudate putamen. Autoradiographic evidence for presynaptic D_2 receptors on corticostriate terminals. *Synapse*, 2(5): 521-531.

Fishell, G., & van der Kooy, D. (1987) Pattern formation in the striatum: Developmental changes in the distribution of striatonigral neurons. *J. Neurosci.*, 7: 1969-1978.

Fredriksson, A., Plaznic, A., Sundstrom, E., Jonnson, G. & Archer, T. (1990) MPTP-induced hypoactivity in mice: reversal by L-DOPA. *Pharmacol. Toxicol.*, 67: 295-301.

Gad, S.C. (1982) A neuromuscular screen for use in industrial toxicology. *J. Toxicol. Envir. Health*, 9: 691-704.

Gauchy, C., Desban, N., Krebs, M.O., Glowinski, J. & Kemel, M.L., 1991, Role of dynorphin- containing neurons in the presynaptic inhibitory control of the evoked acetylcholine-release of dopamine in the striosomes and the matrix of the cat caudate nucleus. *Neurosci.*, 41: 449-458.

Gerfen, C.R. (1984) The neostriatal mosaic: Compartmentalisation of the corti**costriatal** and striatonigral output system. Nature (London), 311: 461-464.

Gerfen, C.R. (1985) The neostriatal mosaic: Compartmentalisation of the projections from the striatum to the substantia nigra in the rat. *J. Comp. Neurol.*, 236: 454-476.

Gerfen, C.R., Baimbridge, K.G. & Miller, J. J. (1985) The neostriatal mosaic: Compartmental distribution of calcium binding protein and parvalbumin in the basal ganglia of the rat and monkey. *Proc. Natl. Acad. Sci.* USA, 82: 8780-8784.

Gerfen C.R. (1989) The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. *Science*, 246: 385-388.

Gerfen, C. R., Engber, T. M., Mahan, L. C., et al. (1990) D_1 and D_2 dopamine receptors regulated gene expression of striatonigral and striatopallidal neurons. *Science*, 250: 1429-1432.

Gerfen, C.R. (1992) The neostriatal mosaic: Multiple levels of compartmental organisation in the basal ganglia. *Annu. Rev. Neurosci.*, 15: 285-320.

Gerfen, C.R. (1992) The neostriatal mosaic: multiple levels of compar**tmenetal** organisation. *Trends Neurosci.*, 15(4): 133-139.

Gnalalingham, K.K., Milkowski, N.A., Smith, L.A. et al. (1995) Short- and long-term changes in striatal and extrastriatal dopamine uptake sites in the MPTP-treated common marmoset. *Eur. J. Pharmacol.*, 277: 235-241.

Graybiel, A.M. & Ragsdale, C.W. (1978) Histochemically distinct compartments in the striatum of human, monkey and cat. *Proc. Natl. Acad. Sci.*, 75: 5723-5726.

Graybiel, A.M. (1990) Neurotransmitters and neuromodulators in the basal ganglia. *Trends Neurosci.*, 13(7): 244-254.

Halliday, G.M., and Tork, I. (1986) Comparative anatomy of the ventromedial mesencephalic tegmentum in the rat, cat, monkey and human. *J. Comp Neurol.*, 252(4): 423-445.

Hard, E. & Larsson, K. (1975) Development of air righting in rats. *Brain Beh. Evol.*, 11(1): 53-59.

Hedreen, J.C. & Folstein, S.E. (1995) Early loss of neostriatal striosome neurons in Huntington's disease. *J Neuropath Exp Neurol*, 54: 105-120.

Hefti, F., Melemed, E,. Shahakian, B.J. & Wurtman, R.J. (1980) Circling behaviour in rats with partial, unilateral nigrostriatal lesions: effects of amphetamine, apomorphine and DOPA. *Pharmacol. Biochem. Behav.*, 12: 185-188.

Herkenham, M & Pert, C. (1981) Mosaic distribution of opiate receptors, parafasiculatar projections and acetylcholinesterase in the rat striatum. *Nature* (London), 291: 41-418.

Herrero, M.T., Hirsch, E.C., Kastner, A. et al. (1993) Does neuromelanin contribute to the vulnerability of catecholinergic neurons in monkeys intoxicated with MPTP. Neurosci., 56: 499-511.

Hokfelt, T., Elde, R., Johansson, O. & Terenius, L. (1977) The distribution of enkephalin-immunoreactive cell bodies in the rat nervous system. *Neurosci. Lett.*, 5: 25-31.

Hoover, J. E. & Strick, P. L. (1993) Multiple output channels in the basal ganglia. *Science*, 259: 819-821.

Huganir, R.L., Greenyard, P. (1990) Regulation of Neurotransmitter Receptor Desesitization by Protein Phosphorylation. *Neuron* 5 :555-567.

Ivens, I. (1990) Neurotoxicity testing during long-term studies. *Neurotox. and teratol.* 12: 637-641.

Jolicoeur, F.B., Rondeau, D.B., Hamel, E., Butterworth, R.F. & Barbeau, A. (1979) Measurement of ataxia and related neurological signs in the laboratory rat. *Can. J. Neurol. Sci.*, 6(2): 209-215.

Kano, M., Hashimoto, K., Chen, C et. al., (1995) Impaired Synapse Elimination During Cerebellar Development in PKC-γ mutant mice. *Cell* 83:1223-1231

Kemp, J.M & Powell, P.S (1971) The connections of the striatum and globus pallidus: synthesis and speculation. *Philos. Trans. R. Soc. London* Sre. B 262: 441-457.

Kish, S.J., Shannak, k. & Hornykiewicz, O. (1988) Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's Disease: pathophysiologic and and clinical implications. *N. Eng. J. Med.*, 318: 876-880.

Krebs, M.O., Trovero, F., Desban, M., Gauchy, C., Glowinski, J. & Kemel, M.L. (1991) Distinct presynaptic regulation of dopamine release through NMDA receptors in striosome- and matrix- enriched areas of the rat striatum. *J Neurosci.*, 11: 1256-1262.

Kunzle, H. (1975) Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. An autoradiographic study in macaca fascicularis. *Brain Res.* 38: 65-73.

Kunzle, H. (1977) Projections from the primary somatosensory cortex to basal ganglia and thalamus in the monkey. *Exp. Brain Res.*, 30: 481-92.

Lam, A.G.M., Campbell, J.M., Bennet, N.K. et al (1998) Local cerebral glucose utilisation in the AS/AGU rat: a mutant with movement disorders. *Eur. J. Neurosci., 10: 1963-1967.*

Lang, A. E. & Lozano, A.M. (1998) Parkinsons Disease (pt 1) *N. Eng. J. Med.*, 339(15): 1044-1053.

Lemon, R.N., Johansson, R.S. & Westling, G. (1996) Modulation of corticospinal influence over hand muscles during gripping tasks in man and monkey. *Can. J. Physiol. Pharmacol.*, 74(4): 47-558.

Levy, L., Hazrati, L.N., Herrero, M.T. et al. (1997) Re-evaluation of the functional anatomy of the basal ganglia in normal and Parkinsonian states. *Neurosci.*, 76(2): 335-343.

Marconi, R., Lefebvre-Caparrós, D., Bonnet, A.M. et al. (1994) Levodopa-induced dyskinesias in Parkinson's Disease phenomenology and pathophysiology. *Mov. Disord.*, 9: 2-12.

Marshall, J.F. & Teitlebaum, P. (1974) Further analysis of sensory inattentionfollowing lateral hypothalamic damage in rats. *J. Comp. Physiol. Psychol.* 86(3): 375-395.

Mayeux, R., Chen,J., Mirabello, E et al. (1990) An estimate of the incidence of dementia in idiopathic Parkinson's disease. *Neurology* 40:1513-1517.

Mead, L.A., Hargreaves, E.L., Ossenkopp, K.P., Kavaliers, M. (1994) A multivariate assessment of spontaneous locomotor activity in the Mongolian gerbil (meriones unguiculatus): influences of age and sex. *Physiol. Behav.*, 57(5): 893-899.

Misu, y., Goshima, Y., Ueda, H.. & Okamura, H. (1996) Neurobiology of L-DOPAergic systems. *Prog. Neurobiol.*, 49: 415-454.

Moratalla, R., Quinn, B., DeLanney, L.E. et al. (1992) Differential vulnerability of primate caudate-putamen and striosome-matrix dopamine systems to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci.*, 89: 3859-3863.

Mukhida, K., Baker, A.& Mendez, I. (2001) Enhancement of sensorimotor behavioural recovery in hemiparkinsonian rats with intrastriatal, intranigral and intrasubthalamic nucleus dopminergic transplants. *J. Neurosci.*, 21(10): 3521-3530.

Nishi, K., Kondo, T. & Narabayashi, H. (1991) Destruction of norepinephrine terminals in of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice reduces locomotor activity induced by L-DOPA. *Neurosci. Lett.*, 123: 244-247.

Oertel, W.H. & Mugnaini, E. (1984) Immunocytochemical studies of GABA-ergic neurons in rat basal ganglia and their relations to other neuronal systems. *Neurosci. lett.*, 47: 233-238.

Olanow, C.W. & Tatton, W.G. (1999) Etiology and pathogenesis of Parkinson's Disease. *Ann. Rev. Neurosci.*, 22: 123-144.

Parent, A.(1990) Extrinsic connections of the basal ganglia. *Trends Neurosci.*, 13(7): 254-258.

Parent, A., Smith, Y. & Bellefeuille, L. (1984) The output organisation of the pallidum and substantia nigra in primate as revealed by a retrograde double labelling method. In *The basal ganglia, structure and function.* eds. J.S. McKenzie, R.E. Kemm, L.N. Wilcock. pp147-60. New York: Plenum.

Parker, A.J. & Clarke, K.A. (1989) Gait topography in rat locomotion. *Physiol. Behav.*, 48: 41-47.

Paxinos, G. & Watson, C. (1982) *The rat brain in stereotaxic coordinates.* Academic Press.

Payne, A.P., Sutcliffe, R.G., Campbell, J.M. et al. (1998) Disordered locomotion in the AS/AGU mutant rat and the effects of L-DOPA or fetal midbrain grafts. *Movement Disorders* 13(5): 832-834.

Payne, A.P., Campbell, J.M., Russell, D. et al. (2000) The AS/AGU rat: a spontaneous model of disruption and degeneration in the nigrostriatal dopaminergic system . *J. Anat.* 196: 629-633.

Pellis, S.M., Pellis, V.C. & Teitelbaum, P. (1991) Air righting without the cervical righting reflex in adult rats. *Behav. Brain Res.*, 45(2): 185-188.

Reddy, P.H., Williams, M. & Tagle, D.A. (1999) Recent advances in understanding the pathogenesis of Huntington's disease. *Trends Neurosci.*, 22: 248-255.

Ribak, C.E., Vaughn, J.E. & Roberts, E. (1979) The GABA neurons and their axonal terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. *J. Comp. Neurol.*, 187: 261-284.

Rosas, G., Lopez-Martin, E., Guerra, M.J. & Labandiera,-Garcia, J.L. (1998) The overall rod performance test in the MPTP-treated –mouse model of Parkinsonism. *J. Neurosci. Methods*, 83: 165-175.

Rouiller, E.M., Yu, X.M., Moret, V. et al. (1998) Dexterity in adult monkeys following early lesion of the motor cortical hand area: the role of cortex adjacent to the lesion. *Eur. J. Neurosci.*, 10(2): 729-740.

Sakai., K. & Gash, D.M. (1994) Effect of bilateral 6-OHDA lesions of the substantia nigra on locomotor activity in the rat. *Brain Res.*, 633: 144-150.

Schell, G.R. & Strick, P.L. (1984) The origin of thalamic inputs to the arcuate premotor and supplementary motor areas. *J. Neurosci.*, 4(2): 539-560.

Schoen, S. W. & Graybiel, A.M. (1992) 5'-Nucleotidase: A new marker for striosomal organization in the rat caudoputamen. *J. Comp. Neurol.*, 322: 566-576.

Schwarcz, R., Creese, I., Coyle JT. & Snyder SH. (1978) Dopamine receptors localised on cerebral cortical afferents to rat corpus striatum. *Nature*, 271: 766-768.

Setlow, B. & McGauch, J.L. (1999) Involvement of the posteroventral caudate-putamen in memory consolidation in the Morris water maze. *Neurobiol. Learn. Mem.*, 71(2): 240-247.

Shearman, M.S., Sekiguchik, K. & Nishizukay (1989) Modulation of ion Channel Activity: a key function of the protein kinase C enzyme family. *Pharmacol. Rev.* 41: 211-237.

Shoulson, I., Glaubiger, G.A. & Chase, T.N. (1975) On-off response: clinical and biochemical correlations during oral and intravenous levodopa administration in Parkinsonian patients. *Neurology*, 25: 1144-1148.

Smith, Y., Shink, E. & Sidibe, M. (1998) Neuronal circuitry and synaptic connectivity of the basal ganglia. *Neurosci. Cli. N. Amer.*, 9(2): 203-221.

Ungerstedt, U. (1968) 6-Hydroxy-dopamine Induced Degeneration of Central Monoamine Neurons. *Eur. J. Pharmacol.* 5: 107-110.

Vincent, S.R., Hokfelt, T., Christensson, I. & Terenius, L. (1982) Imunohistochemical evidence for a dynorphin immunreactive striatonigral pathway. *Eur. J. Pharmacol.*, 85: 251-252.

Wenning, G.K., Tison, F., Ben Shlomo, Y., Daniel, S.E,. Quinn, N.P. et al. (1997) Multiple system atrophy: A review of 203 pathologically proven cases. *Mov. Disord.*, 2: 133-147.

Wichmann, T. & DeLong, M.R. (1998) Models of basal ganglia function and pathophysiology of movement disoeders. *Neurosurg. Clin. N. Amer.*, 9(2) 223-235.

Wilson, C. J., & Groves, P. M. (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: A study employing intracellular injection of horseradish peroxidase. *J.Comp. Neurol.*, 194: 599-615.

Wilson, S.A.K. (1914) An experimental research into the anatomy and physiology of the corpus striatum. *Brain*, 36: 437-492.

Wolf, L.W., LaRegina, M.C., Tolbert, D.L. (1996) A behavioural study of the development of hereditary cerebellar ataxia in the shaker rat mutant. *Behav. Brain Res.* 75: 67-81.

Wooten, F.G. (1987) Pharmacokinetics of levodopa. In *Movement Disorders* ed. Marsden and Fahn. Butterworth-Heinemann international medical reviews.

APPENDICES

APPENDIX 1

Method for calculating neurotransmitter concentrations as measured by HPLC peak areas

A response factor (RF), the ratio of the peak areas of the amine of interest and the internal standard, is firstly calculated from the peaks in the composite standard.

Thus:

$$\text{Response Factor} = \frac{\text{Area of Internal Standard in Composite Standard}}{\text{Area of Amine in Composite Standard}}$$

This RF is then applied to a second calculation in order to determine the absolute value of neurotransmitter in the sample. This is repeated for each neurotransmitter found in the sample.

$$\text{Peak Concentration} = \frac{\text{Area of Amine in Sample}}{\text{Area of Internal Standard in Sample}} \times \text{RF} \times 1.11\times10^{-9}\,\text{mg}/\mu\text{l}$$

The figure of 1.11×10^{-9} is a conversion factor based on the known concentrations of the internal standard in the composite, and to account for the DHBA concentration in the sample.

Final Concentrations are expressed as picograms per sample.

APPENDIX 2

Chemicals and Equipment Used in this Study.

Antec INTRO Electrochemical Detector Presearch Limited, System House, 59-61 Knowl Piece, Hitchin, Herts., SG4 0TY, UK.

Antisedan (atipamezole hydrochloride) Pifzer UK Ltd., Canada House, 272 Field End Road, Eastcote, Ruislip, Middlesex, HA4 9NA, UK.

CMA Automatic Microinjector (microdialysis) Biotech Instruments, Biotech House, 75A High Street, Kimpton, Herts., SG$ 8PU, UK.

Drugs and Composite Standard Components
L-Dopa, Benserazide, DHBA, Dopamine, DOPAC, 5HT, 5HIAA, HVA.
Sigma-Aldrich Ltd., Fancy Road, Poole, Dorset, BH12 4NZ, UK.

HPLC Equipment
Gilson 305 Pump, 805 Manometric Module, 7125i Rheodyne sample injector, microbore colum.
Anachem Ltd., 20 Charles Street, Luton, UK.

Infra-red Motion Activity System Coulburn Instruments, 7462 Penn Drive, Allentown, PA 18108, USA.

Jeol Cryoststat Leica Microsystems (UK) Ltd., Davy Avenue, Knowlhill, Milton Keynes, *MK5 8LB, UK.*

Kontron KS400 Image Analysis System Imaging Associates Ltd., Thame Business Park, Wenman Road, Thame, Oxford, OX9 3XA, UK.

Kopf Stereotaxic Frame David Kopf Instruments, 7234 Elmo Street, Tujunga, CA, 91042 USA.

Mobile Phase Components
Citric Acid, EDTA, Disodium Hydrogen Phosphate, Octane Sulphonic Acid, Methanol. Sodium Bisulphite. Sigma-Aldrich Ltd., Fancy Road, Poole, Dorset, BH12 4NZ, UK.

Redifast dental cement liquid Wright Health Group Ltd., Dundee, UK.

Rompun (Xylazine hydrochloride) Bayer Plc., Bayer House, Strawberry Hill, Newbury, Berkshire, RG14 1JA, UK.

RS epoxy resin RS Components, PO Box 99Corby, Northhants, NN17 9RS. UK

Silicon Graphics Computer and Software SGI Ltd., 1530 Arlington Business Park, Theale, Reading, Berkshire, RG7 4SB, UK.

Sony Handicam Video camera Sony UK Ltd., Brooklands, Weybridge, Surrey, UK.

Tubing for Probe Construction RJ Woods, 39 Blavk Sneddon Street, Paisley, PA3 2DE, UK.

Vetalar (ketamine hydrochloride) Pharmacia & Upjohn Ltd (Animal Health), Hunters Road, Weldon North Estate, Corby, Northants, NN17 5JE, UK.

APPENDIX 3

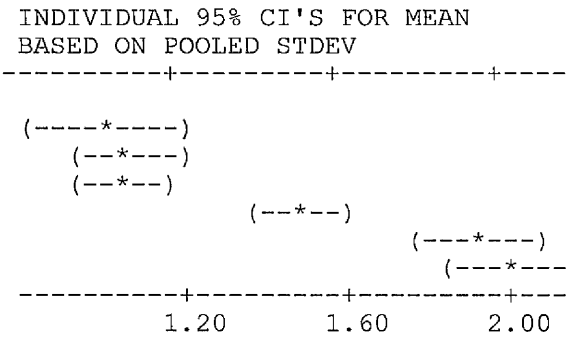
Appendix 3a. Analyses of variance for performance in Inclined Ramp Test for i) AS and ii) AS/AGU rats at different ages. Each Figure shows the relevant analysis of variance (F) together with the means and 95% confidence intervals displayed pictorially. Age groups differ if the confidence intervals do not overlap

i) AS rats

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F	p
FACTOR	5	24.340	4.868	33.23	<0.001
ERROR	158	23.147	0.147		
TOTAL	163	47.488			

AGE	N	MEAN	STDEV
20-30	17	1.0000	0.0000
40-50	35	1.0571	0.2355
60-70	37	1.0270	0.1644
100-130	34	1.4706	0.6147
140-150	22	1.9091	0.4264
365	19	2.0000	0.4714

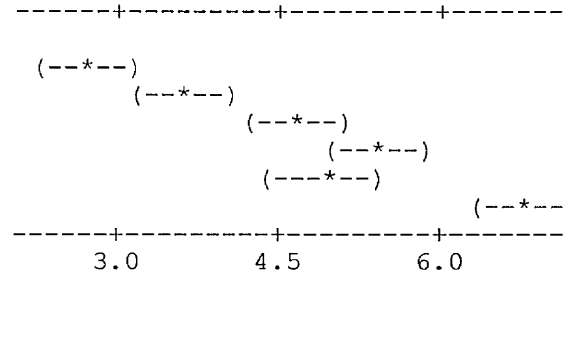


ii) AS/AGU rats

ANALYSIS OF VARIANCE

SOURCE	DF	SS	MS	F	p
FACTOR	5	203.36	40.67	35.17	<0.001
ERROR	129	149.19	1.16		
TOTAL	134	352.55			

LEVEL	N	MEAN	STDEV
20-30	17	2.706	0.686
40-50	27	3.593	1.185
60-70	24	4.625	0.875
100-130	27	5.407	1.394
140-150	20	4.900	1.334
365	20	6.800	0.410



Appendix 3b. Comparisons of the performance on the Inclined Ramp test of AS and AS/AGU rats at the ages shown. The tests performed were student's t-tests and all tests assume a two-tailed distribution.

		N	MEAN	STDEV	SE MEAN
AS	30-40	35	1.057	0.236	0.040
AGU	30-40	27	3.59	1.19	0.23

T= -10.95 P < 0.001 DF= 27

		N	MEAN	STDEV	SE MEAN
AS	60-70	37	1.027	0.164	0.027
AGU	60-70	24	4.625	0.875	0.18

T= -19.91 P < 0.001 DF= 24

		N	MEAN	STDEV	SE MEAN
AS	100-130	34	1.471	0.615	0.11
AGU	100-130	27	5.41	1.39	0.27

T= -13.66 P < 0.001 DF= 34

		N	MEAN	STDEV	SE MEAN
AS	140-150	22	1.909	0.426	0.091
AGU	140-150	20	4.90	1.33	0.30

T= -9.59 P < 0.001 DF= 22

		N	MEAN	STDEV	SE MEAN
AS	365	19	2.000	0.471	0.11
AGU	365	20	6.800	0.410	0.092

T= -33.84 P < 0.001 DF= 35

APPENDIX 4

Age	AS		AS/AGU		χ^2	df	p
	Turned	Fell	Turned	Fell			
20-30	17	0	10	7	6.48	1	< 0.05
40-50	35	0	14	13	18.52	1	< 0.001
60-70	34	3	9	15	18.17	1	< 0.001
80-90	23	0	17	22	17.72	1	< 0.001
100-130	34	0	11	16	24.36	1	< 0.001
140-150	21	1	4	16	21.72	1	< 0.001
365	13	6	0	20	17.56	1	< 0.001

APPENDIX 5

Comparisons between gait analysis data for AS and AS/AGU male rats. Comparisons are made by Student's t-test between the two groups for a) the best recorded footfall b) the worst recorded footfall and c) the difference between the best and worst recorded footfalls. In each case, a "perfect" footfall is held to be one where weight distribution averaged over the period of contact is 50% on each side of the mid-line.

a) The best recorded footfall

	N	MEAN	STDEV	SE MEAN
AS best	8	1.44	1.87	0.66
AGU best	7	3.10	5.33	2.0

T= -0.78 P=0.46 DF= 7

b) The worst recorded footfall

	N	MEAN	STDEV	SE MEAN
AS worst	8	11.0	10.3	3.7
AGU worst	7	24.8	11.2	4.2

T= -2.47 P=0.029 DF= 12

c) Best minus worst recorded footfall

	N	MEAN	STDEV	SE MEAN
AS b-w	8	11.83	9.58	3.4
AGU b-w	7	22.68	7.65	2.9

T= -2.44 P=0.031 DF= 12

APPENDIX 6

Comparisons were made between both 9 month and 12 month male AS and AS/AGU rats for both small and large movements, in both 12-minute and 22-hour sessions.

Comparisons were made using Student's t-tests.

12-minute sessions (9 month animals)

(a) Small movements

Two sample T for C1 vs C2

	N	Mean	StDev	SE Mean
AS	12	18.7	12.1	3.5
AS/AGU	10	27.5	13.3	4.2

95% CI for mu C1 - mu C2: (-20.3, 2.7)
T-Test mu C1 = mu C2 (vs not =): T = -1.62 P = 0.12 DF = 18

(b) Large movements

Two sample T for C3 vs C4

	N	Mean	StDev	SE Mean
AS	12	6.00	6.52	1.9
AS/AGU	10	10.70	7.04	2.2

95% CI for mu C3 - mu C4: (-10.8, 1.4)
T-Test mu C3 = mu C4 (vs not =): T = -1.61 P = 0.12 DF = 18

12-minute sessions (12 month animals)

(a) small movements

Two sample T for C5 vs C6

	N	Mean	StDev	SE Mean
AS	11	17.8	14.2	4.3
AS/AGU	10	23.6	18.8	5.9

95% CI for mu C5 - mu C6: (-21.3, 9.7)
T-Test mu C5 = mu C6 (vs not =): T = -0.79 P = 0.44 DF = 16

(b) Large movements

	N	Mean	StDev	SE Mean
AS	11	2.45	3.62	1.1
AS/AGU	0	8.9	10.7	3.4

95% CI for mu C7 - mu C8: (-14.3, 1.4)
T-Test mu C7 = mu C8 (vs not =): T = -1.82 P = 0.099 DF = 10

22-hour sessions (9 month animals)

(a) Small movements

	N	Mean	StDev	SE Mean
AS	10	628	136	43
AS/AGU	8	978	376	133

95% CI for mu C1 - mu C2: (-672, -28)
T-Test mu C1 = mu C2 (vs not =): T = -2.50 P = 0.037 DF = 8

(b) Large movements

	N	Mean	StDev	SE Mean
AS	10	104.0	35.7	11
AS/AGU	8	206.1	85.1	30

95% CI for mu C3 - mu C4: (-176, -28)
T-Test mu C3 = mu C4 (vs not =): T = -3.18 P = 0.013 DF = 8

22-hour sessions (12 month animals)

(a) Small movements

	N	Mean	StDev	SE Mean
AS	11	544	189	57
AS/AGU	9	1059	259	86

95% CI for mu C5 - mu C6: (-737, -294)
T-Test mu C5 = mu C6 (vs not =): T = -4.98 P = 0.0002 DF = 14

(b) Large movements

Two sample T for C7 vs C8

	N	Mean	StDev	SE Mean
AS	11	86.7	44.6	13
AS/AGU	9	235.3	83.4	28

95% CI for mu C7 - mu C8: (-217, -81)
T-Test mu C7 = mu C8 (vs not =): T = -4.81 P = 0.0005 DF = 11

APPENDIX 7

Appendix 7 (a) Two-way analysis of variance comparing treatment (saline v. L-DOPA) and week of treatment (week 3 v. week 4) for the performance of AS/AGU rats in the Inclined Ramp test. This shows a significant variance for both treatment and week of treatment

SOURCE	DF	SS	MS	F	p
week	1	11.02	11.02	7.39	< 0.001
treatment	1	18.23	18.23	12.23	< 0.001
INTERACTION	1	2.03	2.03	1.36	ns
ERROR	36	53.70	1.49		
TOTAL	39	84.97			

Appendix 7 (b) Comparisons of performance in the Inclined Ramp test of AS/AGU rats treated with L-DOPA (25mg/kg) or saline i) on week 3 of treatment, ii) on week 4 of treatment. From this it will be seen that differences in performance are not significant by week 3, but are significant by week 4.

i) Week 3 of treatment

	N	MEAN	STDEV	SE MEAN
L- DOPA	10	5.30	1.49	0.47
Saline	10	6.20	1.03	0.33

T= -1.57 P=0.14 DF= 16

ii) Week 4 of treatment

	N	MEAN	STDEV	SE MEAN
L-DOPA	10	3.80	1.32	0.42
Saline	10	5.60	0.96	0.31

T= -3.49 P=0.0031 DF= 16

APPENDIX 8

The numbers of AS/AGU animals treated with daily injections of saline or L-DOPA (25mg/kg) which can successfully right themselves in mid-air after being held upside down and dropped from 50 cm above a soft landing. Tests were carried out weekly.

Week	Saline	L-DOPA	(χ^2)	df	p
1	3/10	2/10	0.20	1	ns
2	2/10	5/10	1.28	1	ns
3	0/10	8/10	8	1	< 0.01
4	0/10	9/10	9	1	< 0.01