CAUDATE NUCLEUS AND PROGRAMMING BEHAVIOUR IN CATS

ROLE OF STRIATAL DOPAMINE AND GLUTAMATE, AND THEIR INDIRECT EFFECTS ON GABA OF THE SUBSTANTIA NIGRA PARS RETICULATA AND THE DEEPER LAYERS OF THE COLLICULUS SUPERIOR

ROB JASPERS

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CAUDATE NUCLEUS AND PROGRAMMING BEHAVIOUR IN CATS:

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Promotor: Prof. Dr. A.R. Cools

Voor Hanneke

Voor Tessa en Rianne

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Jaspers, R., Schwarz, M., Sontag, K.-H. and Cools, A.R. **(1984)** Caudate nucleus and programming behaviour in cats: role of dopamine in switching motor patterns. *Behav. Brain Res., 14, 17-28.*

Jaspers, R. and Cools, A.R. **(1985)** GABA-specificity of behaviour responses to picrotoxin injected into the colliculus superior of cats. *Behav. Brain Res., 18, 63-69.*

Jaspers, R.M.A. and Cools, A.R. **(1988)** Behavioural correlates of a progressive dysfunctioning of the caudate nucleus: effects of apomorphine. *Behav. Brain Res., 27, 193-204.*

Jaspers, R.M.A., Berkelbach van der Sprenkel, J.W. and Cools, A.R. **(1989)** Progressive pathology of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the colliculus superior: acute behavioural and metabolic effects of intrastriatal kainic acid. *Neuroscience, 28, 159-169.*

Jaspers, R.M.A., Berkelbach van der Sprenkel, J.W., Tulleken, C.A.F. and Cools, A.R. **(1990)** Local as well as remote functional and metabolic changes after focal ischemia induced by occlusion of the middle cerebral artery in cats. *Brain Res. Bull., 24, 23-32.*

Jaspers, R.M.A. and Cools, A.R. **(1990)** Behavioural correlates of a progressive dysfunctioning of the deeper layers of the colliculus superior: effects of picrotoxin. *Pharm., Biochem. Behav.,* 37, 1-29.

Jaspers, R.M.A., de Vries, T.J. and Cools, A.R. **(1990)** Enhancement in switching motor patterns following local application of the glutamate agonist AMPA into the cat caudate nucleus. *Behav. Brain Res., 37, 237-246.*

Jaspers, R.M.A., de Vries, T.J. and Cools, A.R. **(1990)** Effects of intrastriatal apomorphine on changes in switching behaviour induced by the glutamate agonist AMPA injected into the cat caudate nucleus. *Behav. Brain Res., 37, 247-254.*

Jaspers, R.M.A., Sontag, K.-H. and Cools, A.R. Caudate nucleus and muscle activity: intracaudate haloperidol and forelimb extensor activity during jump-downs in cats. *Subm.*

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1 SCOPE AND INTENT

The caudate nucleus has been the subject of extensive research during the past decades. The finding that there is a dramatic loss of the neurotransmitter dopamine in forebrain structures such as the caudate nucleus in patients suffering from Parkinson's disease (Hornykiewicz, 1966) has stimulated experimental studies at the animal level on the role of the caudate nucleus in behaviour. Dysfunction of the caudate nucleus may result in sensory neglect, motor disorders and/or cognitive disturbances. Reviewing the literature, Öberg and Divac have tried to formulate a unifying concept of the role of the caudate by stating that the caudate nucleus 'participates in cognitive functions', i.e. functions in associative, mnemonic or complex perceptual processes (Öberg and Divac, 1979).

The broad range of behavioral effects following experimentally induced changes of the caudate nucleus, ranging from sensory and motor effects to cognitive alterations, becomes understandable when one assumes that this nucleus is actually involved in a universal process of behaviour programming which *per se* is not restricted to certain categories of observable behaviour. Evidence in favour of such a universal function is derived from animal and human studies. Since this matter is discussed elsewhere (Cools et al., 1984a; see also Chapter 3), only two studies will be mentioned here. In 1984, Cools and colleagues showed that parkinsonian patients suffered from a so-called 'shifting aptitude disorder' which was manifested in a motor task as well as in a

cognitive task (Cools et al., 1984b). Primates, treated intracaudately with cholinergic agents, show an essentially similar shifting aptitude disorder as seen in parkinsonian patients, but now restricted to social interactions (van den Bercken & Cools, 1982). Evidence in favour of the hypothesis that the caudate nucleus is selectively involved in the programming ability underlying the above-mentioned 'shifting aptitude' would be provided by showing that in animals a dopaminergic deficiency at the level of the caudate nucleus may also result in a comparable shifting aptitude disorder in motor behaviour.

At least some of the observed behavioral changes following a disturbed function of the caudate nucleus may actually result from dysfunction of other brain regions that are (in)directly affected by abnormal neural information derived from the caudate. Experimentally induced changes at the level of the caudate nucleus may alter neuronal activity in other brain regions that are (indirectly) innervated by the caudate nucleus (cf. Chevalier et al., 1985; Gale & Casu, 1981; Kelly & McCulloch, 1984, 1987; Patino & Garcia-Munoz, 1985; Scheel-Krüger, 1986). Distortion of the transmission and/or transformation of cortico-caudate signals may result in a reorganization of cortical activity affecting parallel (extracaudate) pathways (see Figure 1.1). In any case, the degree to which extracaudate brain regions become affected may depend on the degree of disturbance induced in the caudate. Evidence in favour of the hypothesis that dysfunction of the caudate nucleus may affect normal function of extracaudate brain regions would be provided by showing that experimentally induced alterations at the level of the caudate nucleus produce behavioral effects similar to that produced by experimentally induced changes in extracaudate brain areas. Taking these points into consideration, the scope of this thesis was twofold: First, it was investigated whether the caudate nucleus may indeed be involved in the programming ability underlying a 'shifting aptitude' in motor behaviour. Second, it was investigated whether an experimentally-induced dysfunctioning caudate nucleus may indirectly produce disturbances at the level of other brain regions as well.

One feature of the caudate nucleus which must be taken into consideration is its

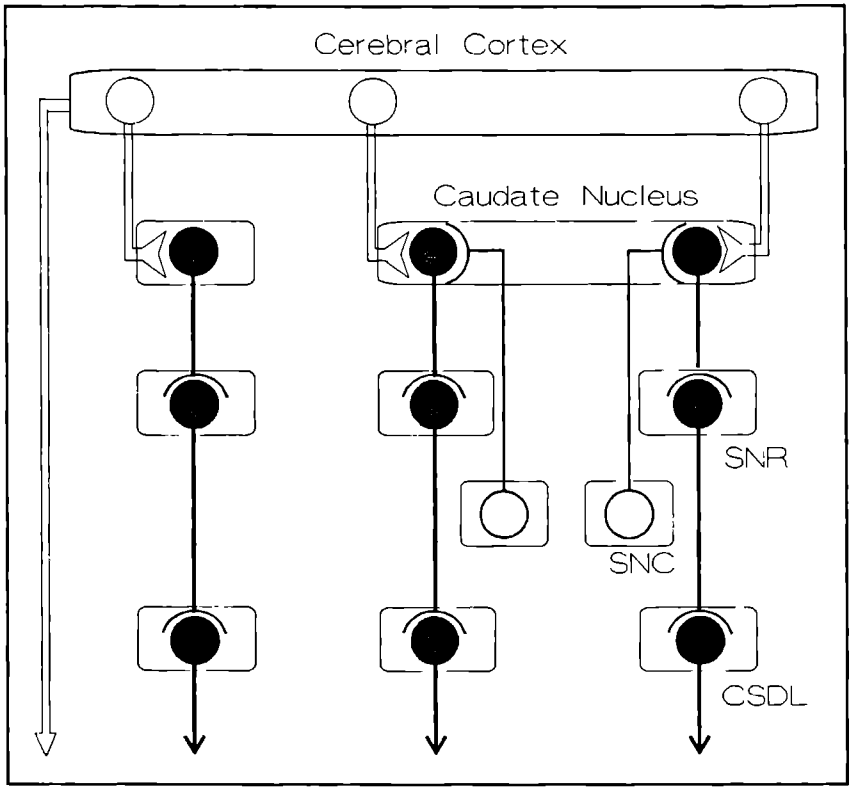

Figure 1.1 Simplified scheme of neuronal circuits involving the caudate nucleus. Two functional subregions are shown (A, B), one of which (B) projects to the substantia nigra pars reticulata (SNR). Functional disturbances at the level of the latter caudate subregion may result in an abnormal activation or inhibition of caudato-nigral fibres, affecting in turn SNR (output) neurons. Since the latter neurons project (among others) to the deeper layers of the colliculus superior (CSDL), neurons located in the latter region may also be affected. In addition, functional disturbances at the level of the caudate nucleus may result in a reorganization of cortical activity which in turn may activate parallel pathways (A, C).

heterogeneous nature. At present, morphological, anatomical, neurological, physiological, pharmacological and behavioral data are available showing that the caudate actually contains several subregions. Although distinct experimental approaches produce

subdivisions which do not completely overlap, it seems likely that this intrinsic heterogeneity in fact reflects the presence of several functional subentities within the caudate nucleus. In view of the above-mentioned heterogeneity, we have limited our experiments to one caudate subregion including its output pathway (see below).

In the caudate nucleus of cats, two distinct subregions are distinguished on the basis of differential behavioral changes following local application of dopaminergic drugs (Cools, Struyker Boudier & Van Rossum, 1976). In this thesis, attention is focused in particular on one subregion, namely the rostromedial part of the caudate nucleus (Cools, Struyker Boudier & Van Rossum, 1976). In Chapter 2, the heterogeneous character of the caudate nucleus with respect to its intrinsic organization, its afferent and efferent connections, and its role in different behaviours are discussed. Chapter 3 describes experiments in which the role of the rostromedial part of the caudate nucleus in the patterning of motor behaviour is studied. In particular, the behavioral effects of alterations in caudate dopaminergic and glutamatergic neurotransmission are emphasized. Chapters 4, 5, and 6 are devoted to the second goal of this thesis. Chapter 4 presents experiments in which the behavioral effects were analyzed following pharmacological treatment of a brain region innervated by the caudate nucleus, i.e. the substantia nigra pars reticulata (Section 4.1), and of a brain region indirectly innervated by the caudate, i.e. the deeper layers of the colliculus superior (Section 4.2). The latter structure receives fibres from the substantia nigra pars reticulata. Many caudato-nigral as well as nigro-collicular fibres contain the neurotransmitter gamma-aminobutyric acid (GABA). In order to gain insight into the behavioral changes associated with inhibition or excitation of these GABAergic fibres, the experiments described in Chapter 4 were performed. Chapter 5 presents behavioral experiments investigating intra- and extracaudate functional consequences of a potent, but reversible activation of dopamine receptors in the caudate nucleus (Section 5.1). The outcome of this study indicated that a strong activation of caudate dopamine receptors resulted also in functional changes at the level of the deeper layers of the colliculus superior. In order to verify this possibility, it was investigated in a subsequent study whether manipulation of collicular GABAergic activity produces behavioral changes similar to those observed following

stimulation of dopamine receptors in the caudate nucleus (Section 5.2). Chapter 6 presents experiments investigating the behavioral and metabolic consequences of intra- and extracaudate functional alterations following hyperactivation of caudate neurons induced by the application of a potent neuro-excitatory drug to the caudate nucleus (Section 6.1). Further, intra- and extracaudate functional and metabolic effects following permanent occlusion of the middle cerebral artery were also analyzed (Section 6.2). In addition to the data presented in Chapter 3, Chapter 7 also considers the function of the caudate nucleus in the programming of behaviour. In the experiments described in this chapter, a first attempt was made to determine whether the role of the caudate nucleus extends even to the programming of electromyographic activity *per se*. In addition, data are presented demonstrating that the deeper layers of the colliculus superior are involved in the caudate-mediated control of muscular activity.

1.2 CATS AS EXPERIMENTAL ANIMALS

In all experiments presented in this thesis, cats were used as experimental animals. The reason for choosing the cat as the experimental animal in these studies included the following:

1. In contrast to other laboratory animals such as, for instance, albino rats, cats exhibit a rich repertoire of behaviours allowing a detailed analysis of subtle changes in behaviour following experimentally induced intracerebral changes in neuronal activity.
2. Motor behaviour of cats has been analyzed in detail resulting in a great amount of knowledge in this respect (cf. Armstrong, 1986; Halbertsma et al., 1976).
3. Behavioral changes following intracerebral application of drugs has been studied intensively in cats during the past, especially with respect to the locus and neurotransmitter specificity of the chosen tools. By using the same tools, extensive control studies could be avoided thereby limiting the number of animals in the present investigations.
4. Due to the relatively large dimensions of the cat brain it became possible to use local drug application as a reliable tool to induce subtle behavioral changes, even when

the drugs had to be administered in caudate subregions. In fact, the existence of distinct functional subregions within the caudate nucleus was first established in cats (Cools, Struyker Boudier & Van Rossum, 1976).

The animals were derived from a breeding stock maintained by the Catholic University of Nijmegen. At least one week before the start of the behavioral studies, the cats were transported from the breeding colony, which was housed on a farm, to the central animal laboratory of the medical faculty where they were housed in a specially designed room. Their age was between 10 and 12 months. In most investigations, only male cats were used because at this age female animals often weighed less than 2.5 kg. A minimum body weight of 2.5 kg was a prerequisite for implanting cannulas in a reliable way (see below). In practice, cats weighing at least 2.8 kg were used for the implantation of cannulas. The cats were housed in groups in iron wire mesh cages (length x width x height: 190 x 120 x 160 cm: maximum number of animals per cage = 8) which were duplicates of those used in the breeding colony. The condition of all animals was checked regularly by a veterinarian. In behaviour research in general, the well-being of the animals is of vital importance since any form of discomfort might directly affect the results of the behaviour analysis. Therefore, a number of measures were taken to avoid any discomfort as much as possible. For instance, the animals were extensively habituated to all activity associated with the actual experiments, such as handling by the experimenter, transport to the experimental set-up, injection procedures, and, where appropriate, the behavioral procedures themselves.

Prior to cannulation experiments, cats were equipped with intracerebrally implanted guide cannulas. The cannulas were implanted under pentobarbitone anaesthesia (40-50 mg/kg) with the help of a stereotaxic instrument. The cannulas were mounted on the skull with help of dental acrylic cement. Postoperative control of the cats showed that, after a short recovery period, the behaviour was not affected as a result of the implantation. They showed normal righting reflexes, orienting responses and pupil reflexes; they reacted in the same way to the experimenter as before the operation. As a rule, animals were tested once a week, and they participated in maximally 5 experi-

ments. After the final experiment, the cats were deeply anaesthetized and transcardially perfused with saline followed by 4% formaldehyde solution for subsequent analysis of the injection loci.

CHAPTER 2

NEUROBIOLOGICAL ASPECTS OF THE CAUDATE NUCLEUS

2.1 INTRODUCTION

In rodents, the caudate nucleus is not a distinct cytoarchitectical entity, but rather forms together with the putamen the caudate-putamen complex or neostriatum, i.e., the core cell mass of the basal ganglia. In primates and carnivores, the striatum is divided into the caudate nucleus and the putamen by the capsula interna (Figure 2.1.1) (cf. Nieuwenhuys, 1977; 1988). In addition to the (neo)striatum, the globus pallidus together with the substantia nigra and the subthalamic nucleus are also part of the basal ganglia. Despite the relatively homogeneous appearance of striatal tissue - more than 95% of the neurons belong to the medium-sized (12-18 μm) spiny type (Bishop, Chang & Kitai, 1982; Dray, 1980; Groves, 1983) - biochemical, anatomical, physiological, metabolic and behavioural studies have revealed a very heterogeneous character of this part of the brain. Since this feature of heterogeneity has important implications when studying the role of the caudate nucleus in the programming of behaviour, this chapter will review some data concerning the functional diversity of the caudate nucleus in relation to its intrinsic organization, afferents and projections. For data based on primate or carnivore studies, the term caudate nucleus will be used and only in those cases where this particular structure is meant by the author; otherwise the more general term striatum will be employed. For studies using rodents, the term neostriatum will be used.

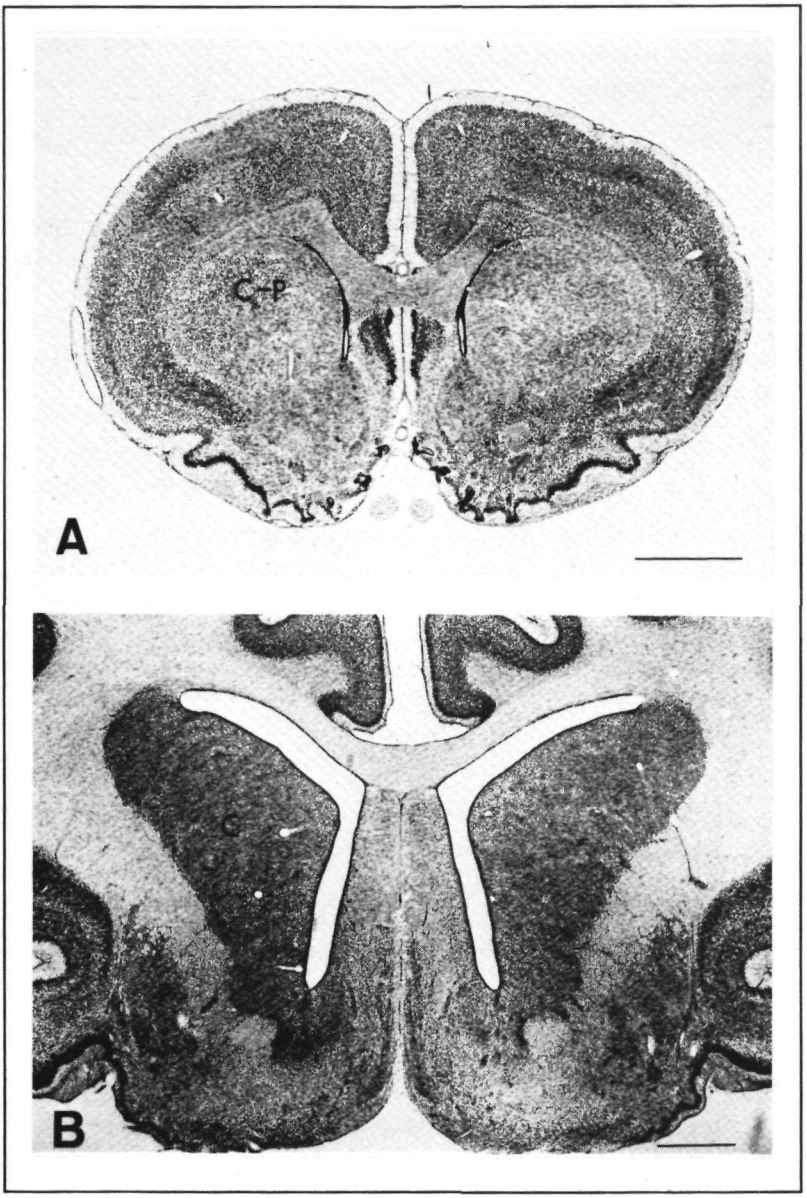

Figure 2.1.1 *Cross sections from the rat (A) and cat (B) illustrating the relative differentiation of the striatum. C-P, caudate-putamen complex; CN, caudate nucleus; P, putamen. (Nissl staining; bar indicates 2 mm). (from: Graybiel & Ragsdale, 1979; with permission).*

2.2 NEUROCHEMICAL COMPARTMENTALIZATION

On the basis of neurochemical markers striatal tissue appears to be segregated into distinct compartments: islands of cell clusters labelled as 'patches' or 'striosomes', and a surrounding substrate labelled as 'matrix'. This was shown first in the rat neostriatum (Pert et al., 1976; Herkenham & Pert, 1981) but was soon this also established in other species such as cats, monkeys and man (Graybiel & Ragsdale, 1978). In fact, the rat neostriatum contains a labyrinthine system of patches marked by dense concentrations of opiate receptors. In 1981, Herkenham and Pert showed in the rat that these patches coincide with regions marked by low acetylcholinesterase (AChE) activity. In the cat, the striatal compartmentalization is similarly characterized by striosomes marked by a relatively low AChE activity and, complementary to these, a matrix marked by a relatively dense AChE-activity (Graybiel & Ragsdale, 1978; Graybiel & Ragsdale, 1979). In subsequent studies, it was found that patches can be distinguished from the surrounding matrix by relatively high concentrations of substance P or relatively high enkephalin-like immunoreactivity (Graybiel et al., 1981; Gerfen, 1984; Penny, Afsharpour & Kitai, 1986). Furthermore, the AChE-rich matrix expresses a relatively high NADPH-(dihydronicotinamide adenine dinucleotide phosphate) diaphorase activity (Sandell, Graybiel & Chesselet, 1986). Diaphorase positive neurons are also marked by somatostatin- and avian pancreatic polypeptide/neuropeptide Y-like immunoreactivity (Vincent & Johansson, 1983; Vincent et al., 1983). Finally, in a computer-assisted morphometrical study it has recently been shown that tyrosine hydroxylase-, DARPP-32(dopamine- and cyclic AMP-regulated phosphoprotein, M_r= 32000)- and enkephalin-like immunoreactivity identifies patches in the rat neostriatum that overlap differentially in distinct parts of this nucleus. For example, the putative markers for presynaptic dopaminergic fibres and postsynaptic dopaminoceptive neurons, i.e., fibres and cells showing immunoreactivity to tyrosine hydroxylase and DARPP-32, respectively, overlap completely in a small marginal neostriatal zone, whereas any consistent overlap between all three markers is absent in the central part (Agnati et al., 1988). An example of the three dimensional clustering of patches is illustrated in Figure 2.2.1.

However, the functional significance of the compartmentalization into patches and matrix is far from clear. As described below, there are indications that the distinct compartments are differentially innervated by the three major striatal afferent systems, i.e. the mesostriatal, the corticostriatal and the thalamostriatal projections.

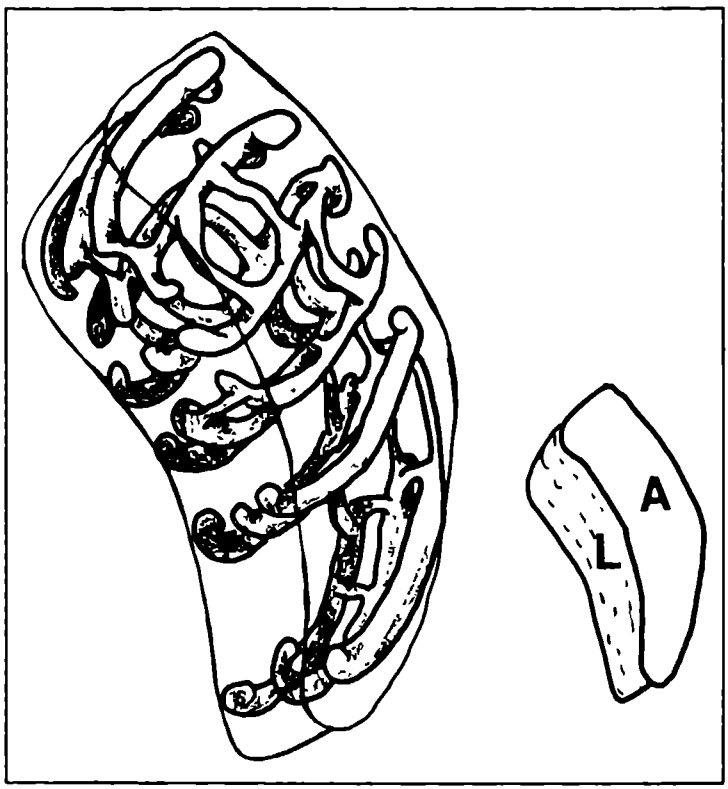

Figure 2.2.1 Simplified three-dimensional reconstruction of the 'patch'-network marked by a dense enkephalin-like immunoreactivity in the caudate nucleus of the cat. The small outline illustrates the orientation of the figure. The network is based on computer-assisted reconstruction derived from serial coronal, horizontal and sagittal sections. L, lateral; A, anterior (from Groves et al., 1988; with permission).

2.3 MOSAIC DISTRIBUTION OF CAUDATE AFFERENTS AND EFFERENTS

Afferent connections

The dopaminergic mesostriatal projection originates in three mesencephalic cell groups labelled as A8, A9 and A10 according to the terminology of Dahlström and Fuxe (1964). As described by Ungerstedt in 1971, dopaminergic cells located in the substantia nigra pars compacta (A9) together with cells located in the retrorubral area (A8) project to the neostriatum of the rat, whereas the dopaminergic nerve endings in the nucleus accumbens and olfactory tubercle originate from cells located in the ventral tegmental area (A10). More recent studies have refined our knowledge concerning the afferent projections of the caudate nucleus. For instance, terminations of A10 dopaminergic neurons are also found in the caudatoputamen complex, especially, but not exclusively, in more ventral regions (cf. Fallon & Moore, 1978; Moore & Bloom, 1978; Szabo, 1980). These data imply that, in fact, the rat neostriatum is only partly 'striatal': another part can be labelled as 'limbic' as a result of its dopaminergic innervation from the ventral tegmental area (cf. Jayaraman, 1985). Below, more detail regarding the projection areas of the distinct dopamine cell groups is presented.

In 1984, Graybiel showed that in the cat caudate nucleus the pattern of immunoreactivity in tyrosine hydroxylase, the enzyme which synthesizes dopamine, changes during ontogeny from 'islands' to a relatively diffuse distribution (Graybiel, 1984). In the mature brain, tyrosine hydroxylase-like immunoreactivity is relatively low in striosomes compared to the surrounding matrix (Graybiel, Hirsch & Agid, 1987). These data suggest that distinct dopamine subsystems develop successively (Graybiel, 1984; Graybiel, Hirsch & Agid, 1987). Additional evidence in favour of this suggestion was presented by Gerfen and coworkers in the rat (Gerfen, Herkenham & Thibault, 1987; Gerfen, Baimbridge & Thibault, 1987; see also Loopuijt, Sebens & Korf, 1987; Murrin & Zeng, 1989). In the rat mesencephalon, two distinct populations of dopamine neurons can be distinguished on the basis of a number of factors : (1) different periods of development, (2) location in the midbrain, (3) presence of histochemical markers and (4) neostriatal termination zones. Dopamine neurons located in the substantia

nigra pars compacta (i.e., the 'ventral tier groups' or ventral A9 together with 'displaced' neurons in the substantia nigra pars reticulata) project to the patch compartment. They also express no Ca-binding protein immunoreactivity and develop relatively early during ontogeny. In contrast, dopamine cells located in the retrorubral area (A8), the ventral tegmental area (A10 area or 'dorsal tier cell groups', including neurons located in the dorsal A9 area) project to the striatal matrix. These cells express Ca-binding protein immunoreactivity and develop later during ontogeny (see Figure 2.3.1). In the cat and monkey, a more or less similar distinction exists: a restricted zone within the medial part of the substantia nigra pars compacta, the 'densocellular zone', contains cells projecting to the striatal patch compartment, whereas dopamine cells located in the remaining lateral parts of the reticular pars compacta project preferentially to the matrix of the caudate nucleus. The latter also holds true for dopamine neurons of the A8 and A10 group. These cells project to the matrix of the dorsal and the ventral striatum, respectively (Jimenez-Castellanos & Graybiel, 1987; Feigenbaum Langer & Graybiel, 1989).

The cerebral cortex is the source of the largest group of striatal afferents (Graybiel & Ragsdale, 1979). In general, all regions of the cerebral cortex project to the striatum. For example, the neocortex projects to the striatum via a projection originating in the supragranular (II and III) and infragranular (V and VI) cortical layers (Royce, 1982; Tanaka, 1987). Via this projection system, the sensorimotor cortical areas impinge directly on the dorsolateral striatum while the visual cortical area projects to the dorsomedial part of the striatum. In addition, the mesocortex projects predominantly to the medial and ventral part of the striatum with the lateral mesocortical areas projecting to ventral striatal areas. Finally, the allocortex projects mainly to the nucleus accumbens and the olfactory tubercle. In the case of the entorhinal cortex, an additional projection exists to the ventromedial striatum and in the case of the piriform cortex, subiculum and hippocampus, an additional projection to the medial part of the (neo)striatum has been described (McGeorge & Faull, 1989, see also Arikuni & Kubota, 1986; Faull, Nauta & Domesick, 1986).

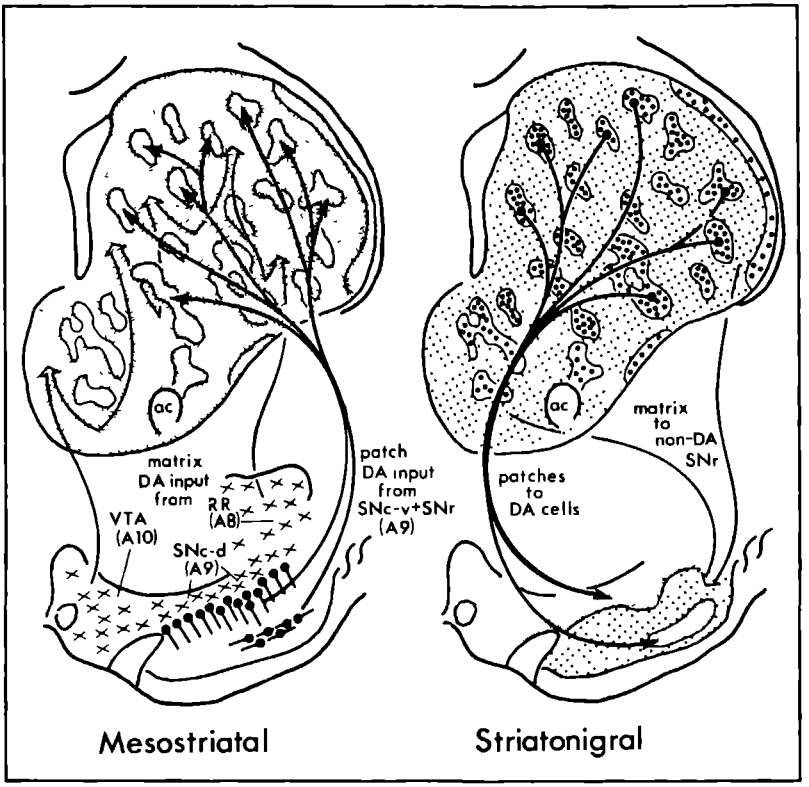

Figure 2.3.1 *Schematic illustration of the compartmental organization of the mesostriatal innervation (left) and striatonigral projection (right) in the rat neostriatum. Dopaminergic afferents to the neostriatal matrix originate from a dorsal set of midbrain neurons (x) located in the ventral tegmental area (A10 cells in the VTA), the dorsal tier of the substantia nigra pars compacta (dorsal A9 cells in the SNc-d), and the retrorubral area (A8 cells in the RR). Dopaminergic afferents to the neostriatal patches originate from the ventral tier of the substantia nigra pars compacta (ventral A9 cells in the SNc-v), and from the A9 dopaminergic cells located in the substantia nigra pars reticulata (SNr). Neurons located in the neostriatal matrix provide inputs to the substantia nigra pars reticulata that avoid the location of the dopaminergic cells in both the SNc and the SNr. Neurons in the neostriatal patches provide inputs to the location of the dopaminergic cell bodies. (From: Gerfen, Herkenham & Thibault, 1987; with permission).*

In addition, the patch and the matrix compartment are differentially innervated by

the cortex (Gerfen, 1984). The patches are innervated by prelimbic cortical areas, i.e., parts of the cortex innervated by the amygdala and hippocampus. In contrast, the matrix receives afferents from the sensorimotor cortex. In addition, the corticocaudate projections are also topographically organized. For instance, Kubozono et al. (1986) found that the dorsolateral caudate nucleus of the cat receives fibres from area 4, whereas more central caudate parts are innervated by area 6. Finally, recent evidence suggests that at least in the rat both the patch and matrix compartments are innervated by each cortical region, but cortical neurons located in the supragranular layers project mainly to the matrix, while output cells located in infragranular layers send their axons to the striosomes (Gerfen, 1989).

The third major innervation, and in fact the second largest, of the striatum is derived from the thalamus. Thalamostriatal afferents originate predominantly in the intralaminar nuclei (Beckstead, 1984; Jones & Leavitt, 1974; Macchi et al., 1984) and appear to terminate in clusters in the striatum (Kalil, 1978; Royce, 1978). In addition, the ventromedial-ventrolateral complex and the dorsomedial nucleus also project to the caudate nucleus (Fisher et al., 1983). In the cat, the supragenicular nucleus projects selectively to the medial and intermediate regions (Hu & Jayaraman, 1986). The rat thalamostriatal projection originating in the parafascicular nucleus appears to avoid striosomes marked by a high opiate binding and a low acetylcholinesterase activity (Herkenham & Pert, 1981). This finding has also been confirmed in the cat where the thalamic centre median/parafascicular complex distributes its axons into the matrix compartment of the caudate nucleus (Beckstead, 1985). This innervation therefore appears to be complementary to the projection derived from the medial substantia nigra (Beckstead, 1985; Jimenez-Castellanos & Graybiel, 1987; see above). Finally, these thalamic nuclei project preferentially to patches of the ventral striatum and nucleus accumbens.

Apart from the three afferent systems described above, the striatum also receives fibres from the pontine and caudal mesencephalic reticular formation, locus coeruleus, raphe nuclei, globus pallidus, subthalamic nucleus and amygdala (Dray, 1980; Fisher et

al., 1983; Graybiel & Ragsdale, 1979). The bulk of the amygdalostriatal fibres are derived from the basolateral amygdala which appears to innervate predominantly striosomes. In addition, the basomedial nucleus of the amygdala innervates the extrastriosomal matrix compartment (Ragsdale & Graybiel, 1988).

Efferent connections

Neurons of the caudate nucleus/neostriatum send their axons to two brain regions: the globus pallidus and the substantia nigra pars reticulata (Dray, 1980; Faull, Nauta & Domesick, 1986; Graybiel & Ragsdale, 1979; Nieuwenhuys, 1977; Percheron, Yelnik & François, 1984; Royce & Laine, 1984; Tulloch, Arbuthnott & Wright, 1978; for review: Scheel-Krüger, 1986). In addition, striatal efferents project to the entopeduncular nucleus, which is the homologue to the inner and medial segment of the globus pallidus in primates (Graybiel & Ragsdale, 1979). The majority of striatal neurons (medium-sized spiny cells; Somogyi & Smith, 1979) are output neurons (Fisher et al., 1986a; Kitai & Kocsis, 1979; Pasik et al., 1988). Many caudate output neurons contain GABA as revealed by immunocytochemistry (presence of glutamate decarboxylase, the biosynthetic enzyme for GABA: Fisher et al., 1986b; Kubota et al., 1987; Kita & Kitai, 1988; Oertel & Mugnaini, 1984). Other putative neurotransmitters that may be used by caudate efferents are substance P (Kanazawa et al., 1980; Somogyi et al., 1982), enkephalin (Johnson, Sar & Stumpf, 1980) and dynorphyn (Vincent et al., 1982). Recent evidence suggests that each of the output pathways is characterized by its own set of (putative) transmitters (for ref., see Graybiel, 1990; Smith & Bolam, 1990). The medium spiny output neurons have axons which form extensive collateral networks before they leave the (neo)striatum (Katayama, Miyazaki & Tsubakawa, 1981; Penny, Wilson & Kitai, 1988; Preston, Bishop & Kitai, 1980; see Figure 2.3.2). In addition, the striatal output neurons have large dendritic fields as illustrated in Figure 2.3.2. As is schematically depicted in Figure 2.3.3, the dendritic fields of the output neurons extend into the striosomes containing part of the nigrostriatal dopaminergic nerve endings. These connections provide a basis for a network in which the dopaminergic pars compacta cells are able to affect the activity of the caudate GABAergic output neurons.

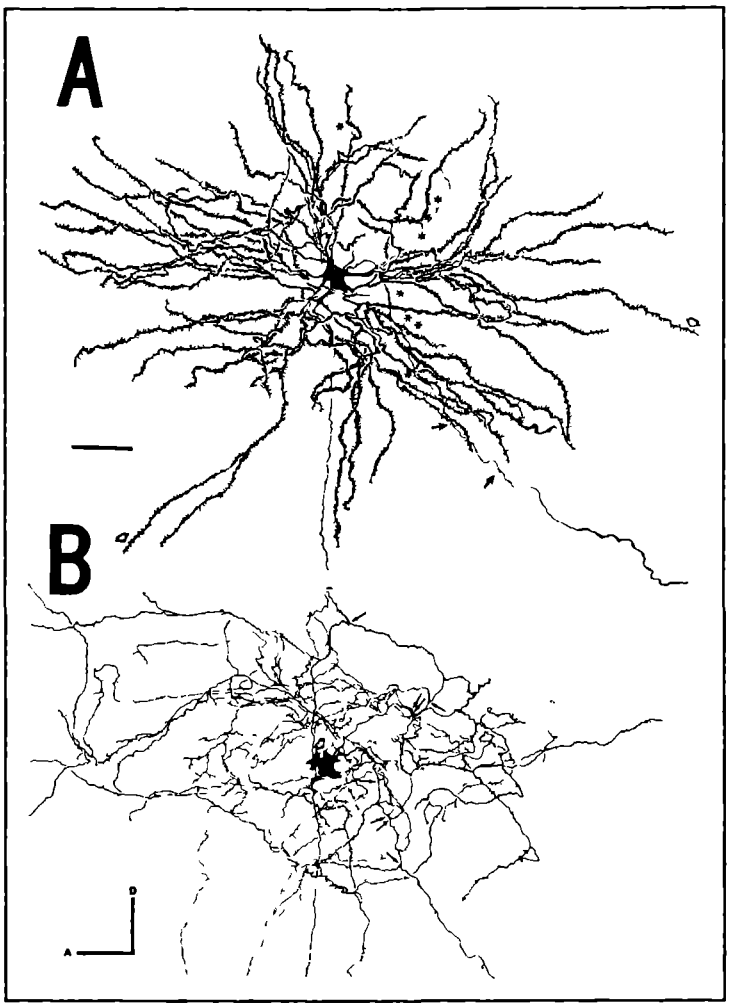

Figure 2.3.2 Reconstruction of a neostriatal projection neuron A: Complete somatodendritic morphology and partial illustration of the axon system. B: The axon-collateral plexus superimposed on the cell's dendritic field (stippled). Calibration bar in A is 40 μm. Anterior (a) and dorsal (d) orientations are indicated in B. (From: Preston, Bishop & Kitai, 1980; with permission).

The striatonigral fibres originating in the patch compartment, and those derived

from the matrix, project to distinct divisions of the substantia nigra (Figure 2.3.1). Cells, located in neostriatal patches (marked by dense concentrations of opiate receptors, or substance P- and leu-enkephalin-like immunoreactivity) project to the dopaminergic cells of the substantia nigra pars compacta, whereas neostriatal neurons located in the matrix provide input to the substantia nigra pars reticulata (Gerfen, 1985; Gerfen, Herkenham & Thibault, 1987; see Figure 2.3.1). The latter findings are consistent with the results reported by Gustafson and coworkers who used dopamine and cyclic AMP-

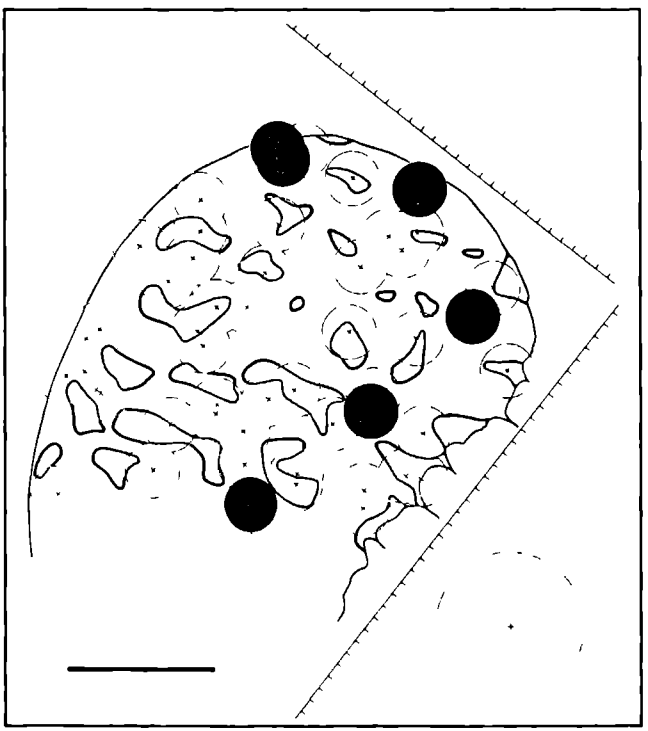

Figure 2.3.3 *Hypothetical distribution of caudate output neurons with their dendritic fields depicted as stippled circles The heavy contours show the actual location of the dopamine islands Dark circles show dendritic fields that did not overlap (about 10 % of randomly generated cell positions within the border of the caudate nucleus (From: Graybiel, 1984; with permission).*

regulated phosphoprotein (DARPP-32 which labels neostriatal dopaminoceptive neurons and their projections) and tyrosine hydroxylase (which labels dopamine synthesizing neurons) immunoreactivity (Gustafson, Ouimet & Greengard, 1989). Finally, Desban and coworkers were able to show in the cat that the caudate matrix, as defined by its dense AChE-labelling, indeed is the source of the projection to the substantia nigra pars reticulata by using a combination of autoradiography and acetylcholinesterase staining (Desban et al., 1989).

2.4 FELINE CAUDATE NUCLEUS: FUNCTIONAL IMPLICATIONS

In 1976, Cools and coworkers described two subregions within the caudate nucleus of the cat (Cools & Janssen 1976; Cools, Struyker Boudier & Van Rossum, 1976). Both regions were characterized on the basis of functional and pharmacological features: the so-called caput nuclei caudati rostromedialis (CRM region) located in the rostromedial part of the caudate nucleus and the extra-CRM region of the caudate nucleus (rCRM

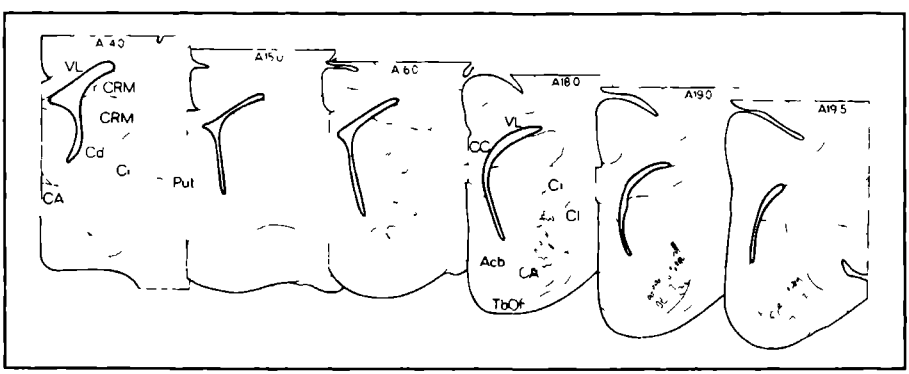

Figure 2.4.1 Semi-diagrammatic outline of the functionally and pharmacologically distinct rostromedial part of the caudate nucleus (CRM: shaded area) and anterodorsal part (rCRM: open area) of the caudate nucleus (From: Cools, Struyker Boudier & van Rossum, 1976; with permission).

region), which dominates the anterodorsal part of the caudate (Figure 2.4.1). Each region is characterized by its own dopamine receptor type. The CRM region contains so-called DAe receptors which are selectively stimulated by apomorphine and selectively inhibited by low doses of butyrophenones such as haloperidol. In contrast, the rCRM region contains so-called DA_1 receptors which are selectively activated by (3,4-dihydroxy-phenylamino)-2-imidazoline (DPI) and selectively inhibited by ergometrine and piribedil. Activation of DA_2 receptors produces characteristic abnormal head movements, whereas stimulation of DAi receptors induces orofacial dyskinetic movements (Cools, Struyker Boudier & Van Rossum, 1976; Cools et al., 1989). In rats,

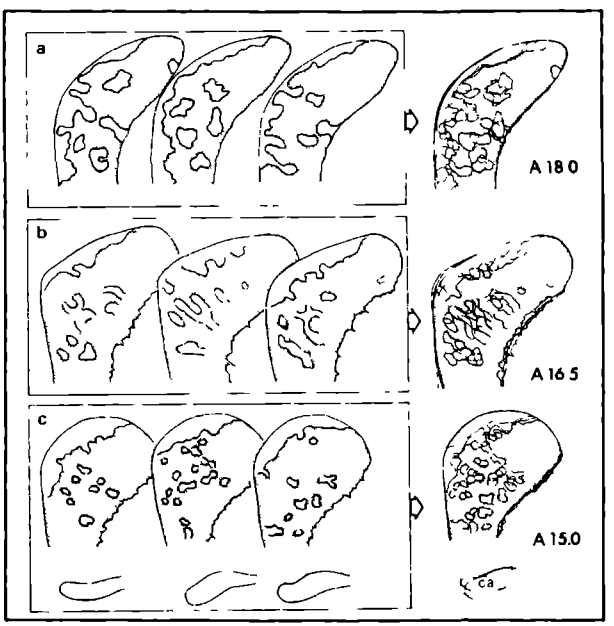

Figure 2.4.2 Schematic localization of the striosomes (white areas) and matrix (dotted areas; AChE staining) zones on rostral (A) medial (B) and caudal (C) frontal sections of the caudate nucleus in three different animals. The superimposition of the individual localizations of main striosomal areas in a given frontal plane is represented on the right part of the figure. ca = anterior commissure.(From Desban et al., 1989; with permission).

dopamine may also act differentially in distinct neostriatal regions as suggested by the finding that systemic administration of apomorphine produces regional differences in activity in the neostriatum. For example, apomorphine decreases glucose utilization in dorsomedial regions, but increases glucose utilization in the ventromedial region, but it does not change metabolism in other neostriatal regions (Brown, Wolfson & Feldman, 1987). The detailed study of Desban and colleagues (1989) makes it possible to directly compare the functionally and pharmacologically distinct regions as defined by Cools and coworkers on the one hand, and the biochemical compartmentalization as found by AChE-staining on the other hand (see Figures 2.4.1 and 2.4.2). The rostromedial CRM region appears to be dominated by AChE-poor striosomes, whereas the anterodorsal rCRM region appears to be dominated by the AChE-rich matrix compartment. In view of the notion that the striosomes are selectively innervated by the densocellular zone of the substantia nigra pars compacta (see Section 2.3), it seems likely that the rostromedial CRM area of the caudate nucleus is directly controlled by these dopaminergic nigrostriatal fibres. On the other hand, data reported by Desban and coworkers suggest that this part of the caudate nucleus selectively projects to the caudal (lateral) part of the substantia nigra pars reticulata (Desban et al., 1989). In contrast, the anterodorsal rCRM area, in which the matrix zone is most prominent, seems to be directly controlled by the dopaminergic fibres originating in the retrorubral area (A8) and ventral tegmental area (A10) (see Section 2.3).

CHAPTER 3

CAUDATE NUCLEUS AND THE PROGRAMMING OF MOTOR BEHAVIOUR IN CATS

3.0 GENERAL INTRODUCTION

Recent neurobehavioural studies have revealed that the caudate nucleus is involved in programming social behaviour in monkeys (Van den Bercken & Cools, 1982). Cholinergic agents injected into the caudate nucleus of Java monkeys living in a social group have been found to produce changes in the balance between social behaviour directed by the treated monkey and social behaviour directed by the partners of the treated monkey. Moreover, additional experiments in rats have indicated that the neostriatum determines the degree in which the animal itself directs its own behaviour; the experimentally-induced changes in behaviour which was directed by exteroceptive stimuli turned out to be the consequence of the former effect. This was demonstrated in experiments in which rats were forced to switch behavioural strategies in a so-called 'swimming without escape' test. Intrastriatal injections of the dopaminergic antagonist haloperidol, for example, reduced the programming of behaviour strategies which were not directed by external stimuli. In contrast, haloperidol did not attenuate the programming of behaviour strategies which were directed by external stimuli. Further, the dopamine agonist apomorphine increased the ability to programme behaviour strategies which were not directed by external stimuli. According to these studies the rat neostriatum and the monkey caudate nucleus, respectively, appear to be selectively involved in the process of ordering and sequencing behaviour that is not directed by exteroceptive stimuli. This conclusion holds true for social behaviour repertoires of Java

monkeys and behaviour strategies of rats. In man, a deficient dopaminergic function at the level of the caudate nucleus also results in analogous deficits. Patients suffering from Parkinson's disease, for example, show a reduced ability to switch behavioural programmes without the help of external information. This "shifting aptitude disorder" is manifested at the level of both shifting motor pattern sequences as well as at the level of switching problem-solving strategies in a cognitive task (Cools et al., 1984).

Because the caudate nucleus is known to be involved in motor behaviour, the question arises as to whether the caudate's role in the ability to shift behaviours not directed by exteroceptive stimuli also extends to the programming of motor behaviour in animals. During the past decade many workers postulated that the caudate nucleus is an important substrate for programming complex motor behaviour (Cools, Lohman & Van den Bercken, 1977; Olmstead et al., 1976; Schmidt, 1983; Teitelbaum, Schallert & Whishaw, 1983). In Section 3.1 data are presented showing that inhibition of caudate (rostromedial part) dopamine receptors selectively reduces the ability to switch motor patterns which are not directed by exteroceptive stimuli.

Apart from the mesostriatal dopaminergic innervation, the caudate nucleus receives its principal afferents from the thalamus and the neocortex (see Chapter 2). The most extensive projection originates in the cortex. It is generally accepted that this input, which is derived from almost all parts of the neocortex (see Chapter 2; see also Arikuni & Kubota, 1986; Graybiel & Ragsdale, 1979; Jinnai & Matsuda, 1979; McGeorge & Faull, 1987) is excitatory and glutamatergic (Carter, 1982; Cotman et al.,1987; Fonnum, 1984; Hassler et al.,1982; Kerkerian, Nieoullon & Dusticier, 1983; Nieoullon & Dusticier, 1983; Spencer, 1986; Updyke & Lyles, 1987; Young, Bromberg & Penney jr., 1981). In Section 3.2 data are presented showing that stimulation of caudate (rostromedial part) glutamate receptors with help of the quisqualate receptor agonist dl-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) enhances switching motor patterns in a receptor-specific way. Finally, the interaction between dopamine and glutamate with respect to the animal's ability to switch motor patterns is studied in Section 3.3. This section presents data showing that apomorphine prevents

only the AMPA-induced increase in switching behaviour, but not the AMPA-induced incorrect limb placing movements. The data are discussed in view of the known importance of the caudato-nigro-collicular pathway in switching behaviour. Further, the data suggest that the dopaminergic modulation of glutamate activity within the rostromedial caudate nucleus is restricted to functional changes mediated by quisqualate receptors.

Summary

Cats were trained to walk on a specially designed treadmill: the cats were able to collect food pellets by switching motor patterns with or without the help of exteroceptive stimuli inherent to the treadmill. To study the involvement of the rostromedial part of the caudate nucleus in switching motor patterns cats received bilateral intracaudate injections of haloperidol. In addition, in a final series of experiments, EMG recordings of two antagonistic muscles, together with recordings of characteristic changes in the length of one muscle, were made before and after the haloperidol treatment. Haloperidol resulted in a decreased number of motor patterns which were not directed by exteroceptive stimuli (non-exteroceptively directed motor patterns). This haloperidol-induced effect was dose-dependently counteracted by the additional intracaudate injections of apomorphine which *per se* remained ineffective.

Haloperidol neither affected the number of food collecting attempts nor reduced the number of exteroceptively directed motor patterns. Furthermore, haloperidol did not affect the capacity to switch to proprioceptively directed motor patterns. Finally, haloperidol did not produce abnormalities in EMG and length signals recorded from hindlimb muscles.

It is concluded that haloperidol selectively reduced the animal's capacity to 'programme non-stimulus directed motor behaviour'. The data are discussed in view of their significance for therapy of patients with basal ganglia disorders, such as patients suffering from Parkinson's Disease.

3.1.1 INTRODUCTION

The purpose of the present study is twofold. First, it describes a new experimental approach to study alterations in the ability of cats to switch to motor patterns which

are not directed by exteroceptive stimuli. And second, it provides evidence that the caudate nucleus, especially the dopaminergic neurotransmission in the rostromedial part, is indeed involved in programming the ordering and sequencing of motor patterns which are not directed by exteroceptive stimuli. Switching motor patterns was investigated in cats trained to walk on a specially designed treadmill: the cats were able to collect food pellets by switching motor patterns with or without the help of exteroceptive stimuli inherent to the treadmill. To study the involvement of the rostromedial caudate nucleus in programming motor patterns cats received intracaudate bilateral injections of an agent known to temporarily produce a dysfunctioning of this structure. For this purpose the dopaminergic antagonist haloperidol was chosen in view of its known efficacy to selectively inhibit the transmission of information from the dopaminergic, nigrostriatal fibres towards their corresponding postsynaptic receptors within the rostromedial part of the caudate nucleus (for ref. see Cools, Struyker Boudier & Van Rossum, 1976). The ability of the dopaminergic agonist apomorphine to counteract the haloperidol-induced effect was studied in order to establish the dopaminergic nature of the latter effect. In a final series of experiments, EMG recordings of two antagonistic muscles, together with recordings of characteristic changes in the length of one muscle, were made before and after the haloperidol treatment of unrestrained cats walking on the treadmill in order to detect the possible occurrence of motor deficits.

3.1.2 EXPERIMENTAL PROCEDURES

Apparatus

Studies on motor behaviour of cats have shown that locomotor patterns including changes in gait and/or coordination of limbs can be studied in cats walking on a motor-driven treadmill. For the purpose of the present study a special apparatus was designed. A motor-driven treadmill of 120 cm length and 20 cm width was used; the treadmill was placed in a perspex enclosure (120 x 20 x 65 cm; see Figure 3.1.1). One long wall was made of transparent perspex to enable the recording of motor patterns

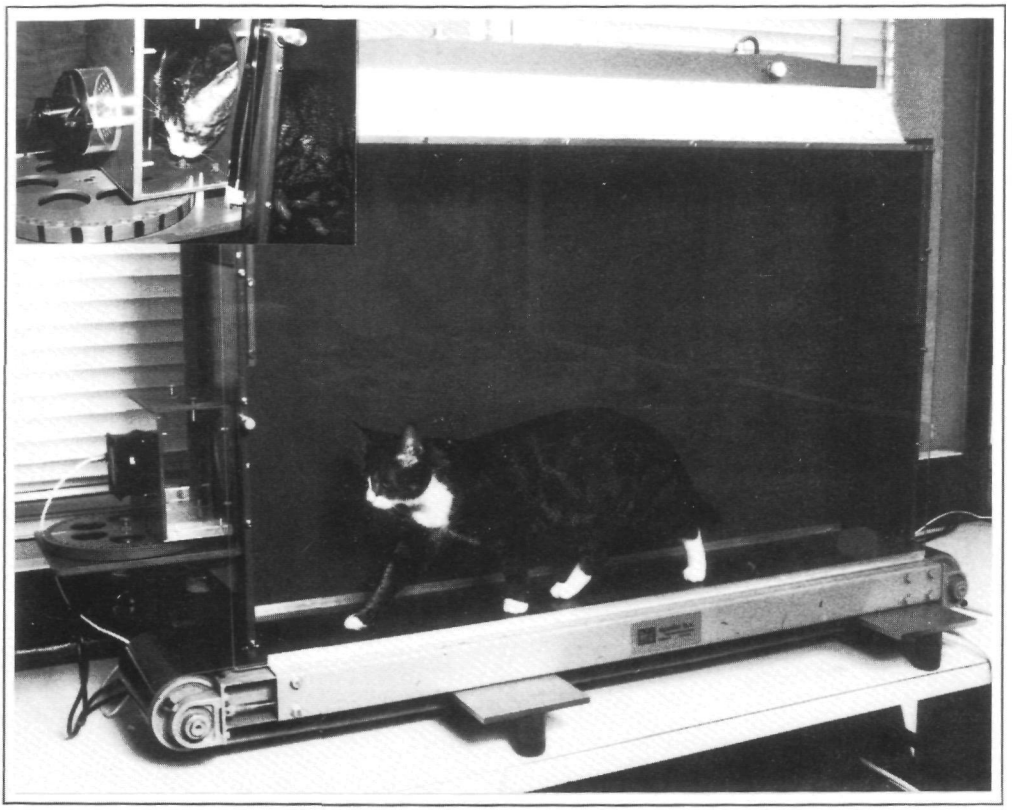

Figure 3.1.1 *Treadmill and food dispenser (inset). For details, see text.*

by means of a closed video circuit. The wall in front of the cat's head contained a window (10 cm width, 12 cm hight at a distance of 18 cm from the bottom). A food dispenser was mounted below the window at the back of the front panel (see Figure 3.1.1, inset). The food dispenser was placed in a box fitted with a ventilator (System PAPST type 900) to remove odour inherent to the food (specially shaped pellets; Hope Farms) and to produce a constant background noise. The rater manually directed the motor-driven food dispenser which delivered one pellet per time. In general, the treadmill was designed in such a way that the stepping cat was unable to see, smell or

note the delivery of a food pellet as long as the cat was stepping in the middle of the treadmill. Thus, switching from stepping to collecting food could never be directed by exteroceptive stimuli inherent to the delivery of the pellet.

Animals

Male and female cats weighing 2.5-4.5 kg were selected according to their stepping performance on the treadmill (1.0-1.25 km/ hr). Next, only cooperative cats were trained to reach the criterion, i.e. walking in the middle of the treadmill. During that period, the cats were rewarded initially by pellets offered manually through the window and later on, by pellets delivered by the food dispenser. The training was stopped as soon as the cat was able to collect food pellets from the food dispenser. Only cats which collected food in a random manner at random time-intervals were included in the present study.

After the training cats were anaesthetized with sodium pentobarbitone (40-50 mg/kg) and stereotaxically equipped with stainless steel cannulas (outer diameter 0.8 mm; outer diameter of the inner cannula that extended 1 mm below the tip of the outer cannula: 0.55 mm) into the rostromedial part of the caudate nucleus. The coordinates were: A 15.0, L 5.0 and H 5.0 (Snider & Niemer, 1964). For further details of the method, see Cools, Struyker Boudier & Van Rossum, 1976). In a subgroup of six cats, EMG electrodes were also chronically implanted into the lateral gastrocnemius and anterior tibial muscles. The connecting cables were passed subcutaneously to the head and their sockets were embedded in acrylic cement. Additionally, metal pins were inserted percutaneously into the cat's calcaneus and head of tibia to allow attachment of a mercury-in-rubber length gauge during recording sessions. EMG and length signals were transmitted with the help of two battery-driven FM transmitters to FM receivers, decoded, amplified and recorded on magnetic tape (for further details of method, see Prochazka, Stevens & Wand, 1979). After two weeks, the animals were retested on the treadmill to check their recovery; none of the cats had to be discarded.

Motor behaviour on the treadmill

In general, cats which were trained on the treadmill displayed one or more of the following motor patterns.

A) Walking. The cat simply walked with constant speed somewhere in the middle of the treadmill. Thus, the cat maintained its ongoing motor program.

B) Gait accelerations, type 1. The cat visually fixated a particular part of the window or treadmill and approached the window by accelerating its gait, thereby continuously fixating its originally selected target. Thus, the cat switched motor patterns thereby continuously matching stimuli inherent to the treadmill. These motor patterns are denoted as 'exteroceptively directed gait accelerations'.

C) Gait accelerations, type 2. The cat accelerated its gait without visual fixating anything which could serve as an exteroceptive stimulus. Thus, the cat switched to motor patterns which were not directed by exteroceptive stimuli. These motor patterns are denoted as 'non-exteroceptively directed gait accelerations'.

D) Gait transitions, type 1. The cat visually fixated the belt immediately in front of its forelegs and/or tactually fixated the front panel by making contact with its forelegs, thereby altering its interlimb coordination by decreasing the steplength of the forelegs and increasing the steplength of the hindlegs. Thus, the cat switched motor patterns, thereby continuously matching exteroceptive stimuli inherent to the treadmill. These motor patterns are denoted as 'exteroceptively directed gait transitions'.

E) Gait transitions, type 2. The cat altered its interlimb coordination without visually or tactually fixating any observable, exteroceptive stimulus. Thus, the cat switched motor patterns which were not directed by exteroceptive stimuli. These motor patterns are denoted as 'non-exteroceptively directed gait transitions'.

F) Eating behaviour. Once the cat had approached the window, the animal bent its head through the window in order to collect a pellet. These motor patterns are denoted as 'food collecting attempts'.

Cats which performed a whole sequence as soon as they were confronted with a running belt or the back panel were discarded in order to exclude animals using conditioned stimuli for directing their motor patterns.

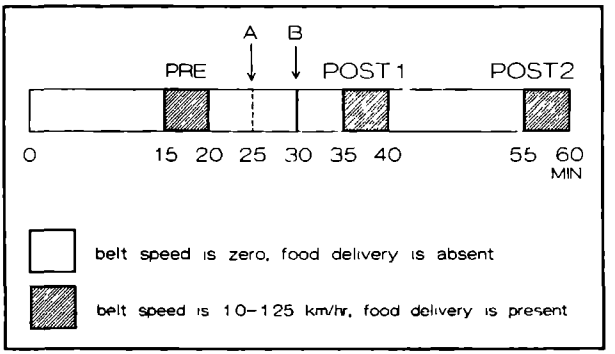

Figure 3.1.2 Experimental paradigm. A: point of injection time of haloperidol in case apomorphine is given at B. B: point of injection time in case a single drug is given. PRE, pre-injection test period. POST, post-injection test periods.

Design

Twenty-four hours prior to the experiments the cats were food deprived. Each experiment consisted of three walking periods, each lasting 5 minutes (see Figure 3.1.2). The first walking period served as the control period. The second and third walking periods, i.e. 5 and 25 min after intracaudate injections of solvent, viz. distilled water (control), haloperidol (Haldol, Janssen Pharmaceutica) or apomorphine hydrochloride (Brocades), served as post-injection periods 1 and 2, respectively (POST 1 and POST 2 in Figure 3.1.2). According to former experiments, in which open-field behaviour was analyzed, haloperidol and apomorphine are effective, dopamine-specific and locus-specific in a dose of 12.5 μg/5.0 μl and 0.6 μg/5.0 μl, respectively (for ref. see Cools, Struyker Boudier & Van Rossum, 1975, 1976). The doses were freshly prepared immediately before the injections were given. Dopamine specificity of the observed effects was tested in an additional series of experiments in which haloperidol was injected 5 minutes prior to apomorphine. All substances were bilaterally injected in a volume of 5.0 μl with a Hamilton syringe (outer diameter of injection needle: 0.4 mm) extending 2 mm below the tip of the embedded guide cannula.

The motor patterns on the running belt were recorded on videotape by means of a closed video-circuit, and subsequently analyzed. The number of each distinct type of motor pattern was counted. Apart from the fact that a number of experiments were analyzed independently by two observers, the remainder of the experiments was analyzed by a single observer after having reached a sufficiently high inter-rater reliability. Pre-injection scores were expressed as the absolute number of observed motor patterns. In contrast, the post-injection scores were expressed as percentage of the amount of motor patterns observed during the pre-injection period; in this manner it became possible to solve the problem of the rather large intra- and inter-individual variability (see Table 3.1.1 in which both the median and range of the pre-injection scores are presented). In case an animal failed to display a particular motor pattern during the pre-injection period, that animal was excluded from the evaluation of the drug-induced effects on that movement. The Mann-Whitney U-test was used for statistical analyses (two-tailed, unless otherwise indicated; Siegel, 1956). Group differences with $p<0.05$ were considered to be significant.

Given a drug-induced effect on motor behaviour, it is evident that such a change might be attributed to a motor deficit. For this purpose, EMG and length signals were recorded before and after the treatment which produced such a change, i.e. haloperidol injections.

In general, cats were used maximally five times with a minimum interval of one week between the trials. After finishing the experiments the cats were deeply anaesthetized with sodium pentobarbitone and perfused intracardially with a 4 % formaldehyde solution. The brains were removed and the target sites were verified according to previously described procedures (Cools, Struyker Boudier & Van Rossum, 1976).

3.1.3 RESULTS

Histological verification revealed that all injections were properly placed within the rostromedial part of the caudate nucleus; the coordinates found were: A 13.5-15.0, L 5.5-6.5 and H 5.5-7.0 (see Figures 2.4.3 and 4.1.3).

In general, the cats showed the following sequence during the test periods.
(1) Walking in the middle of the treadmill.
(2) Approaching the front panel by accelerating its gait in one of the two alternatives mentioned in the section Experimental Procedures (viz. non-exteroceptively or exteroceptively directed gait accelerations).
(3) Adopting and maintaining a position as close as possible to the front panel by altering its interlimb coordination in one of the two alternatives mentioned in the section Materials and Methods (viz. non-exteroceptively or exteroceptively directed gait transitions).
(4) Bending the head through the window in order to collect a food pellet.
(5) Collecting the food pellet.
(6) Either drifting backwards on the running belt in a passive manner, or decelerating its gait, meanwhile eating the collected pellet as quickly as possible.
It must be taken into account that the given description portrays a full-blown sequence; however, cats may also 'plug-in' at different stages of this sequence. Normally, the cats approached the front panel several times during one test period. Since the distinct motor patterns were differentially affected by the chosen treatments, the results will be separately presented.

Walking

All cats showed this behaviour before and after their treatment. the limb, head and body movements remained undisturbed by any treatment. None of the cats showed either incorrect adjustments of their body positions and postures on the running belt or abnormal postures and positions on the standing belt at any time. The absence of motor impairments was confirmed by recordings of hindlimb EMG and length signals

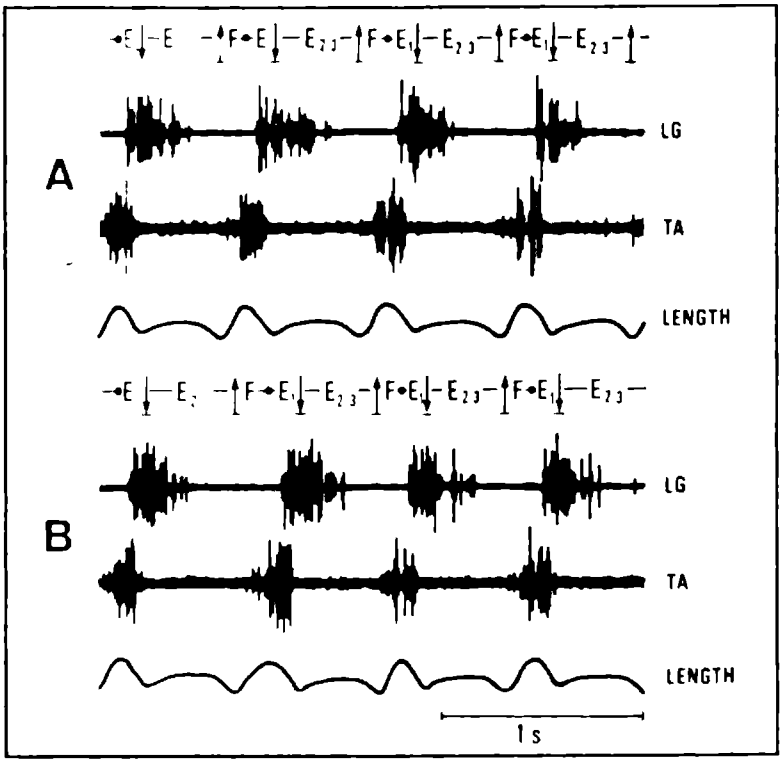

Figure 3.1.3 Four steps during unobstructed walking on a treadmill before (A) and after (B) the bilateral intracaudate injection of 12.5 µg haloperidol/5 µl. First trace: phases of step-cycle according to Philippson (1905). Second trace: lateral gastrocnemius (LG) EMG. Third trace: tibialis anterior (TA) EMG. Fourth trace: monitored length of ankle extensors.

in cats walking on the running belt (n=6). Since intracaudate haloperidol was the only effective treatment which altered the animal's ability to switch to motor patterns that are not directed by exteroceptive stimuli (see below), the EMG-implanted cats received haloperidol only. None of the six cats exhibited detectable changes of the innervation patterns of the recorded pair of muscles and of the muscle length after the chosen treatment. A typical recording is demonstrated in Figure 3.1.3.

Non-exteroceptively directed gait accelerations

Since the cats did not show a significant amount of this behaviour during the pre-injection period (Table 3.1.1), drug-induced changes could not be detected in this respect. However, it should be noted that the vast majority of the tested cats were able to show this behaviour (see Table 3.1.1).

Exteroceptively directed gait accelerations

The data are shown in Figure 3.1.4. This figure shows that the injection of solvent produced a small reduction of the pre-injection scores (15%). Neither haloperidol nor apomorphine produced effects different from those produced by the solvent. Also halo-

Table 3.1.1 Pre-injection median values + range (in parentheses) of gait accelerations (exteroceptively directed and non-exteroceptively directed), gait transitions (exteroceptively directed and non-exteroceptively directed) and food collecting attempts of all cats tested.

SLV, solvent (5 μl); HAL, haloperidol (12.5 μg/5 μl); A(0.3), apomorphine (0.3 μg/5 μl); A(0.6), apomorphine (0.6 μg/5 μl)

	SLV	HAL	A(0.3)	A(0.6)	HAL+ A(0.3)	HAL+ A(0.6)
non-exteroceptively directed gait accelerations	4 (1-15)	3 (1-5)	4 (2-8)	5 (1-14)	2 (1-5)	3 (1-8)
exteroceptively directed gait accelerations	11 (3-28)	15 (2-43)	9 (2-13)	10 (4-18)	18 (8-25)	9 (3-15)
non-exteroceptively directed gait transitions	9 (1-26)	10 (2-26)	17 (2-40)	9 (1-24)	23 (13-48)	22 (11-34)
exteroceptively directed gait transitions	13 (2-20)	7 (2-22)	10 (3-23)	7 (5-25)	5 (3-14)	6 (4-22)
food collecting attempts	34 (9-60)	22 (6-59)	42 (13-72)	35 (21-68)	26 (18-68)	43 (19-53)

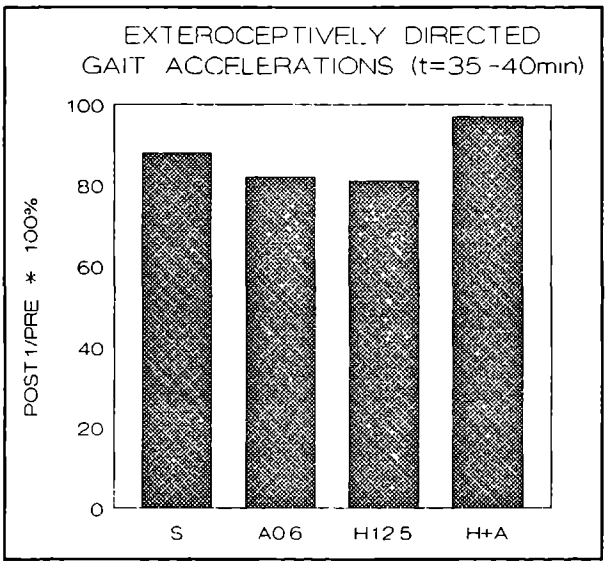

Figure 3.1.4 Median value of exteroceptively directed gait accelerations 5-10 min after bilateral intracaudate injection of solvent 5 µl (S), apomorphine 0.6 µg/5 µl (A0.6), haloperidol 12.5 µg/5 µl (H12.5) and haloperidol 12.5 µl/5 µl plus apomorphine 0.6 µg/5 µl (H+A).

peridol given 5 min prior to apomorphine did not produce any significant effect in this respect.

Non-exteroceptively directed gait transitions

This motor behaviour was strongly affected by haloperidol; moreover, this haloperidol effect was abolished in a dose-dependent manner by apomorphine which *per se* remained ineffective. As shown in Figure 3.1.5, the scores of the haloperidol-treated cats were significantly lower than those of the solvent-treated cats ($p < 0.02$). The latter haloperidol effect was abolished by 0.6 µg apomorphine, but not by 0.3 µg apomorphine (Figure 3.1.5). The time schedule for the latter experiment, in which haloperidol was given 5 min prior to apomorphine and, accordingly, 10 min prior to the first post-injection test period, differed from that for the former experiments, in which haloperi-

dol was given 5 min prior to the first post injection period. Accordingly, it became necessary to provide evidence that haloperidol was still effective during the chosen recording period in the combined haloperidol-apomorphine experiments. Therefore, data collected in the second post-injection period were also analyzed. As shown in Figure 3.1.6 haloperidol was still effective at that time; apomorphine also abolished this effect at that time.

Since the number of food collecting attempts was unaffected by haloperidol in comparison with solvent (see below), the observed decrease in the number of non-exteroceptively directed gait transitions allows the prediction that the number of

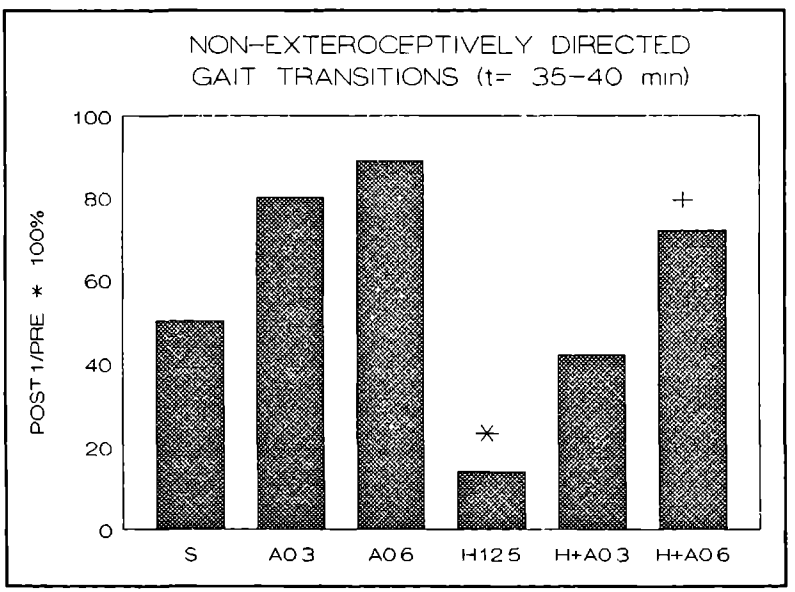

*Figure 3.1.5 Median value of non-exteroceptively directed gait transitions 5-10 min after bilateral intracaudate injections of solvent 5 µl (S), apomorphine 0 6 µg/5 µl (A0.6), haloperidol 12.5 µg/5 µl (H12.5) and haloperidol 12.5 µl/5 µl plus apomorphine 0 3 µg/5 µl or 0.6 µg/5 µl (H+A0.3 and H+A0.6, respectively). *, p<0.02, drug vs solvent. +, p<0 02, drug combination vs haloperidol (Mann Whitney U-test, two tailed).*

exteroceptively directed gait transitions, i.e. the only alternative to collect food, should be increased. For this reason, the one-tailed Mann Whitney U-test was used for statistical analysis in the latter case (next paragraph).

Exteroceptively directed gait transitions

This motor behaviour was also affected by haloperidol, although this effect was not abolished by apomorphine which perse remained ineffective. As shown in Figure 3.1.7 solvent produced a 55% reduction of the pre-injection scores. However, the post-injection scores of haloperidol-treated cats were significantly higher than those of the solvent-treated cats ($p<0.05$, one-tailed). The latter effect was not significantly counteracted by 0.6 μg apomorphine (Figure 3.1.7). Apomorphine itself remained without any effect (Figure 3.1.7).

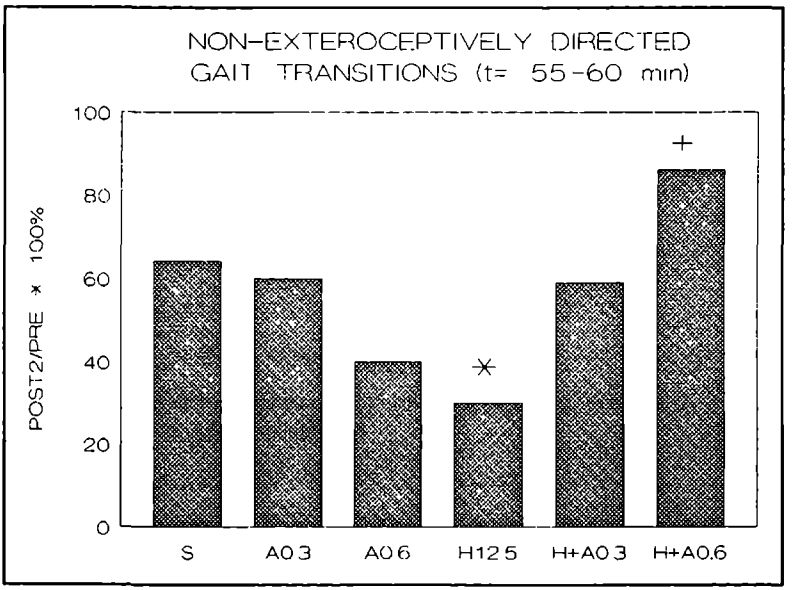

Figure 3.1.6 Median value of non-exteroceptively directed gait transitions 25-30 min after bilateral intracaudate injections. For abbreviations, see legend Figure 3.1.5.

Food collecting attempts

As shown in Table 3.1.2 none of the tested cats showed any significant difference during both post-injection test periods. However, the post-injection scores of all treated cats were about 50% lower than their corresponding pre-injection scores.

Miscellaneous

As mentioned in Section 3.1.2 (Experimental Procedures), cats which performed a whole sequence as soon as they were confronted with a running belt or the back panel were discarded in order to exclude animals using conditioned stimuli for directing their motor behaviour. On the basis of this criterion three haloperidol-treated cats were excluded from the above-mentioned analysis: these animals immediately performed a

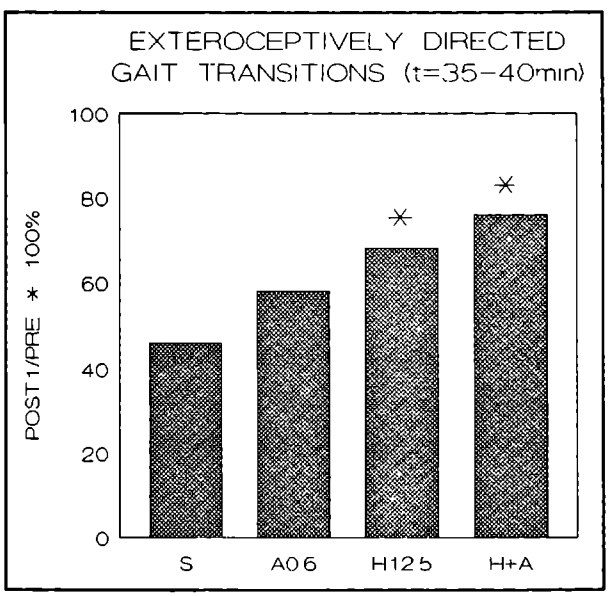

*Figure 3.1.7 Median value of exteroceptively directed gait transitions 5-10 min after bilateral intracaudate injections. For abbreviations, see legend Figure 3.1.4. *, $p<0.05$, drug or drug combination vs solvent (Mann Whitney U-test, one tailed).*

Table 3.1.2 Mean values (± SEM) of the ratio of post-injection scores (numerator) and pre-injection scores (denominator) of food collecting attempts The onset of post-injection period 1 (POST 1) and post-injection period 2 (POST 2) was, respectively, 5 and 25 min after intracaudate administration of various drugs.

The number in parentheses are the number of cats.

DRUG	POST 1	POST 2
Solvent	0.5 ± 0.3 (19)	0.6 ± 0.3 (18)
Haloperidol 12.5	0.4 ± 0.3 (10)	0.5 ± 0.3 (18)
Apomorphine 0.3	0.6 ± 0.4 (9)	0.6 ± 0.3 (9)
Apomorphine 0.6	0.6 ± 0.2 (9)	0.5 ± 0.2 (9)
Haloperidol 12.5 + Apomorphine 0.3	0.6 ± 0.2 (5)	0.5 ± 0.3 (5)
Haloperidol 12.5 + Apomorphine 0.6	0.7 ± 0.3 (7)	0.7 ± 0.3 (7)

whole sequence as soon as the standing belt started to run at the beginning of the first and second post-injection period. In contrast to the afore-mentioned haloperidol-treated cats, these cats had post-injection scores of non-exteroceptively directed gait transitions (median value 220%, n=3), that were significantly higher than those of the solvent-treated cats (median value 50%, n=19; $p<0.02$). Furthermore, these cats had post-injection scores of food collecting attempts (median value 100%, n=3) that were significantly higher than those of the solvent-treated animals (median value 50%, n=19; $p<0.02$).

3.1.4 DISCUSSION

Treadmill design

The present design makes it possible to distinguish motor patterns which are fully

dictated by exteroceptive stimuli (exteroceptively directed motor patterns) from motor patterns which are not dictated by these stimuli (non-exteroceptively directed motor patterns; see Section 3.1.2, Experimental Procedures). As described in the Section 3.1.3 (Results), cats display both exteroceptively and non-exteroceptively directed gait accelerations and/or transitions. Thus deficits due to a reduced capacity to switch to exteroceptively directed motor patterns can be differentiated from deficits due to a reduced capacity to switch to non-exteroceptively directed motor patterns.

In this context it is useful to recall that there are, in principle, various sources for directing motor behaviour: (a) exteroceptive stimuli, i.e. stimuli that are emitted by the physical environment of the organism and detected by sensory receptors for pressure, light, etc.; (b) proprioceptive stimuli, i.e. stimuli that are emitted by muscles, tendon organs and joints; they are detected by sensory receptors for the position of limbs and body, length of striate muscles, etc.; and (c) brain signals that are not dictated by any of the above-mentioned stimuli; to which degree these brain signals are anyhow deduced from the mentioned stimuli is still a matter of debate (Roland 1978, Teitelbaum, Schallert & Whishaw, 1983). Motor behaviours that are directed by mechanisms within the brain should be labelled as 'non-stimulus directed'. In the case of conditioning one must include here conditioned stimuli, i.e. stimuli that direct a particular chain of motor patterns as a consequence of a learning process. In this case one can label such a chain of motor patterns: 'stimulus-triggered'. In practice the patterning of motor behaviour is likely to be derived from more than one of these sources. First, cats execute exteroceptively directed gait accelerations and transitions; thus, deficits due to a reduced capacity to switch to exteroceptively directed motor patterns can be detected. Secondly, cats are permanently forced to adjust their postures and positions during walking; thus, deficits due to a reduced capacity to switch to proprioceptively directed motor patterns can be detected. Third, cats can show stimulus-triggered motor patterns. As shown in Section 3.2.3 (Results: Miscellaneous), certain haloperidol-treated cats immediately performed a whole sequence of motor patterns as soon as the belt started to run. Since the sudden change in exteroceptive stimuli inherent to the belt was sufficient to trigger the whole sequence, it is evident that

these cats showed a response that was conditioned by the available stimulus complex. The validity of the latter statement is underlined by the finding that these cats showed a significant increased number of food collecting attempts. Given this notion, the present design also allows the evaluation of a reduced capacity to switch to stimulus-triggered motor patterns. Fourth, cats with a normal capacity to switch to proprioceptively directed and/or stimulus-triggered motor patterns can still show a reduced capacity to switch to non-exteroceptively directed gait accelerations and transitions (see below, Caudate nucleus and programming motor behaviour). Since such a decrease can only be due to a reduced capacity to switch to motor patterns that are directed by brain signals, that are not dictated by exteroceptive and/or proprioceptive stimuli, such cats apparently suffer from a reduced capacity to switch to non-stimulus directed motor patterns. Thus, deficits due to a reduced capacity to switch to non-stimulus directed motor patterns can also be detected.

Apart from the mentioned advantages it is important to note the following. First, experimentally induced decreases in the number of a particular motor behaviour only reflect a reduced capacity to switch to that motor pattern in case the animals have the disposal of a normal capacity to execute that behaviour. In this context, it should be mentioned that only deprived cats display food collecting attempts; cats that are not deprived do not display such eating behaviour (data not shown). In other words, experimentally induced decreases only reflect a reduced capacity to switch to that motor pattern in case the state inherent to the food deprivation remains unaffected. Second, experimentally induced increases in the number of a particular motor behaviour simply reflect the increased degree in which the organism appeals to that motor pattern; they do not reflect an improved capacity to switch to that motor behaviour. Given the above-mentioned possibilities and restrictions, the present design creates the opportunity to study the neuronal circuitry underlying each distinct type of motor behaviour. According to our knowledge it is the first time that switching to 'non-stimulus directed motor programmes' can be studied in a quantitative manner.

Caudate nucleus and programming motor behaviour

Following intracaudate injections of solvent, a remarkable decrease in the number of non-exteroceptively and exteroceptively directed gait transitions was found: in both cases the reduction amounted to 50-55%. In view of the fact that caudate injections of solvent normally produce a very short-lasting effect (for ref., see Cools, Struyker Boudier & Van Rossum, 1976), it can be excluded that the noted decrease was due to the solvent injections, because this decrease was still present during the second post-injection period. Since all treatments produced comparable decreases during both post-injection periods, it is quite likely that the chosen procedure itself caused this phenomenon.

Intracaudate injections of haloperidol affected two out of the six different types of motor patterns. The number of non-exteroceptively directed gait transitions was significantly decreased, whereas the number of exteroceptively directed gait transitions was significantly increased. The haloperidol-induced decrease in the number of non-exteroceptively directed gait transitions was dose-dependently abolished by apomorphine that *per se* remained devoid of any significant effect. This finding showed that the haloperidol-induced decrease was dopamine-specific (see Section 3.1.1, Introduction); this fits in with earlier reported data about the potency of the chosen dose (12.5 μg) and volume (5 μl; see also Section 3.1.2, Experimental Procedures) to reduce the functional involvement of the dopamine neurotransmission. In contrast, the haloperidol-induced increase in the number of exteroceptively directed gait transitions was not attenuated by 0.6 μg apomorphine indicating that the latter effect was not dopamine-specific. This finding underlines the above-mentioned notion that drug-induced increases in the number of a particular motor pattern simply reflect the increased degree in which the organism applies to the capacity to switch to that motor pattern. Both sets of data together suggest that the haloperidol-induced increase in the number of exteroceptively directed gait transitions was due to alterations in brain processes different from the striatal, dopaminergic process.

Apomorphine neither increased nor decreased the number of any type of the motor

patterns studied. Since the present design is not suitable for the detection of the occurrence of an improved capacity to switch motor patterns (see above: Treadmill design) no definite conclusion can be drawn in this respect. On the other hand, it can be concluded that apomorphine does not reduce the capacity to switch motor patterns.

Considering the present data, it becomes evident that inhibiting caudate dopamine receptors by 12.5 μg haloperidol selectively reduces the number of non-exteroceptively directed gait transitions. This effect is highly specific in terms of programming distinct movements because of the following. First, haloperidol did not alter the number of food collecting attempts, indicating that haloperidol did not influence the state inherent to food deprivation. Second, haloperidol did not alter the number of exteroceptively directed gait accelerations, indicating that haloperidol did not influence the capacity to switch to exteroceptively directed motor patterns. Third, haloperidol did not affect the execution of proprioceptively directed motor patterns as shown by the absence of (a) incorrect adjustments of body postures and positions on the running belt, and (b) abnormal body postures and positions on the standing belt, indicating that haloperidol did not influence the capacity to switch to proprioceptively directed motor patterns. In this context, it is useful to recall data reported about the substantia nigra, pars reticulata (see Section 4.1; Wolfarth, Kolasiewicz & Sontag, 1981; Heim et al. 1986), i.e. one of the main output stations of the caudate nucleus (Chapter 2). Intranigral injections of picrotoxin have been found to produce both incorrect body postures and positions on a running belt and abnormal postures and positions on firm ground. From this point of view, it is important to realize that, in contrast to intranigral injections of picrotoxin, intracaudate injections of haloperidol did not produce these symptoms. Fourth, haloperidol did not produce abnormalities in EMG and length signals recorded from the hindleg muscles, providing additional evidence that haloperidol did not produce motor deficits *per se*.

On the basis of these data, it can be concluded that the chosen manipulation, intracaudate administered haloperidol (12.5 μg), decreases the number of non-exteroceptively directed motor patterns by selectively reducing the animal's capacity to

switch to non-stimulus directed motor patterns. It is proposed to denote this capacity as 'the ability to switch arbitrarily motor programmes'. The present study implies that the function of the caudate nucleus in programming non-stimulus directed behaviour is not limited to complex behaviour such as social interactions in Java monkeys or behavioral strategies in rats (see Section 3.0): this function also extends to motor behaviour *per se*. In other words, the caudate nucleus plays a crucial role in the cerebral organization of behaviour in its broadest sense, because it determines the animal's ability to switch arbitrarily behavioral programmes.

The clinical impact of the present findings is evident: patients in whom the dopaminergic activity in the basal ganglia is diminished, should also have an impaired ability to switch arbitrarily their behaviour. Indeed, it has recently been found that patients suffering from Parkinson's disease show a so-called 'shifting aptitude' disorder that manifests itself both at the motor level and at the cognitive level (Cools et al., 1984). Such patients are less able to switch motor, and even cognitive, programmes that are not directed by available, exteroceptive information. Additional evidence in this respect has been provided by others who have found that parkinsonian patients suffer from a deficient ability to shift set without external 'cues', compared to set-shifting with the help of exteroceptive information in eye-tracking tasks (Crawford, Henderson & Kennard, 1989; White et al., 1988), motor pattern tasks (Rogers & Chan, 1988; Benecke et al., 1987; Flowers, 1976), memory tasks (Helkala et al., 1988; Brown & Marsden, 1987) and cognitive tasks (Brown, 1989; Lees & Smith, 1983; Flowers & Robertson, 1985; Taylor, Saint-Cyr and Lang, 1986). In conclusion, the available data from animal and human studies together strongly suggest that the function of the caudate nucleus in programming behaviour arbitrarily is not limited to certain behavioral categories; the way in which disturbances in this universal capacity is manifested depends on the constraints of the test used.

As shown in the present animal study, an impaired ability to switch to non-exteroceptively directed motor patterns could be compensated by increasing the degree of switching to exteroceptively directed motor patterns. This finding opens the

perspective that 'learning to use exteroceptive and/or proprioceptive stimuli for directing behaviour' may be therapeutically effective in terms of compensating the reduced ability to switch arbitrarily behavioural programmes. Apart from numerous histories dealing with Parkinson patients, which spontaneously assess this principle, their exists no systematic study in this respect. Given the observations of Stern and colleagues (Stern, Lander & Lees, 1980) that certain patients with Parkinson's disease even create imaginary stimuli for that purpose, it is believed that the therapeutic approach mentioned may be a powerful supplement to the present day treatment of patients suffering from Parkinson's disease.

Summary

The effect of intracaudate (rostromedial part) injections of the glutamate agonist dl-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), viz. an agonist of quisqualate receptors, on switching behaviour was investigated. First, cats had to switch from hanging with the forepaws on the bar to climbing on the bar; then, they had to switch to walking; finally, they had to switch to jumping off the bar. AMPA induced limb deficits, i.e. unilateral incorrect or absent placing of the fore- and/or hindlimb, in part of the tested cats; in the remainder of the tested animals AMPA reduced climbing time. Limb deficits were prevented by the broad-spectrum glutamate antagonist kynurenic acid (KYN) and by the selective NMDA antagonist d-2-amino-7-phosphonoheptanoate.

In all cats AMPA increased the number of head movements as well as that of walking-restarts. These effects were counteracted only by KYN. These data show that part of the AMPA-induced effects were selectively mediated by quisqualate receptors. The present data are discussed in view of the role of the rostromedial caudate nucleus in switching behaviour.

3.2.1 INTRODUCTION

With respect to excitatory amino acids, at least three receptor subtypes have been postulated; they are labelled according to distinct agonists that bind preferentially to one subtype: the NMDA receptor, the quisqualate receptor and the kainate receptor (Cotman et al., 1987; Fagg, 1985; Foster & Fagg, 1984). Given the notion that glutamate is not an endogenous ligand for NMDA or kainate receptors (Fagg, 1985; Foster & Fagg, 1984; see also Olsen, Szamraj & Houser, 1987), it is likely that glutamate, released from corticocaudate fibres may activate quisqualate receptors. The

latter suggestion is underlined by two other sets of data. First, cortical stimulation induces excitatory responses of caudate neurons that are not mediated by NMDA receptors (Herrling, 1985). Second, intracaudate responses to cortical stimulation can be blocked by glutamate-diethyl-ester (Spencer, 1986), a substance that is reported to inhibit selectively quisqualate receptors (Foster & Fagg, 1984; Krogsgaard-Larsen, Honoré & Hansen, 1980; Krogsgaard-Larsen et al., 1982).

Behavioral studies investigating the role of glutamate neurotransmission at the level of the caudate nucleus are scarce. According to Schmidt and coworkers, injections of relatively high doses of the selective NMDA receptor antagonist dl-2-amino-5-phosphonovaleric acid in the rat neostriatum result in stereotyped movements resembling those following stimulation of dopamine receptors (Schmidt, 1986; Schmidt, Krähling & Ruhland, 1987). On the other hand, intrastriatal application of relatively low doses of the quisqualate-selective agonist dl-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA; see Krogsgaard-Larsen, Honoré & Hansen, 1980; Krogsgaard-Larsen et al., 1982) in rats tested in a 'forced-swimming-with-escape' test has been reported to reduce dose-dependently the number of animals being able to escape (Cools & Peeters, 1987). AMPA-treated rats went on with switching to different swimming behaviours. As shown by Cools and Peeters, the latter effect could be attenuated by pretreatment with the aselective glutamate antagonist kynurenic acid (KYN; see Stone & Connick, 1985; Turski, Herrling & Do, 1987) as well as by the selective NMDA receptor antagonist d-2-amino-7-phosphonoheptanoate (AP7; see Cotman et al., 1987; Fagg, 1985; Foster & Fagg, 1984).

In Section 3.1, it has been hypothesized that the rostromedial caudate nucleus is involved in the organism's ability to switch arbitrarily from one behavioral pattern to the next pattern, i.e. to switch to behavioral patterns which are not continuously directed by exteroceptive or proprioceptive stimuli (see also Wegener, Schmidt & Ehret, 1988). The reports of Schmidt (1986) and of Cools and Peeters (1987) suggest that excitatory amino acid neurotransmission processes may also be involved in the latter function. In order to investigate the role of rostromedial caudate quisqualate

receptors in switching motor patterns we have injected AMPA bilaterally into the caudate nucleus of cats. Drug effects were analyzed in a relatively simple paradigm, i.e. the 'bar-test', in which the animals had to switch to different motor patterns (see Section 3.2.2, Experimental Procedures).

Since there are no selective quisqualate receptor antagonists available, we performed two sets of experiments to investigate the receptor specificity of AMPA-induced behavioral changes: First, it was tested whether KYN, which is known to block all glutamate receptor subtypes (see above), was able to block AMPA-induced behavioral effects. In addition, the efficacy of the NMDA-selective antagonist AP7 in counteracting AMPA-induced effects was investigated in order to study the possible involvement of NMDA receptors in AMPA-induced effects.

3.2.2 EXPERIMENTAL PROCEDURES

Subjects

Male cats (weighing 3.5-5.0 kg) were selected from a laboratory breeding colony of the University of Nijmegen; they were 12-15 months old. The cats were housed in iron cages (1.9 x 1.2 x 1.6 m) in groups of 4-8 animals; food (Hope Farms) and water were available ad libitum.

Surgical procedures

Under sodium pentobarbitone anaesthesia (45 mg/kg i.p.) the animals were stereotaxically equipped with double-barrelled, stainless steel cannulas (outer diameter 0.8 mm; outer diameter of inner cannula which extended 1 mm below the tip of the outer cannula: 0.55 mm) into the rostromedial part of the caudate nucleus (coordinates [Snider & Niemer, 1964]: A 14.5, L 5.0 and H 5.0) according to previously described methods (see Section 3.1.3).

Apparatus and procedures

The cats were habituated during 4-6 sessions to the following procedures and set-up: the forepaws of the cat were placed on one end of a wooden bar (5 x 5 x 200 cm) situated 2 m above the ground while its hindlimbs were hanging freely. Next, the cat had to climb on the bar and, subsequently, to walk on the bar to the other end. At that end, the cat was able to jump down on a wooden platform (72 x 107 cm) 25 cm below the bar; at the platform, some food pellets were offered (Brekkies, Effem B.V., Veghel). Each session consisted of 3 test periods that were spaced by an interval of 15 min. During each test period, the cat had to execute 3 trials in a row; a trial consisted of climbing on the bar, walking towards the end of the bar and jumping off the bar. During training sessions, cats were also habituated to the injection procedure by inserting the injection needle into the cannulas without performing an actual injection. As soon as the cat was able to display complete trials during 3 successive test periods per session, training was stopped. One week later, the experiments were started. During an experiment, drug injections (volume 5.0 μl) were given bilaterally in the caudate nucleus of hand-fixed cats with help of a Hamilton syringe (diameter of the tip of the injection needle: 0.4 mm). Five min before drug-application, the first test period (PRE) was started. Five and 20 min after the end of the injections, the second and the third test period were started (POST1 and POST2, respectively). Each test period lasted maximally 5 min. This time-schedule was based on the outcome of pilot studies. All trials were recorded on video-tape with help of a closed video-circuit to allow subsequent detailed analysis. During recording, the cat was not able to see the experimenter; care was taken to avoid the occurrence of changes in the surroundings that could direct the behaviour of the animal. In experiments, in which the receptor specificity of AMPA-induced effects was analyzed, cats received 5 min prior to AMPA either AP7 or KYN (see below). The time-schedule of these experiments was the same as that of the experiments described above, except for the start of the PRE test period: this was started 5 min before the caudate nucleus injections of the AP7 or KYN.

Drugs

Cats (n=28) participated in maximally 5 experiments that were spaced by 1 week.

Most animals (n=21) received solvent (distilled water) during the first experiment, whereas the remainder of the cats (n=7) received 0.5 μg AMPA (gift from Dr. P. Krogsgaard-Larsen); during the second experiment, part of the solvent-treated cats received 1.0 μg (n=11) or 0.1 μg AMPA (n=7). During the next experiments, cats received one or more of the following treatments: KYN 1.0 μg (Sigma) plus AMPA 1.0 μg (n=12); KYN 1.0 μg (n=10); AP7 1.0 μg (gift from Dr. J. Scheel-Krüger) plus AMPA 1.0 μg (n=8); and AP7 1.0 μg (n=8). Whenever necessary, the pH of the solutions was adjusted to 7.

Dependent variables

1. Climbing (first phase). Climbing was considered to start as soon as the cat was released by the experimenter while it was hanging with its forepaws on the bar. Climbing was considered to be finished as soon as the animal reached a stable position on the bar. In case the animal was able to execute complete climbing, the time required to climb on the bar was expressed in seconds. Moreover, it was noted when the cat displayed unsuccessful climbing attempts or no hindlimb movements at all.

2. Head movements (second phase). Following climbing, the animals executed a variable number of normal, non-forced head, torso and body movements before they started to walk towards the other end of the bar. In practice, a torso or a body movement was always preceded by a head movement. Therefore, only the number of head movements was counted.

3. Walking (third phase). Walking was considered to start as soon the cat made its first step towards the other end of the bar. Walking was considered to be completed as soon as the cat jumped off the bar. In case the cats walked, it was analyzed how many times they interrupted this behaviour, viz. switched to other kinds of movements such as normal, non-forced head, torso and/or body movements, bar-scratching, licking the fur, etc.. In the present study, only the number of walking-restarts was taken into account. In case the cat did not start walking within 90 s, the trial was broken off.

4. Jumping (final phase). At the end of the bar, the cats could collect food pellets by jumping down on a platform. It was recorded whether they landed correctly on the

platform.

During each test period, three trials were executed. Accordingly, each variable was measured three times. At the end of each experiment, mean climbing-time during each test period was calculated per cat by averaging the three climbing-time scores obtained during that period. In order to obtain sufficient numbers per test period, the frequency parameters head movements and walking restarts were calculated for each cat as follows: at the end of each experiment, the total number of head movements and walking restarts were calculated by adding the three scores of each parameter obtained during that period. In order to reduce the interindividual variability, the ratio of the difference and the sum of the mean climbing-time, total number of head movements and total number of walking restarts during POST1 and PRE was computed (cf. Section 3.1.3): POST1-PRE/POST1+ PRE. The same ratio was computed for the measures obtained during the POST2 test period.

Statistics

The data were statistically analyzed with help of the Mann Whitney U-test, two tailed, unless otherwise mentioned (Siegel, 1956). Experimental groups were considered to differ significantly in case of $p<0.05$.

Histological verification

After the end of the final experiment, the cats were deeply anaesthetized with pentobarbital and perfused transcardially with a 4% formaldehyde solution. Subsequently, the brains were removed and cross-sections were cut with help of a cryostat (30 μm slices). The slices were stained with cresyl violet to allow the exact location of the injection loci.

3.2.3 RESULTS

Histology

All injections were placed in the rostromedial part of the caudate nucleus,

coordinates (Snider & Niemer, 1964) A 14.0-15.0, L 4.8-5.9 and H 4.4-5.4. A representative example of the injection sites is illustrated in Figure 3.2.1.

Control tests

During control experiments, in which the solvent was injected, all tested cats were able to execute complete trials in a normal way during both POST1 and POST2; their performance was comparable to that of the cats during PRE test periods of all experiments. Climbing was executed without any difficulty; the cats executed 2-3 head movements before they started normal walking; finally, the cats jumped on the platform without making any misplacement. Absolute values (median plus range) of climbing (duration), head movements (number) and walking restarts (number) during the PRE test period of all experiments are presented in Table 3.2.1.

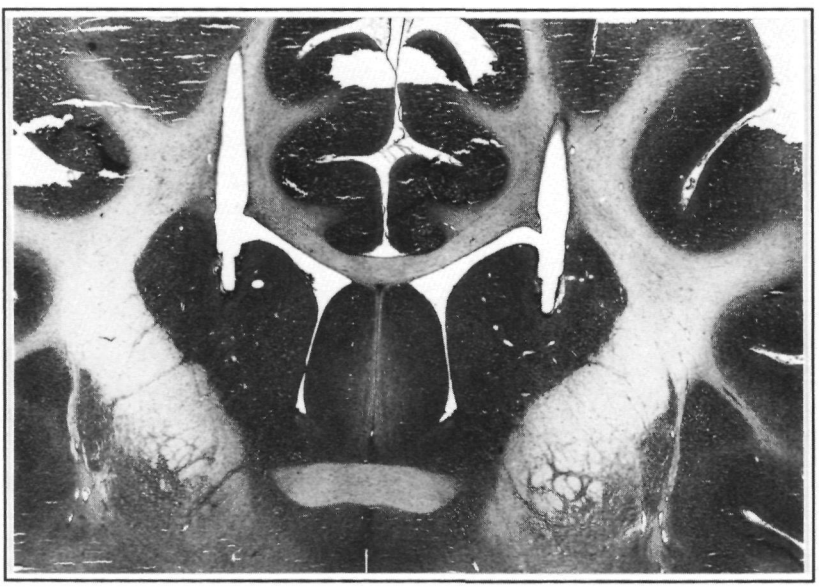

Figure 3.2.1 *Picture of a representative example of injection sites in the rostromedial part of the caudate nucleus.*

Table 3.2.1 Median (+ 25-75 % range) values of mean climbing-time, of total number of head movements and of total number of walking-restarts during pre-injection (PRE) tests of all experimental groups. SLV= solvent (distilled water); AMPA=dl-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid; KYN=kynurenic acid; AP7=d-2-amino-7-phosphonoheptanoate.

(n)=number of cats.

DRUG (μg/5.0 μl)	(n)	CLIMBING (s)	HEAD MOVEMENTS (n)	RESTARTS (n)
SLV	(21)	1.8 (1.7-2.7)	9 (3-15)	0 (0-4)
AMPA 0.1	(7)	1.7 (1.5-2.2)	9 (4-9)	1 (0-5)
AMPA 0.5	(7)	3.6 (2.7-4.5)	8 (2-10)	1 (1-3)
AMPA 1.0	(11)	2.1 (1.8-2.2)	5 (3-13)	0 (0-3)
KYN 1.0 +AMPA 1.0	(12)	1.6 (1.3-2.5)	5 (3-11)	1 (1-3)
AP7 1.0 +AMPA 1.0	(8)	1.8 (1.6-1.9)	6.5 (5-7)	0.5 (0-2)
KYN 1.0	(10)	1.7 (1.5-1.8)	9 (3-13)	0.5 (0-2)
AP7 1.0	(8)	1.7 (1.5-1.9)	4.5 (3-9)	0 (0-2)

In general, the AMPA-induced effects occurred only during POST1 test periods. Furthermore, AMPA-induced changes were not limited to either one of the three trials during POST1. Therefore, the description of the results given below accounts for the whole POST1 period.

AMPA-induced limb deficits

In contrast to 0.1 and 0.5 μg AMPA, a dose of 1.0 μg AMPA dramatically affected normal limb movements in the bar-test (Table 3.2.2). Abnormal limb movements were present in 6 out of 11 AMPA (1.0 μg)-treated cats. The limb deficits occurred only

unilaterally: both fore- and hindlimb could be affected. During climbing, these animals were able to retract only one hindlimb in a normal way; they did not succeed in placing the other hindlimb on the bar until they made at least several attempts. However, after some time, the cats ultimately succeeded in climbing on the bar (see below). While sitting or standing on the bar, often one fore- or hindlimb slowly slipped off the bar. While control cats immediately retracted a freely hanging limb, these AMPA-treated animals did not retract the affected limb; instead, that limb gradually slipped further down until it was completely extended. In case the upper part of that limb accidently touched the bar as a result of a body movement, the limb was retracted and correctly placed on the bar. During walking the affected fore- and/or hindlimb was occasionally

Table 3.2.2 *Percentage of animals showing unilateral limb deficits during POST1 tests: 5-10 min after the intracaudate injection of SLV, 1.0 μg AMPA, 1.0 μg KYN or 1.0 μg AP7 plus AMPA 1.0 μg (KYN and AP7 were injected 5 min before AMPA); 10-15 min after the injection of 1.0 μg KYN or 1.0 μg AP7.*

(n)=number of animals. For abbreviations see legend of Table 3.2.1.

DRUG (μg/5 μl)	ANIMALS WITH LIMB DEFICITS (n)	(%)
SLV	(21)	0
AMPA 0.1	(7)	0
AMPA 0.5	(7)	0
AMPA 1.0	(11)	55*
KYN 1.0 +AMPA 1.0	(12)	0+
AP7 1.0 +AMPA 1.0	(8)	0+
KYN 1.0	(10)	0
AP7 1.0	(8)	0

*$p<0.05$, AMPA vs. SLV.
+$p<0.05$, AMPA + KYN and AMPA + AP7 vs. AMPA
(Fisher exact probability test, two tailed).

placed alongside the bar. As a result, that limb again slipped down; often the cats made several stepping movements 'in the air' before they managed to correctly place the limb on the bar. Although the occurrence of limb deficits clearly affected the ability to walk on the bar, it did not affect the number of restarts (see below: walking). At the end of the bar, they always succeeded in jumping on the platform without making any misplacement. The occurrence of limb deficits was prevented by the pretreatment of KYN or AP7; the antagonists themselves did not induce motor disturbances when injected alone (Table 3.2.2).

Climbing

Climbing-time was not affected by 0.1 or 0.5 μg AMPA. However, the ratio of climbing-time of those cats that showed limb deficits after 1.0 μg AMPA was significantly different from that of the remainder of cats treated with this dose ($p=0.008$). The ratio of climbing-time of cats displaying limb abnormalities was significantly increased ($p<0.04$) compared to that of control animals (median climbing-time during POST1 of solvent- and AMPA-treated cats showing limb deficits: 2.3 and 3.1 s, respectively). In contrast, the ratio of climbing-time of cats lacking limb deficits after 1.0 μg AMPA was significantly reduced (see Figure 3.2.2). As shown in Figure 3.2.2, the latter decrease in climbing-time was significantly attenuated by pretreatment of KYN. In contrast, AP7 was unable to counteract this AMPA-induced effect; both KYN and AP7 were ineffective in this respect when tested alone.

Head movements

AMPA 0.1 or 0.5 μg did not affect the number of head movements. Cats showing limb deficits after 1.0 μg AMPA did not differ from those showing no deficits after this dose ($p>0.05$). For that reason, both subgroups were pooled. As shown in Figure 3.2.3, the ratio of the number of head movements of both subgroups together was significantly increased compared to that of control cats. In contrast to AP7, KYN was able to reduce this AMPA-induced increase. Both AP7 and KYN did not affect the number of head movements when tested alone (see Figure 3.2.3).

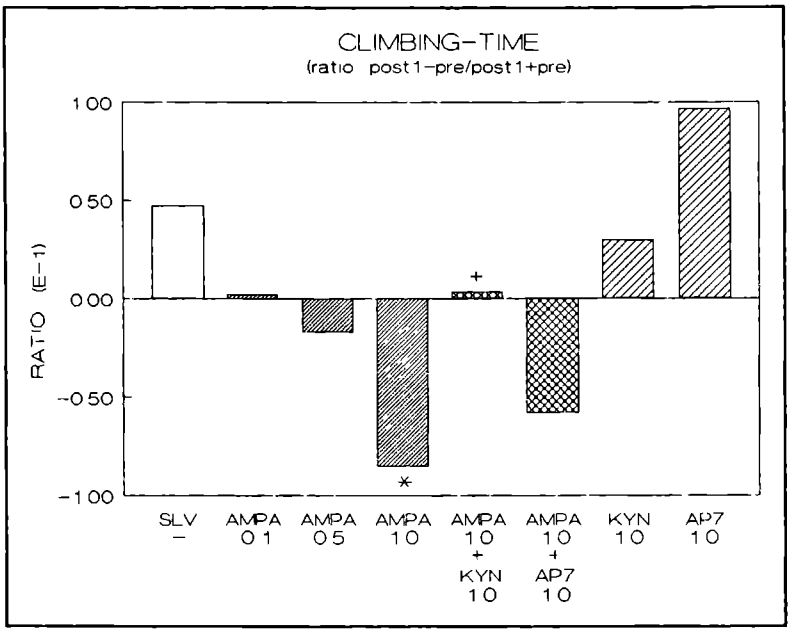

*Figure 3.2.2 Ratio of the mean climbing-time (POST1-PRE / POST1+PRE) during POST1: 5-10 min after solvent (SLV), 0.1, 0.5 or 1.0 μg AMPA, 1.0 μg KYN or 1.0 μg AP7 plus 1.0 μg AMPA (KYN and AP7 were injected 5 min before AMPA); 10-15 min after 1.0 μg KYN or 1.0 μg AP7. For abbreviations see legend of Table 3.2.1. * $p<0.02$: drug vs. SLV. + $p<0.05$: 1 0 μg AMPA plus Kyn vs. 1.0 μg AMPA.*

Walking

After the intracaudate application of AMPA 0.1 μg, 6 out of 7 tested cats did execute normal walking; the remaining cat did not walk at all. This dose did neither affect the number of restarts (see Figure 3.2.4). AMPA 0.5 μg did neither affect the ability to execute normal walking. As illustrated in Figure 3.2.4, the number of restarts was significantly enhanced following 0.5 μg AMPA. After 1.0 μg AMPA, 10 out of 11 tested cats did execute walking during POST1. Interestingly, 6 of these cats showed limb deficits as described above (see: AMPA-induced limb deficits). Apparently, the occurrence of these deficits did not prevent these cats to walk on the bar. Cats

displaying limb deficits following 1.0 μg AMPA required significantly more time for walking (after subtracting the time when walking was interrupted) than the remaining animals of this test group (5.4 vs. 3.3 s: $p=0.01$). However, both subgroups did not differ with respect to the number of restarts ($p>0.05$); therefore, they were pooled for the subsequent analysis: as shown in Figure 3.2.4, 1.0 μg AMPA significantly enhanced the number of restarts. KYN significantly attenuated the AMPA-induced increase in spite of the finding that KYN itself also enhanced the number of restarts. The KYN-induced increase was also counteracted by AMPA. Walking was not interrupted by specific behaviours; before restarting cats could execute a variety of different behaviours

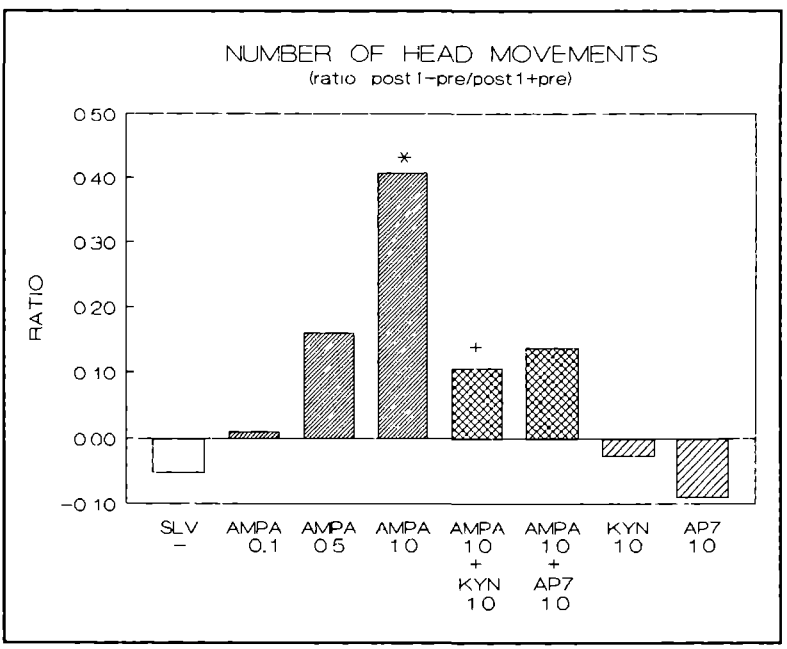

Figure 3.2.3 *Ratio of the total number of head movements (POST1-PRE / POST1+PRE) during POST1: 5-10 min after solvent (SLV), 0.1, 0.5 or 1.0 μg AMPA, 1.0 μg KYN or 1.0 μg AP7 plus 1.0 μg AMPA (KYN and AP7 were injected 5 min before AMPA); 10-15 min after 1.0 μg KYN or 1.0 μg AP7. For abbreviations see legend of Table 3.2.1. * $p<0.002$: drug vs. SLV. + $p<0.02$: 1.0 μg AMPA plus KYN vs. 1.0 μg AMPA.*

such as non-forced head, torso and/or body movements, licking the fur or the forelimbs, scratching the bar, etc.; sometimes they made no movement at all. In contrast to KYN, AP7 was unable to reduce the AMPA-induced increase; moreover, this antagonist was ineffective in changing the number of restarts when tested alone (see Figure 3.2.4).

Jumping

All tested cats except from those (n=2) that did not walk at all (see above) were able to jump down on the platform without making any misplacement. In addition, these cats always collected the food pellets offered on the platform.

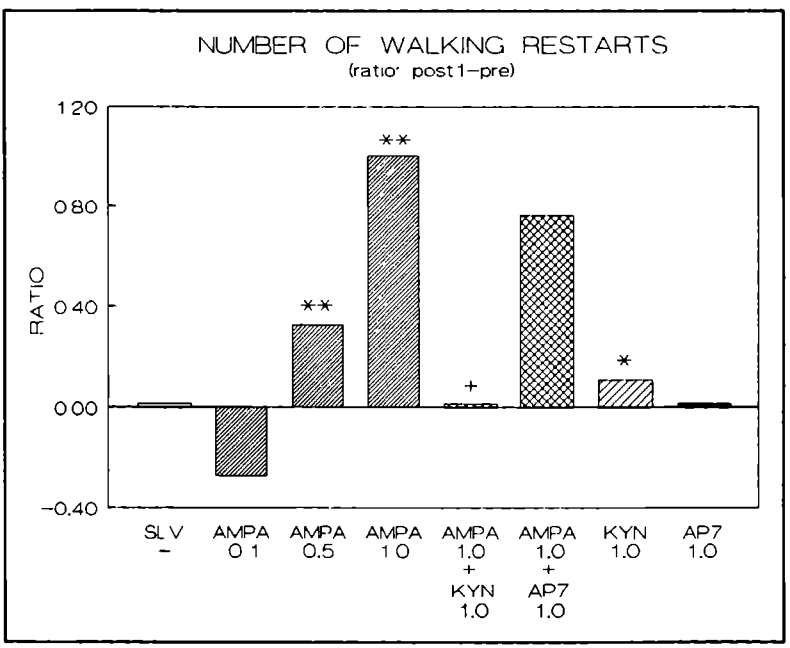

*Figure 3.2.4 Ratio of the total number of walking-restarts (POST1-PRE) during POST1: 5-10 min after solvent (SLV), 0.1, 0.5 or 1.0 μg AMPA, 1.0 μg KYN or 1.0 μg AP7 plus 1.0 μg AMPA (KYN and AP7 were injected 5 min before AMPA); 10-15 min after 1.0 μg KYN or 1.0 μg AP7. For abbreviations see legend of Table I. * $p<0.05$, ** $p<0.02$: drug vs. SLV. + $p<0.002$: 1.0 μg AMPA plus Kyn vs. 1.0 μg AMPA.*

3.2.4 DISCUSSION

The present results show that the selective quisqualate receptor agonist AMPA elicited two distinct sets of effects:

1. The highest dose of AMPA tested, i.e. 1.0 μg, produced unilateral motor disturbances in fore- and hindlimbs. In contrast to other AMPA-induced effects (see below), these limb deficits could be prevented by KYN as well as by AP7. These findings confirm those of others who also found that certain AMPA-induced behavioral changes were blocked by AP7 (Cools & Peeters, 1987). These data show that both subtypes of glutamate receptors were involved in the display of limb deficits. Whether or not these subtypes function independently of each other remains open for future research (c.f. Cotman et al., 1987). This does not hold true for the second set of AMPA-induced effects: as discussed below, they were apparently mediated by only one subtype of glutamate receptors, viz. the quisqualate receptor. Therefore, it appears that the AMPA-induced limb deficits were mediated by another mechanism than the remaining AMPA effects. Below, we will return to this topic.

2. Cats that were not hampered by any obvious limb deficit following caudate nucleus injection of 1.0 μg AMPA required less time to climb on the bar compared to control animals. Given the otherwise normal motor performance of AMPA-treated cats during climbing these findings suggest that the AMPA-induced reduction in climbing-time was, at least partly, due to an increased ability to switch from hanging to climbing. The latter effect was selectively mediated by quisqualate receptors since KYN, in contrast to an otherwise effective dose of AP7 (see above), counteracted this AMPA-induced effect. AMPA 1.0 μg induced an increase in the number of head movements. Since there were no changes in the environment, there were no exteroceptive stimuli except for those induced by self-motion that could have elicited the latter movements. The latter effect too was specific for quisqualate receptors since KYN, but not AP7, counteracted the AMPA-induced enhancement. As described in Section 3.2.3, AMPA did not significantly affect the ability to execute walking. On the other hand, the number of restarts was enhanced by 0.5 as well as by 1.0 μg. The latter increase was

not due to AMPA-induced abnormal movements: walking was not interrupted by specific kinds of behaviours (see Section 3.2.3). This enhancement was attenuated by the pretreatment of KYN. Again, this AMPA-induced effect was not blocked by AP7, implying that the latter effect too was selectively mediated by quisqualate receptors. Surprisingly, KYN, but not AP7, increased the number of restarts as well. KYN is known to antagonize effects mediated by all three receptor types (Stone & Connick, 1985; Turski, Herrling & Do, 1987) in contrast to AP7 that blocks selectively NMDA receptors (Cotman et al., 1987; Fagg, 1985; Foster & Fagg, 1984). These findings open the perspective that the KYN-induced increase in the number of restarts was mediated via quisqualate or kainate receptors. AMPA in turn counteracted this KYN-induced effect. These data become only understandable if one assumes that the three receptor types are anyhow functionally linked to each other.

In the present study, all injections were placed in the rostromedial part of the caudate nucleus. AP7, a substance that is known to have a low diffusion rate (Millan et al., 1986), was able to block the AMPA-induced limb disturbances. Accordingly, it is not likely that the latter effect was due to leaking of AMPA outside the target area. Furthermore, limb deficits were only present during the first, but not during the second, post-injection test period suggesting that they were not due to irreversible AMPA-induced changes. In view of these considerations, it is important to recall that the noted limb deficits neither occur after intracerebral injections of dopaminergic agents into the rostromedial part of the caudate nucleus nor occur after experimentally-induced alterations in hierarchically lower order output-stations of this part of the caudate nucleus, viz. the substantia nigra, pars reticulata and the deeper layers of the colliculus superior (Section 3.1; see also Chapters 4 and 5; cf. Gelissen & Cools, 1988). In contrast, limb deficits comparable to those reported in the present study do occur in cats with unilateral lesions of the frontal cortex or transection of the pyramidal tract (Armstrong, 1986; Liddell & Phillips, 1944). These findings together suggest that the limb deficits might be due to a drug-induced change in the striato-pallido-thalamo-cortical circuitry rather than due to a change in the striato-nigro-collicular circuitry.

The reverse holds true for the remaining AMPA-induced effects. Ample evidence is now available that both the rostromedial part of the caudate nucleus and its hierarchically lower order output-stations the substantia nigra pars reticulata and the deeper layers of the colliculus superior are essential for 'switching behaviour' (for rev. see Cools, 1986). The present findings suggest that AMPA enhanced the ability to switch behaviours: it produced an increase in switching from hanging to climbing as manifested in the AMPA-induced reduction in climbing-time and it produced an enhancement in switching from walking to other behaviours as manifested in the AMPA-induced increase in the number of restarts. In addition, the increase in the number of head movements might also be due to an AMPA-induced increase in switching behaviours. In other words, these data suggest that alterations in 'switching behaviour' might be due to a drug-induced change in the striato-nigro-collicular circuitry rather than due to a change in the striato-pallido-thalamo-cortical circuitry. The hypothesis about the differential involvement of these circuitries in limb deficits and 'switching behaviour' respectively, is supported by the finding that the distinct subtypes of glutamate receptors were differentially involved in each set of effects: NMDA- as well as quisqualate-receptors were involved in the display of limb deficits, whereas only quisqualate receptors were involved in the effects upon 'switching behaviour'. Considering the present finding that activation of quisqualate receptors produced an increase in switching behaviour, it might be expected that a selective inhibition of quisqualate receptors induces a decrease in switching behaviour. As long as there are no selective quisqualate receptor antagonists available, we are not able to investigate the latter possibility. In this respect, it might be of interest to study the effect of the recently developed aminoacid receptor antagonist 6-cyano-7-nitroquinoxaline-2-3-dione (CNQX) which potently inhibits AMPA binding and selectively inhibits quisqualate and kainate, but not NMDA, induced excitation on spinal neurons (Drejer & Honoré, 1988; Honoré et al., 1988). In the present study, KYN was unable to produce such an effect in spite of the finding that this dose was effective with respect to (1) blocking AMPA-induced effects and (2) producing an increase in the number of head movements. Since KYN is known to antagonize not only quisqualate receptors, but also NMDA and kainate receptors, it underlines the notion mentioned above that these receptors

mediate different, but related functions.

Summary

Bilateral intracaudate (rostromedial part) application of the glutamate agonist dl-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), viz. an agonist of quisqualate receptors, affects switching behaviour in cats that have to climb on a small wooden bar and, subsequently, to switch to distinct patterns (see Section 3.2): it produces increases in switching from one pattern to another pattern (1) and it induces limb deficits, i.e. unilateral deficient placing of the fore- and/or hindlimb. In the present study, the effect of stimulating rostromedial caudate dopamine receptors on behavioral changes induced by caudate injections of AMPA was investigated. Therefore, the dopamine agonist apomorphine was injected into the rostromedial part of the caudate nucleus 5 min before the caudate injection of 1.0 μg AMPA. AMPA-induced increases in switching behaviour were prevented by 0.6 μg, but not 0.3 μg, apomorphine. In contrast, AMPA-induced limb deficits were not prevented by pretreatment of apomorphine. In view of the notion that the dopaminergic rostromedial caudate nucleus, its output station the substantia nigra, pars reticulata and the nigral output station the deeper layers of the colliculus superior are essential for switching behaviour, but not for the display of disturbances like AMPA-induced limb deficits, the present data strongly suggest that only AMPA-induced changes in switching, but not AMPA-induced limb deficits, are mediated by the caudato-nigro-collicular circuitry. The glutamate receptor-selectivity of the modulatory action of dopamine is discussed.

3.3.1 INTRODUCTION

The interaction between dopaminergic nigrostriatal terminals and glutamatergic corticostriatal afferents has been subjected to extensive research in the past decades (cf. Arbuthnott, 1984; Chesselet, 1984; Cools & Peeters, 1987; Godukhin, Zharikova &

Budantsef, 1984; Hirata, Yim & Mogenson, 1984; Schmidt, 1986; Schmidt, Krähling & Ruhland, 1987). The results of these studies have shown that these neurotransmitter systems are connected to each other in more than one way. Dopaminergic receptors are present on cortico-caudate terminals (Kornhuber & Kornhuber, 1986), although this has been recently disputed (Joyce & Marshall, 1987). On the other hand, evidence has been found that at least part of the striatal glutamate receptor population is located presynaptically on nigrostriatal terminals (Bouyer et al., 1984; Roberts et al., 1982). Changes in dopaminergic activity have been reported to modulate the release of glutamate (Chiodo & Berger, 1986; Kerwin et al., 1984; Rowlands & Roberts, 1980), but evidence for the reverse interaction, i.e. glutamate modulating the release of dopamine, has also been reported (Chéramy et al., 1986; Chesselet, 1984).

The picture becomes even more complicated when taking into account the existence of different subtypes of glutamate receptors. Based on electrophysiological, biochemical and anatomical criteria, at least three glutamate receptor subtypes have been defined and labelled according to specific agonist properties of distinct ligands: the NMDA, the quisqualate and the kainate receptor (see Section 3.2; Cotman et al., 1987; Fagg, 1985; Foster & Fagg, 1984). In Section 3.2, evidence was presented that quisqualate receptors within the rostromedial part of the feline caudate nucleus are involved in programming behaviour. In fact, bilateral intracaudate injections of the selective quisqualate receptor agonist dl-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA; Krogsgaard Larsen, Honoré & Hansen, 1980; Krogsgaard Larsen et al., 1982) have been found to produce two sets of effects, viz. unilateral limb deficits and an increase in 'switching behaviour'. Limb deficits are characterized by inadequate placing responses of one fore- and/or hindlimb during the execution of various motor patterns, and 'switching behaviour' is defined as the transition from one behaviour pattern to the next pattern (see below). Quisqualate as well as NMDA receptors are involved in the display of AMPA-induced limb deficits since the aselective glutamate receptor antagonist kynurenic acid (KYN; Stone & Connick, 1985; Turski, Herrling & Do, 1987) as well as by the selective NMDA receptor antagonist d-2-amino-7-phosphonoheptanoate (AP7; Cotman et al., 1987; Fagg, 1985; Foster & Fagg, 1984) suppress the AMPA-induced

limb deficits. In contrast, only quisqualate receptors are involved in AMPA-induced increases in switching since only KYN, but not AP7, reduces AMPA-induced increases in switching (see Section 3.2).

At the level of the caudate nucleus, dopamine receptors within the rostromedial part of this nucleus are known to control striatally derived neuronal information arriving at hierarchically lower order output stations such as the substantia nigra pars reticulata and the deeper layers of the superior colliculus (Chapters 2, 4 and 5; see also Gelissen & Cools, 1988). Moreover, experimentally-induced changes in caudate dopaminergic activity are known to affect the animal's ability to switch arbitrarily ongoing behaviour (Section 3.1). In contrast, changes in striatal dopamine activity have never been found to result in motor disturbances comparable to the AMPA-induced limb deficits. On the basis of these and related data, we have therefore hypothesi that the AMPA-induced increase in switching behaviour, but not the AMPA-induced limb deficit, is mediated via the caudate output station the substantia nigra pars reticulata and the nigral output station the deeper layers of the colliculus superior.

The present study was undertaken in order to gain insight into the interaction between dopamine and glutamate at the level of the rostromedial part of the caudate nucleus. In view of the above-mentioned hypothesis, it was decided to investigate especially the effects of stimulation of caudate dopamine receptors on AMPA-induced increases in switching behaviour as well as on AMPA-induced limb deficits. For that purpose, we used an experimental design in which the behavioral effects of AMPA as described above occur. In short, cats have to climb on a wooden bar and, subsequently, to switch to distinct motor patterns in order to collect food pellets. As described in Section 3.3.2 (Experimental Procedures), this paradigm allows one to analyze both AMPA-induced limb deficits and AMPA-induced increases in switching behaviour. Five minutes before AMPA, apomorphine was injected into the rostromedial part of the caudate nucleus in order to stimulate the dopamine receptors.

3.3.2 EXPERIMENTAL PROCEDURES

Subjects

In the present study 28 adult male cats were used (weighing 3.5-5.0 kg) part of which also participated in experiments reported in Section 3.2.

Surgical procedure

Under sodium pentobarbitone anaesthesia (45 mg/kg i.p.) the animals were stereotaxically equipped with double-barrelled, stainless steel cannulas (outer diameter 0.8 mm; outer diameter of inner cannula which extended 1 mm below the tip of the outer cannula: 0.55 mm) into the rostromedial part of the caudate nucleus (coordinates: A 14.5, L 5.0 and H 5.0; atlas of Snider & Niemer, 1964) according to previously reported methods (see Section 3.1.2).

Apparatus and procedures

All cats were habituated to the experimental set-up and injection procedures. For an extensive description of the experimental set-up, training and habituation procedures, the reader is referred to Section 3.2.2. During the experiment, each cat had to execute successively the following motor patterns per trial:

1. Climbing on the bar: following the placement of the forepaws on one end of a wooden bar (5 x 5 x 200 cm) situated 2 m above the floor, the cat had to climb on the bar.

2. Walking: after climbing on the bar, the cat had to walk to the other end of the bar.

3. Jumping: at the end of the bar, the cat had to jump off the bar on a wooden platform (72 x 107 cm) 25 cm below the bar in order to receive a reward (food pellets: Brekkies, Effem B.V., Veghel). Each experiment consisted of 3 test periods. A single test period lasting maximally 5 min consisted of 3 trials. Drug-injections (volume: 5.0 μl) were given bilaterally in the rostromedial part of the caudate nucleus with help of a Hamilton syringe (diameter of the injection needle: 0.4 mm). Five min before drug application, the first test period was started (PRE). Five and 20 min after

the end of the final drug injections, the second and third test period was started (POST1 and POST2, respectively). The time-schedule was based on the results of previous studies. All test periods were recorded on video-tape with help of a closed video-circuit to allow subsequent detailed analysis. During recording the cat was not able to see the experimenter. Cats participated in maximally 5 experiments; the experiments were spaced by at least 1 week.

Drugs

The effects of the following drug treatments are presented: solvent (distilled water, n=21); 1.0 μg AMPA (gift from Dr. P. Krogsgaard-Larsen, n=11), injected 5 min before the start of POST1; 0.3 or 0.6 μg apomorphine (Brocades), injected 5 min before the application of 1.0 μg AMPA (n=7 and n=11, respectively); 0.6 μg apomorphine (n=10), injected 10 min before the start of POST1, serving as a necessary control for the latter experiment, in which 0.6 μg, but not 0.3 μg, apomorphine was found to be effective. The highest dose of apomorphine tested, i.e. 0.6 μg, is known to be maximally effective, locus-specific and dopamine-specific in open field tests (see Cools, Struyker Boudier & Van Rossum, 1976). Apart from solvent and 1.0 μg AMPA, which were given in the first and the second experiment respectively, all other treatments including those described in the previous report were given in an at random order. Whenever necessary, the pH of the solutions was adjusted to 7.

Dependent variables

1. Climbing. Climbing was considered to start as soon as the cat was released by the experimenter while it was hanging with its forepaws on the bar. Climbing was considered to be finished as soon as the animal reached a stable position on the bar. In case the animal was able to execute complete climbing, the time required to climb on the bar was expressed in seconds. Moreover, it was noted when the cat displayed unsuccessful hindlimb movements or no hindlimb movements at all.

2. Head movements. Following climbing, the animals executed a variable number of normal, non-forced head, torso and body movements before they started to walk towards the other end of the bar. In practice, a torso or a body movement was always

preceded by a head movement. Therefore, only the number of head movements was counted.

3. Walking. Walking was considered to start as soon as the cat made its first step towards the other end of the bar. Walking was considered to be completed as soon as the cat jumped off the bar. In case the cats walked, it was analyzed how many times they interrupted this behaviour, viz. switched to other kinds of movements such as normal, non-forced head, torso and/or body movements, bar-scratching, licking the fur, etc.. In the present study, only the number of walking-restarts was taken into account. In case the cat did not start walking within 90 s, the trial was broken off.

4. Jumping. At the end of the bar, the cats could collect food pellets by jumping down on a platform. It was recorded whether they landed correctly on the platform.

During each test period, three trials were executed. Accordingly, each variable was measured three times. At the end of each experiment, mean climbing-time during each test period was calculated per cat by averaging the three climbing-time scores obtained during that period. In order to obtain sufficient numbers per test period, the frequency parameters head movements and walking restarts were calculated for each cat as follows: at the end of each experiment, the total number of head movements and walking restarts were calculated by adding the three scores of each parameter obtained during that period. Since AMPA-induced effects were present only during POST1, and not during POST2, the effect of apomorphine on AMPA-induced behavioral effects was analyzed only during POST1. In order to reduce the interindividual variability, the ratio of the difference and the sum of the mean climbing-time, total number of head movements and total number of walking-restarts during POST1 and PRE was computed: POST1-PRE/ POST1+PRE (see Section 3.1.2).

Statistics

The data were statistically analyzed with help of the Mann Whitney U-test, two tailed, unless otherwise mentioned (Siegel, 1956). Experimental groups were considered to differ significantly in case of $p<0.05$.

Histological verification

After the end of the final experiment, the cats were deeply anaesthetized with pentobarbital and perfused transcardially with a 4% formaldehyde solution. Subsequently, the brains were removed and cross-sections were cut with help of a cryostat (30 μm slices). The slices were stained with cresyl violet to allow exact location of the injection loci.

3.3.3 RESULTS

Histology

All injections were placed in the rostromedial part, in the body of the caudate nucleus: coordinates (Snider & Niemer, 1964) A 14.0-15.0, L 4.8-5.9 and H 4.4-5.4 (see Figure 3.2.1).

Control-tests

During PRE tests, all cats were able to execute complete trials in a normal way: all cats were able to climb on the bar (median climbing-time and 25-75% range of solvent-treated cats: 1.8 and 1.7-2.7 s, respectively); they executed several head movements (median number and 25-75% range in solvent-treated cats: 9, 3-15, respectively) before they started walking; the animals hardly interrupted walking (median number of restarts and range in solvent-treated cats: 0 and 0-4, respectively). At the end of the bar, they jumped down on the platform without making any misplacement.

Apomorphine and AMPA-induced limb deficits

AMPA 1.0 μg induced abnormal fore- and/or hindlimb movements in POST1 in 6 out of 11 tested cats (see also Section 3.2.3). As shown in Table 3.3.1, 0.3 and 0.6 μg apomorphine were unable to change this AMPA-induced effect. On the other hand, apomorphine *per se* did not induce limb deficits in any of the tested cats (Table 3.3.1).

Table 3.3.1 Percentage of animals showing unilateral limb deficits during POST1 tests: 5-10 min after the intracaudate injection of solvent (SLV), 1.0 μg dl-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), 0.3 or 0.6 μg apomorphine (APO 0.3 and APO 0.6, respectively) plus 1.0 μg AMPA (APO was injected 5 min before AMPA); 10-15 min after the injection of APO 0.6.

(n) = number of animals.

DRUG (μg/5 μl)	ANIMALS WITH LIMB DEFICITS (n)	(%)
SLV	(21)	0
AMPA	(11)	55*
APO 0.3 +AMPA	(7)	29
APO 0.6 +AMPA	(11)	45
APO 0.6	(10)	0

*p<0.05, AMPA vs. SLV.
APO 0.3 or APO 0.6 plus AMPA vs. AMPA: n.s. (p>0.05).
(Fisher exact probability test, two tailed).

Climbing

The ratio of climbing-time of AMPA-treated cats showing limb deficits differed significantly from that in cats devoid of such disturbances (p=0.008; see Section 3.2.3); in the present study, only the climbing-time of cats showing no limb disturbances was taken into account. In these cats the ratio of climbing-time was significantly reduced by 1.0 μg AMPA (see Figure 3.3.1). As shown, 0.6 μg, but not 0.3 μg, apomorphine attenuated this AMPA-induced effect. Climbing-time was not affected by 0.6 μg apomorphine *per se* (see Figure 3.3.1).

Head movements

With respect to the number of normal head movements, animals showing limb deficits following 1.0 μg AMPA, 0.3 μg apomorphine + 1.0 μg AMPA or 0.6 μg apo-

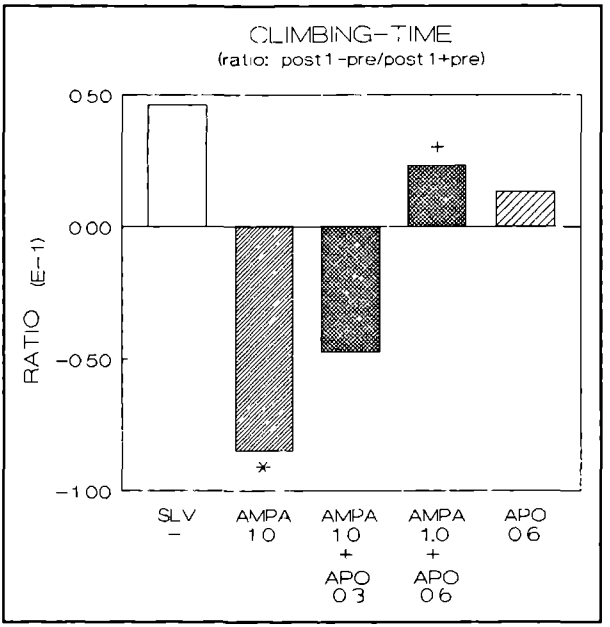

*Figure 3.3.1 Ratio of the mean climbing-time (POST1-PRE / POST1+PRE) during POST1: 5-10 min after Solvent (SLV), 1.0 μg AMPA, 0.3 or 0.6 μg apomorphine (APO) plus 1.0 μg AMPA (APO was injected 5 min before AMPA);10-15 min after 0.6 μg apomorphine. *, p<0.02: drug vs. SLV. +, p<0.02: 1.0 μg AMPA plus 0.6 μg APO vs. 1.0 μg AMPA.*

morphine + 1.0 μg AMPA did not differ from those who received the same treatment but that did not show these disturbances. Therefore, they were pooled. Figure 3.3.2 shows that the ratio of the number of normal head movements was significantly increased after 1.0 μg AMPA. The latter effect was reduced by 0.6 μg, but not by 0.3 μg, apomorphine. On the other hand, 0.6 μg apomorphine *per se* was unable to affect the ratio of the number of head movements (see Figure 3.3.2).

Walking

Regarding the number of walking-restarts, animals showing limb deficits following 1.0

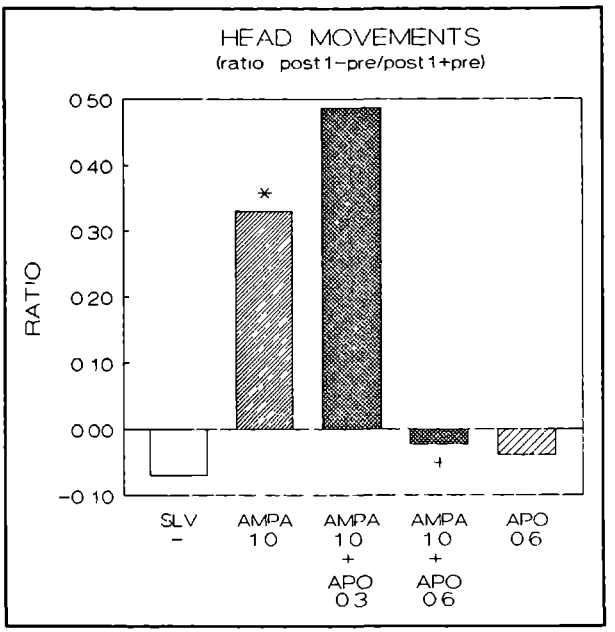

*Figure 3.3.2 Ratio of the total number of head movements (POST1-PRE / POST1+PRE) during POST1. 5-10 min after Solvent (SLV), 1.0 μg AMPA, 0.3 or 0.6 μg apomorphine (APO) plus 1.0 μg AMPA (APO was injected 5 min before AMPA);10-15 min after 0.6 μg apomorphine. * $p<0.02$: drug vs. SLV. + $p<0.02$. 1.0 μg AMPA plus 0.6 μg APO vs 1.0 μg AMPA.*

μg AMPA, 0.3 μg apomorphine + 1.0 μg AMPA or 0.6 μg apomorphine + 1.0 μg AMPA did not differ from those who received the same treatment but that did not show these disturbances. Therefore, they were pooled. As illustrated in Figure 3.3.3, AMPA 1.0 μg significantly increased the number of restarts. Again the latter effect was reduced only by the highest dose of apomorphine, whereas it was unaffected by 0.6 μg apomorphine *per se* (see Figure 3.3.3).

Jumping

As a final remark, none of the treatments was able to affect normal jumping ability;

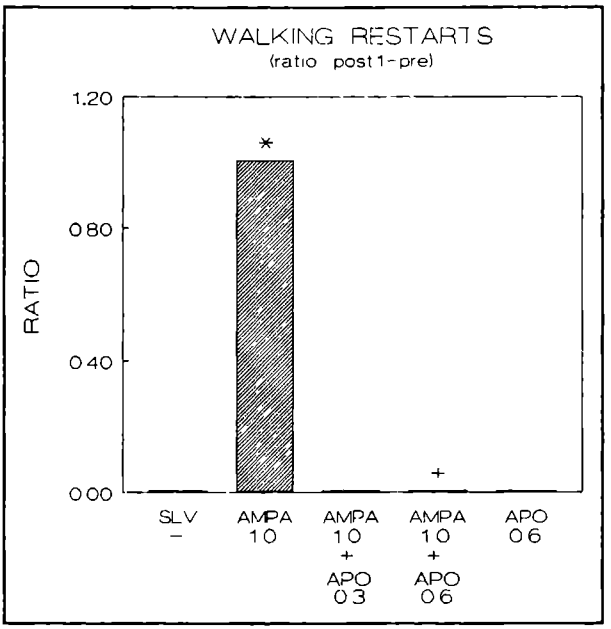

*Figure 3.3.3 Ratio of the total number of walking-restarts (POST1-PRE) during POST1: 5-10 min after Solvent (SLV), 1.0 μg AMPA, 0.3 or 0.6 μg apomorphine (APO) plus 1.0 μg AMPA (APO was injected 5 min before AMPA); 10-15 min after 0.6 μg apomorphine. * $p<0.002$: drug vs. SLV. + $p<0.02$: 1.0 μg AMPA plus 0.6 μg APO vs. 1.0 μg AMPA.*

all cats collected the food pellets offered to them on the platform.

3.3.4 DISCUSSION

As mentioned in Section 3.3.1 (Introduction), bilateral intracaudate injections of 1.0 μg AMPA produced two sets of effects in cats executing a sequence of distinct motor patterns on the bar: it produced unilateral limb deficits in part of the tested cats and it induced an increase in switching behaviour.

AMPA-induced limb deficits

Previously, it has been shown that the AMPA-induced limb deficits are prevented by the aselective glutamate receptor antagonist KYN as well as by the selective NMDA receptor antagonist AP7, showing that quisqualate as well as NMDA receptors are involved in the display of these motor disturbances (see Section 3.2). The present study shows that pretreatment with apomorphine did not affect the AMPA-induced limb disturbances. Furthermore, the bilateral intracaudate application of apomorphine *per se* did not induce comparable deficits in any of the tested cats. Since the latter dose of apomorphine is known to be locus-specific, dopamine-selective and maximally effective in open field tests (for ref., see Cools, Struyker Boudier & Van Rossum, 1976) these data show that dopamine receptors within the rostromedial part of the caudate nucleus were not involved in the display of AMPA-induced limb deficits.

AMPA-induced increase in switching behaviour

Apart from the limb disturbances, 1.0 μg AMPA induced an increase in switching behaviour: switching from hanging to climbing in cats showing no limb deficits was increased as suggested by the reduction in climbing-time, and switching from walking to other behaviours was enhanced as shown by the increase in the number of walking-restarts. In addition, AMPA induced an increase in the number of head movements that might also be due to an AMPA induced increase in switching behaviour. In contrast to AMPA induced limb deficits, changes in switching behaviour are known to be counteracted by KYN, but not by AP7, showing that the latter effect is selectively mediated by quisqualate receptors (Section 3.2). The present study shows that pretreatment with apomorphine attenuated the AMPA induced decrease in climbing-time, counteracted the AMPA induced increase in the number of walking-restarts, and reduced the AMPA induced enhancement in the number of head movements. These data show that apomorphine attenuated AMPA induced increases in switching behaviour. Moreover, only 0.6 μg, but not 0.3 μg, apomorphine affected the latter AMPA induced behavioral changes. In other words, only a dose of apomorphine that is known to be maximally effective in open field tests, i.e. 0.6 μg, turned out to be effective in this respect. The latter finding is in agreement with the results of

iontophoretic experiments of Chiodo and Berger (1986) who found that only relatively high doses, but not lower doses of dopamine were able to reduce glutamate-induced excitatory responses of caudate neurons. The finding that apomorphine *per se* did not alter climbing-time, the number of walking-restarts and the number of head movements is in agreement with the outcome of former studies showing that 0.6 μg apomorphine *per se* did not affect switching motor patterns (Section 3.1, see also Section 5.1).

The caudato-nigro-collicular circuitry and switching behaviour

Inhibition or activation of dopamine receptors within the rostromedial part of the caudate nucleus is known to affect neuronal activity arriving at the level of the substantia nigra pars reticulata and the deeper layers of the superior colliculus (see also Chapter 6). Furthermore, both the substantia nigra pars reticulata and the deeper layers of the superior colliculus are essential in switching behaviour (see Chapter 5; see also Cools, 1986; Gelissen & Cools, 1986). Moreover, inhibition of striatal dopaminergic activity is known to reduce the animal's ability to switch arbitrarily motor patterns (Section 3.1; Wegener, Schmidt & Ehret, 1988). On the basis of these data, it can be concluded that the results of the present study provides evidence in favour of the hypothesis that AMPA-induced changes in switching behaviour, but not AMPA-induced limb deficits, are mediated via the caudato-nigro-collicular circuitry.

As a final remark, the results of the present study together with the fact that the AMPA-induced increase in switching behaviour is selectively mediated by quisqualate receptors suggest that the modulatory role of dopamine on glutamate activity in the rostromedial part of the feline caudate nucleus is restricted to functional changes mediated by quisqualate receptors.

CHAPTER 4

THE CAUDATO-NIGRO-COLLICULAR PATHWAY

4.0 GENERAL INTRODUCTION

As is described in Section 2.3, the caudate nucleus projects to the globus pallidus and to the substantia nigra pars reticulata. Many caudatopallidal and caudatonigral fibres contain the neurotransmitter GABA. In turn, the substantia nigra pars reticulata projects to the mesencephalic reticular formation, the ventrolateral and ventromedial thalamic nuclei, and to the deeper layers of the colliculus superior (see Section 2.3). Since the colliculus superior is known to project directly as well as indirectly to the spinal cord (see Graham, 1977), one way in which caudate neuronal signals may ultimately reach the effector organs is the caudato-nigro-collicular pathway. Classically, the GABAergic caudatonigral pathway is considered to function as a feedback pathway to control the dopaminergic nigro(pars compacta)-caudate projection (see Section 4.1). Therefore, the first step to be taken is to investigate whether the substantia nigra pars reticulata indeed may serve as an output station which receives neuronal signals from, among others, the caudate nucleus and which, in turn, sends its information to other brain regions such as the deeper layers of the colliculus superior. The effects of intranigral injections of GABAergic compounds were investigated in the experiments described in Section 4.1. In order to test whether the caudatonigral pathway functions as part of a feedback loop or serves as a caudate output channel, the effects of intracaudate application of dopaminergic substances on the behavioural response induced by intranigral injections of GABAergic substances were also studied.

As mentioned, the reticular substantia nigra projects via GABAergic fibres to the deeper layers of the colliculus superior. In order to investigate the behavioural response of experimentally induced changes in the activity of the nigrocollicular pathway, the experiments described in Section 4.2 were performed. As discussed below (Section 4.1.4 and 4.2.4), both intranigral application and intracollicular injection of GABAergic substances lead to behavioural changes which can be differentiated from each other as well as from those following experimentally-induced changes of dopaminergic or GABAergic activity within the caudate nucleus.

4.1 THE SUBSTANTIA NIGRA PARS RETICULATA, A FIRST ORDER OUTPUT STATION OF THE CAUDATE NUCLEUS: NIGRAL GABA AND MOTOR BEHAVIOUR

Summary

The behavioural consequences of unilateral injections into the substantia nigra pars reticulata of the GABAergic antagonist picrotoxin or the GABAergic agonist were investigated in cats. In addition, the involvement of striatal dopaminergic mechanisms in the behavioural expression of GABAergic mechanisms within the substantia nigra pars reticulata was investigated. Therefore, apomorphine or haloperidol were bilaterally administrated into the rostromedial part of the caudate nucleus of cats pretreated with a unilateral injection of picrotoxin or muscimol into the nigral pars reticulata. The pharmacological treatment of the caudate nucleus did not produce any significant change in the behaviour elicited from the nigra; neither the picrotoxin-induced asymmetric posturing, asymmetric circling, freezing and hindlimb disorder nor the muscimol-induced asymmetric posturing, asymmetric spinning and stereotyped licking were significantly affected. The latter behaviour was absent in animals with a partial or total destruction of the nigral pars reticulata. The present results demonstrate that the nigral pars reticulata does not form part and parcel of a feedback system that simply transmits incoming signals from the caudate nucleus towards the pars compacta, i.e. the origin of the dopaminergic, nigrostriatal fibres. Finally, the present study demonstrates that the dopaminergic activity within the caudate nucleus may only modify, but certainly not determine, the behavioural expression of the nigral pars reticulata. It is concluded that the substantia nigra pars reticulata not only transmits, but also transforms its incoming signals.

4.1.1 INTRODUCTION

Traditionally, the substantia nigra pars reticulata is considered to be an intercalated station between striatonigral, GABAergic and nigrostriatal, dopaminergic fibres (Dray, 1979). Accordingly, this brain region has been believed to act like a part of a feedback system that simply transmits the incoming signals from the striatum towards the nigral pars compacta, i.e. the origin of the nigrostriatal fibres (Andén & Stock, 1973; Dray, 1979; Dray & Straughan, 1976; Figure 4.1.1A). Since the late seventies however, evidence has been presented that the substantia nigra pars reticulata also functions as an output station (Figure 4.1.1B) that connects the neostriatum/caudate nucleus with structures such as the thalamus, colliculus superior and reticular formation (Chevalier et al., 1985; Cools, 1978; De Montis et al., 1979; Di Chiara et al., 1978; Gale & Casu, 1981; Garant & Gale, 1987; Garzia-Munoz, 1977; Grace & Bunney, 1985; Graybiel & Ragsdale, 1979; May & Hall, 1986; Olianas et al., 1978; Scheel-Krüger, Arnt & Magelund, 1977; Schulz, 1986; Starr, Summerhayes & Kilpatrick, 1983; Wolfarth, Kolasiewicz & Sontag, 1981; Williams & Faull, 1985; 1988). The latter finding might imply that the substantia nigra pars reticulata not only transmits, but also transforms input signals (Figure 4.1.1C).

Important evidence for such a proposal would be the demonstration that the behavioural expression of the substantia nigra pars reticulata differs from that of any area sending afferents to this brain region. Recently, we have reported that unilateral injections of the GABA agonist muscimol into the substantia nigra pars reticulata of cats resulted in behavioural phenomena such as vigorous rotating of the head-to-tail type, unilateral twisting of the head and stereotyped licking (Wolfarth, Kolasiewicz & Sontag, 1981). In the same study, it was found that unilateral intranigral injections of GABA antagonists such as picrotoxin and bicucculine produced slow circling, unilateral twisting of the head, immobility and motor disturbances of the hindlimbs. Apart from the twisting behaviour, none of the remaining phenomena have ever been reported after manipulation of the caudate nucleus in cats (Cools & Van Rossum, 1970; Cools, Struyker Boudier & Van Rossum, 1976).

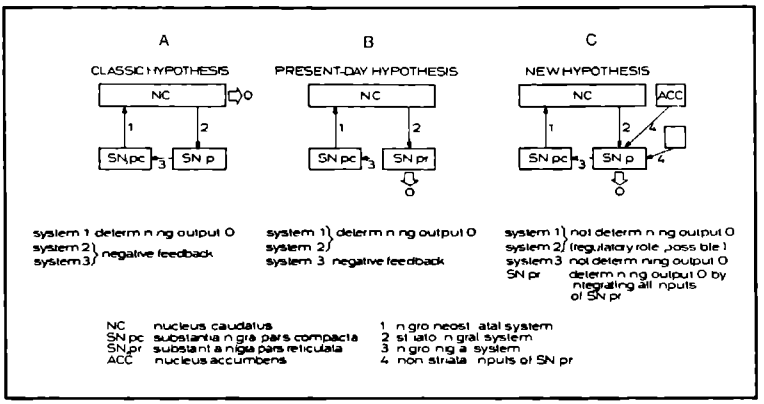

Figure 4.1.1 A classic hypothesis, substantia nigra, pars reticulata as intercalated station between striatonigral and dopaminergic, nigrostriatal fibres B present-day hypothesis, substantia nigra pars reticulata as output station of the neostriatum C new hypothesis proposed in this chapter, substantia nigra pars reticulata as a station that transforms different input signals into new, physiologically meaningful output signals

If indeed the information leaving the substantia nigra pars reticulata originates in this brain region as a result of its properties to transform inputs from one or more brain structures (Figure 4.1.1C), it becomes necessary to investigate the influence of the latter brain structures. In the present study, we focused our attention on the influence of the nigrostriatal and striatonigral system (Figure 4.1.1C, systems 1 and 2) Accordingly, we studied the effects of bilateral administration of the dopaminergic agonist apomorphine and the dopaminergic antagonist haloperidol into the rostromedial part of the caudate nucleus on behaviour elicited by unilateral injections of muscimol or picrotoxin into the substantia nigra pars reticulata of cats (cf. Cools, Struyker Boudier & Van Rossum, 1976, Wolfarth, Kolasiewicz & Sontag, 1981).

Animals and surgical procedure

In order to allow intracerebral injections into conscious, freely moving animals, 25 cats of either sex (2.8-3.5 kg) were prepared as previously described (Cools, Struyker Boudier & Van Rossum, 1976; Wolfarth, Kolasiewicz & Sontag, 1981). Under sodium pentobarbital anaesthesia (35 mg/kg, i.p.), a stainless steel cannula with external diameter of 0.65 mm was stereotactically implanted into the right substantia nigra pars reticulata (coordinates A 3.0, L 5.0, H 2.7, according to the atlas of Snider & Niemer, 1964) in order to allow unilateral intranigral injections (1 μl) through an injection needle protruding 0.1 mm below the tip of the guide cannula (Wolfarth, Kolasiewicz & Sontag, 1981). In addition, two stainless steel cannulas with external and internal diameters of 0.8 and 0.4 mm respectively were implanted into the left and right caudate nucleus (A 14-16, L 4-6, H 6-8, according to Snider & Niemer, 1964) in order to allow bilateral intracaudate injections (5 μl per side) 1.0-2.0 mm below the tip of the guide cannulas. First, the cats were habituated to the experimental cage (90x60x60 cm) and injection procedures during two 1-h sessions on separate days. The experiments, in which the behavioural responses to intracerebral injections were analyzed in conscious, freely moving animals, started at least 7 days after the operation (for details: Cools, Struyker Boudier & Van Rossum, 1976; Wolfarth, Kolasiewicz & Sontag, 1981). In case a cat was used for a second or a third time, the experiments were spaced by at least 4 days.

Drugs

The concentrations of intranigrally injected picrotoxin (2 μg/ μl; Nutr. Biochem. Corp.) and muscimol (0.4 μg/μl; Serva) were chosen on the basis of the fact that the behaviour of these doses could be modified by the systemic administration of apomorphine (Wolfarth, Kolasiewicz & Sontag, 1981). The doses of intracaudate solutions of apomorphine (5 μg/5μl per side; apomorphine hydrochloride, ACF Chemiefarma NV) and haloperidol (12.5 μg/5μl per side, Haldol, Janssen Pharma-

ceutica) were chosen on the basis of the fact that they are maximally effective in eliciting behavioural effects from the caudate nucleus, as shown in several studies (cf. Cools, Struyker Boudier & Van Rossum, 1976).

Experimental design

For reasons given in Section 4.1.3 (Results), the picrotoxin-pretreated cats received their intracaudate injections 50 min after the intranigral injections; the muscimol-pretreated cats received them 90 min after the intranigral injection. The first 5 min of the post-injection period were not taken into account because of injection artifacts; the subsequent 10 min period was used for analyzing the influence of intracaudate injections, as the behavioural responses to such injections in naive cats fade away about 15 min after the injection. Only cats showing the expected response to intranigral injections of picrotoxin and/or muscimol (Wolfarth, Kolasiewicz & Sontag, 1981) were used in the latter series of experiments.

Histological procedures

At the end of the last behavioural test, the cats were deeply anaesthetized with pentobarbital and subsequently transcardially perfused with a 4% formaldehyde solution. The dissected brains were processed to be histologically examined (20 μm slices, Nissl and haematoxylin staining) in order to estimate the location of the tip of the injection needles.

4.1.3 RESULTS

Unilateral intranigral injection of picrotoxin resulted in effects identical to those reported earlier (Wolfarth, Kolasiewicz & Sontag, 1981; Table 4.1.1): (a) contralateral posturing implying static asymmetry of the trunk and/or neck for a minimum period of 30 s (CP); (b) fast contralateral head turnings with eyes fixed in the head (CT); (c) slow contralateral circling without spinning (sCC); (d) immobility or freezing implying

Table 4.1.1 Percentage of animals showing behavioural deficits following unilateral 'effective' intranigral injections of picrotoxin before (pre) and after (post) bilateral intracaudate injections of distilled water, haloperidol and apomorphine.

No significant differences between the distinct 'post values' were found (Mann Whitney U-test, two tailed).

	Solvent (n=6)		Haloperidol (n=6)		Apomorphine (n=7)	
	%pre	%post	%pre	%post	%pre	%post
Contra-posturing CP[a]	100	100	100	100	100	71
Contra-turning CT	83	50	67	67	100	86
Slow-circling sCC	50	33	17	34	0	0
Freezing FR	100	100	100	100	100	100
Hindlimb defic. HD	100	100	100	100	100	86
HD, score[b]	2.2±0.4	2.2±0.4	2.3±0.3	2.8±0.2	2.3±0.4	1.9±0.6
CP, score[c]	-	-	174±20	207±18	160±13	93±27[d]

[a] Implying static asymmetry of the trunk and/or neck for a minimum period of 30 s.
[b] HD, score was measured 10 min before and after the intracaudate treatment (average ± SEM).
[c] CP, score = amount of time (s) during which CP was present in the third 5 min interval before (pre) and second 5 min interval after (post) the intracaudate treatment (mean ± SEM).
[d] apomorphine, post vs apomorphine, pre: $p < 0.05$ (Wilcoxon matched-pairs signed-ranks test, two tailed).

absence of any movement including those of ears, eyes and facial muscles for a minimum period of 30 s (FR); and (e) motor disturbances of the hindlimbs to a greater or lesser extent (HD). The degree of pathology was assesses during 3 series of 3 successive tests: the most serious HD implied absence of any movement of the hindlimbs when the forelimbs were put on a wooden bar (300 x 5 x 5 cm) placed 2 m above the floor, absence of any forward locomotion when all four limbs were placed on the bar, and increased clinging to the bar (HD, score 3); a moderate HD implied presence of

disoriented and unsuccessful movements of the hindlimbs in the above-mentioned situations (HD, score 2); the least HD implied presence of disoriented, but successful, movements of the hindlimbs in these situations (HD, score 1).

The severity of the overall picrotoxin-induced syndrome reached its maximum about 40 min after the injection (at which time the HD was measured) and then remained unchanged for at least 120 min; for this reason, the intracaudate injections discussed below were given 50 min after the picrotoxin administration. histological verification revealed that: (a) 12 out of 14 effective injections were placed in, or within a distance of 1 mm from, the target area; (b) 4 out of the 5 ineffective injections were placed into the substantia nigra, being partly or fully destroyed; and (c) 1 out of the 5 ineffective injections together with 2 out of the 14 effective injections were placed more than 2 mm beyond the borderline of the chosen target area. The label 'ineffective' refers to nigral injections that did not result in the display of CP, FR, and HD; both 'effective' and 'ineffective' injections produced asymmetric head turnings accompanied by asymmetric changes in the musculature of the eyes, ears and face.

Bilateral administration of maximally effective doses of apomorphine (5.O μg) or haloperidol (12.5 μg) was unable to alter the behaviour deficits elicited by an 'effective', intranigral injection of picrotoxin given 50 min prior earlier: the percentage of animals showing behaviour deficits after haloperidol or apomorphine did not significantly differ from that found in control experiments, in which the solvent of the dopaminergic agents (distilled water) was given (Table 4.1.1; Mann Whitney U test, two tailed). Comparing pre- and post-injection values per test-series, it turned out that only the time, during which the animals maintained their contralateral asymmetry, was significantly reduced by apomorphine (CP, score in Table 4.1.1: $p< 0.05$, Wilcoxon matched-pairs, signed-ranks test, two tailed). Histological verification revealed that all injections were placed into the chosen target areas (Figures 4.1.2A and 3.2.1).

Unilateral intranigral injections of muscimol resulted in effects identical to those reported earlier (Wolfarth, Kolasiewicz & Sontag, 1981; Table 4.1.2): (a) contralateral

Table 4.1.2 Percentage of animals showing behavioural deficits following unilateral 'effective' intranigral injections of muscimol before (pre) and after (post) bilateral intracaudate injections of distilled water, haloperidol and apomorphine.

No significant differences between the distinct 'post values' were found (Mann Whitney U-test, two tailed).

	Solvent (n=8)		Haloperidol (n=6)		Apomorphine (n=6)	
	%pre	%post	%pre	%post	%pre	%post
Contra-posturing CP[a]	100	100	100	100	100	100
Contra-turning CT	63	50	100	67	100	100
Fast-circling fCC	100	100	100	100	100	67
Licking	100	87	100	83	83	100
Lick, score[b]	2.1±0.2	2.0±0.4	2.3±0.2	2.2±0.5	2.0±0.5	2.2±0.5
fCC, score[c]	39±14	38±13	20±7	26±10	25±8	12±6[d]

[a] Implying also postural changes into the contralateral direction.
[b] Licking score: average ± SEM (see text).
[c] fCC, score = number of full circles made during the first plus second 5 min interval before (pre) and after (post) the intracaudate treatment (average ± SEM).
[d] apomorphine, post vs apomorphine, pre: $p<0.05$ (Wilcoxon matched-pairs signed-ranks test, two tailed).

posturing (CP); (b) fast contralateral head turnings with eyes fixed in the head (CT); (c) fast contralateral circling of the head-to-tail type (fCC); and (d) stereotyped behaviour such as licking. In Table 4.1.2, the average ±SEM of the maximum score /min throughout the pre- vs post-injection period is given: continuous licking, score 3; discontinuous licking spells, score 2; a single licking spell, score 1.

The overall muscimol-induced syndrome reached its maximum about 60-80 min after the injection and remained then unchanged for several hours; for this reason, the intracaudate injections discussed below were given 90 min after the muscimol

administration. Following histological verification, it turned out that: (a) all 13 effective injections were placed in, or within a distance of 1 mm from, the target area (Figure 4.1.2A); (b) 4 out of 7 ineffective injections were placed into the substantia nigra pars reticulata, being partly or fully destroyed (Figure 4.1.2B); and (c) 3 out of the 7 ineffective injections were placed outside the target area (Figure 4.1.2C). The label 'ineffective' refers to nigral injections that did not result in the display of CP and fCC; both 'effective' and 'ineffective' injections produced asymmetric head turnings by asymmetric changes in the musculature of the eyes, ears and face.

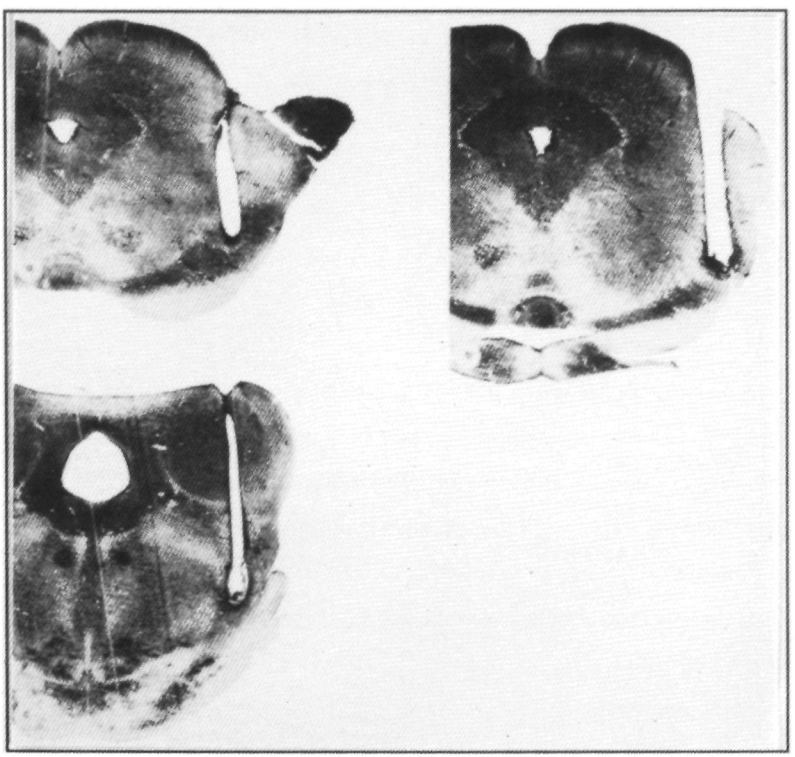

Figure 4.1.2 *A: representative picture of cat brain with an 'effective' injection site of muscimol (0.4 μg/μl) into the substantia nigra. B: representative picture of cat brain with an 'ineffective' injection site of muscimol (0.4 μg/μl) into the nigral pars reticulata being partially destroyed. C: representative picture of cat brain with*

Bilateral administration of maximally effective doses of apomorphine (5.0 μg) or haloperidol (12.5 μg) was unable to affect the behaviour deficits elicited by an 'effective' intranigral injection of muscimol given 90 min earlier: the percentage of animals showing behaviour deficits after haloperidol or apomorphine did not significantly differ from that found in control experiments, in which the solvent of the dopaminergic agents (distilled water) was given (Table 4.1.2; Mann Whitney U test, two tailed). Comparing pre- and post-injection values per test-series, it turned out that only the number of full circles was significantly reduced by apomorphine (fCC, score in Table 4.1.2: $p<0.05$, Wilcoxon matched-pairs signed-ranks test, two tailed). Histological verification revealed that all injections were placed into the chosen target areas (Figures 4.1.2A and 3.2.1).

4.1.4 DISCUSSION

In view of the fact that partial or total destruction of the substantia nigra in 8 cats blocked the potency of intranigrally injected GABAergic agents to elicit the behavioural deficits listed in Tables 4.1.1 and 4.1.2, the present results allow the conclusion that these drug-induced deficits were due to changes occurring within the substantia nigra pars reticulata. Since asymmetric head turnings accompanied by asymmetric changes in the facial musculature, i.e. a characteristic behavioural expression of the caudate nucleus function (Cools & Van Rossum, 1970; Cools, Struyker Boudier & Van Rossum, 1976), appeared not only in animals having a partially or totally destroyed substantia nigra pars reticulata but also in animals receiving muscimol injections outside the substantia nigra, the former conclusion does not hold true for this phenomenon; actually, it favours an important role for structures adjacent to the substantia nigra (cf. Lee, Slater & Crossman, 1981).

The present results also demonstrate that the drug-induced changes in the dopaminergic activity of the rostromedial caudate nucleus do not significantly affect the overall behaviour elicited from the substantia nigra pars reticulata (cf. Arnt & Scheel-Krüger,

1979a; 1979b). This result cannot be attributed to an insufficient efficacy of the intracaudate treatment since the doses selected have been found to be maximally effective in altering the behavioural expression of the caudate function in studies which have been replicated several times (cf. Cools, Struyker Boudier & Van Rossum, 1976). Accordingly, the present study shows that those aspects of the behavioural expression of the reticular function, which are investigated in the present experiments, are not significantly affected by experimentally induced changes in the main afferent input of this brain region (cf. Arnt & Scheel-Krüger, 1979a; 1979b). Although it cannot be excluded that absence of a point-to-point relationship between the chosen area of the caudate nucleus and that of the nigral pars reticulata underlies the present findings, this explanation seems quite unlikely. For the complex neuropil of intracaudate interneurons on the one hand, and the extensive dendritic field of the nigral pars reticulata on the other hand, form an excellent network for cross-talk between the distinct areas within each brain structure. Given the neuroanatomical connection between the caudate nucleus and the substantia nigra pars reticulata on the one hand, and the enormous discrepancy between the behavioural expression of these nuclei on the other hand, (for detailed descriptions see Cools & Van Rossum, 197O; Wolfarth, Kolasiewicz & Sontag, 1981), we therefore reach the conclusion that the substantia nigra pars reticulata not only transmits, but also transforms, its incoming signals into new output signals (Figure 4.1C).

In view of the fact that the intranigral doses selected were rather high and, accordingly, might have masked the presence of any modulating, but not directing or determining, influence of the striatonigral input on the function of the nigra, we cannot ascertain whether or not the feline caudate nucleus exerts a modulating influence; indeed, apomorphine's ability to shorten the duration of the picrotoxin-induced asymmetric posturing certainly does not exclude such a striatal function (cf. Arnt & Scheel-Krüger, 1979a; 1979b). Further studies in which lower doses of muscimol or picrotoxin are used will be required for analyzing the presence of a modulating role of the rostromedial caudate nucleus.

Concerning the classic controversy as to what degree the nigro-striatal dopaminergic fibres are involved in the behavioural expression of the substantia nigra pars reticulata in rats (Arnt & Scheel-Krüger, 1979a, 1979b; Di Chiara et al., 1978; Garcia-Munoz et al., 1977; Lee, Slater & Crossman, 1981; Pycock, 1980; Scheel-Krüger, Arnt & Magelund, 1977; Scheel-Krüger et al., 1978; Scheel-Krüger & Magelund, 1981), the present results provide hard evidence that the behaviour elicited from this region in the feline brain is not mediated via the dopaminergic fibres arising in the nigral pars compacta and terminating within the caudate nucleus (Figure 4.1C, cf. Arnt & Scheel-Krüger, 1979a, 1979b); this fits in with biochemical data showing that the nigral picrotoxin treatment used in the present study does not alter the intracaudate dopamine concentrations (Kolasiewicz & Grabowska-Andén, personal communication). Nevertheless, our conclusion is at variance with feline studies providing evidence that the systemic administration of the dopaminergic agonist apomorphine affects some aspects of the nigral syndrome (Wolfarth, Kolasiewicz & Sontag, 1981). To explain this seeming discrepancy, it is useful to recall that the substantia nigra pars reticulata: (1) also receives non-striatal afferents from dopaminergic structures such as the nucleus accumbens (Troiano & Siegel, 1978; cf. Graybiel & Ragsdale, 1979); and (2) sends efferents to the thalamus, superior colliculus and reticular formation. In view of these anatomical connections we propose that the ability of systemically administrated apomorphine to affect the nigral syndrome is partly due to the apomorphine-induced changes in the overall inflow into the pars reticulata and partly due to the apomorphine-induced changes in structures such as the thalamus, i.e. structures innervated by the substantia nigra pars reticulata as well as the caudate nucleus. This proposal not only underlines the importance of these structures for the behavioural expression of the substantia nigra pars reticulata, but also offers a conceivable explanation for the fact that intracaudate apomorphine did reduce, but not increase (Wolfarth, Kolasiewicz & Sontag, 1981), the number of muscimol-induced circles of the head-to-tail type. For, the latter phenomenon cannot be attributed to an increased striato-nigral input, since such an increase is known to release GABA into the substantia nigra and, accordingly, should potentiate and not attenuate, effects elicited by intranigrally administered muscimol.

4.2 THE DEEPER LAYERS OF THE COLLICULUS SUPERIOR, A SECOND ORDER OUTPUT STATION OF THE CAUDATE NUCLEUS: COLLICULAR GABA AND MOTOR BEHAVIOUR

Summary

The behavioural response to picrotoxin (25-500 ng) injected into the deeper layers of the colliculus superior of freely moving cats was investigated. The maximal response to unilateral injections of picrotoxin (≥ 200 ng) was characterized by the following sequence of behavioural events. During the first 5 min after the injection the cat executed retroflexions of the contralateral ear. After 1-5 min these contralateral ear movements were followed by short, contralateral head movements. As time progressed the front part of the body, including the forelimbs, became involved in the movements resulting in contralateral torso movements. Finally, as the response was maximal, the whole body became involved in the movements resulting in contralateral body movements. Data are shown indicating that most of these behavioural phenomena were (1) dose-dependent, (2) locus-specific, and (3) GABA-specific. Bilateral injections of picrotoxin resulted in similar characteristic movements, but now directed towards both sides and/or directed 'ventrocaudally'. Finally, it was found that blindfolding the animals did not change the response to unilaterally injected picrotoxin. As the behavioural phenomena described here are dissimilar to the effects observed after experimentally-induced alterations in GABAergic activity at the level of the substantia nigra, pars reticulata, it is concluded that the deeper layers of the colliculus superior, being an output station of the substantia nigra pars reticulata, transforms its input signals into new output signals. Finally, it is suggested that picrotoxin resulted in a fixed code at the level of the colliculus superior, forcing the animals to execute characteristic motor patterns.

4.2.1 INTRODUCTION

The deeper layers of the colliculus superior are considered to be one of the main output stations of the basal ganglia (Edwards et al., 1979; Graybiel, 1978; Scheel-Krüger, 1983). There is substantial evidence that descending fibres from the substantia nigra, pars reticulata impinge upon the deeper layers of the colliculus superior (Chevalier et al., 1985; Deniau et al., 1977; Graybiel, 1978; May & Hall, 1986; Vincent, Hattori & McGeer, 1978; Williams & Faull, 1985, 1988). The deeper layers of the colliculus superior gives rise to ascending projections to the pretectum, the medial geniculata complex, the intralaminar nuclei of the thalamus, the fields of Forel, and the zona incerta. Descending fibres terminate in the pontine nuclei, the raphe nuclei, the reticular formation, and the spinal cord (for rev., see Graham, 1977).

The substantia nigra pars reticulata receives GABAergic fibres from the caudate nucleus (for rev., see Scheel-Krüger, 1983). The behavioural effects of GABAergic substances injected into the reticular substantia nigra of cats have been reported in the preceding section (Section 4.1; see also Wolfarth, Kolasiewicz & Sontag, 1981). Muscimol, a potent GABA agonist, elicited asymmetric responses such as fast, contralateral head-to-tail circling, fast contralateral head turning, contralateral posturing, and stereotyped licking. On the other hand, picrotoxin, an agent that closes the chlorid channels that are opened by GABA, resulted in a characteristic behavioural state denoted as 'freezing', viz. absence of any movement for a period of at least 30 s. Furthermore, picrotoxin elicited asymmetric posturing, static head turning, and an inability to lift the hindlimbs when the forelimbs were put on a bar placed 2 m above the floor (Cools et al., 1983). Because of the dissimilarity of the behavioural response evoked in the caudate nucleus (Cools, Struyker Boudier & Van Rossum, 1976) and the substantia nigra pars reticulata, respectively, it could be concluded that the substantia nigra not only transmits it's incoming signals, but also transforms them into new output signals (Cools et al., 1983).

As we are interested in the way in which information leaving the basal ganglia is

mediated downstream towards the level of the spinal cord, we focused our attention on the colliculus superior. The deeper layers of the colliculus superior receive GABAergic fibres from the substantia nigra pars reticulata (Chevalier et al., 1981; Vincent, Hattori & McGeer, 1978). Furthermore, there is evidence that striatonigral fibres project monosynaptically upon nigrocollicular output neurons (Scheel-Krüger, 1983; Williams & Faull, 1985), at least part of which are also GABAergic (Mize, 1988). In order to gain insight into the behavioural significance of this nigrocollicular GABAergic pathway we injected GABAergic substances into the deeper layers of the colliculus superior of cats. The results of these experiments may indicate whether information arriving at this level is also transformed into new output signals, in accordance to the concept obtained from the nigra studies (Section 4.1).

4.2.2 EXPERIMENTAL PROCEDURES

Cats of either sex (weighing 2.5-4.5 kg) were prepared as described previously (Cools, Struyker Boudier & Van Rossum, 1976). In order to allow intracerebral injections, two stainless steel cannulas (outer diameter 0.8 mm; diameter of the inner cannula that extended 1 mm below the tip of the outer cannula: 0.55 mm) were stereotactically implanted under sodium pentobarbital anaesthesia (40-50 mg/kg, i.p.) in each cat. In order to avoid damage to the tectum, the tip of the cannula was implanted into the corpus callosum (A 1.5, L 3.5, H 6.5; according to the atlas of Snider and Niemer, 1964). With help of a 5 μl Hamilton syringe (diameter if the injection needle with sharpened tip: 0.35 mm) small volumes (0.5-1.0 μl) were injected into the deeper layers (A 1.5, L 3.5, H 2.5) and superficial layers (A 1.5, L 3.5, H 4.5) of the colliculus superior. The colliculus superior is divided into superficial vs deeper layers according to the terminology of Kanaseki and Sprague (1974). One week after the implantation the cats were habituated to sound-tight wooden observation cage (90 x 60 x 60 cm) and the injection procedure during two 1-h sessions on separate days. Two weeks after the implantation the experiments were started, in which the behavioural responses to intracollicular injected substances were analyzed in freely

moving cats. The dependent variables in this analysis were operationally defined items, viz. isolated, forced, unidirectional movements of ears, head, torso (front part of the body including the forelimbs) or body. These movements were labelled as being present/absent during a certain time-interval (see below). All data are presented as percentage of cats showing a particular behavioural item. Each experiment was recorded on video-tape with help of a closed video-circuit. Fifteen minutes before the intracerebral injections were administrated the cat was placed in the observation cage in order to readapt to the experimental surroundings. Injections were given to conscious animals; the injection needle was not removed until 30 s after finishing the injection. After the injections, the behavioural response was observed and analyzed for 30 min. Picrotoxin (25-500 ng/0.5 μl; Serva) was injected unilaterally or bilaterally. Bilateral injections of distilled water, viz. the solvent of picrotoxin, served as control. In case picrotoxin (or muscimol, see below) was given unilaterally, the solvent was injected contralaterally. In case an animal was used again, the experiments were spaced by at least 7 days.

In an additional series of experiments muscimol (50 ng/1.0 μl) was injected ipsilaterally 20 min after the picrotoxin injection in order to determine whether muscimol counteracts the picrotoxin-induced behavioural response. The potency of muscimol in blocking the picrotoxin-induced response was analyzed from 25 till 60 min after the initial picrotoxin injection.

In a final series of experiments cats were blindfolded prior to the picrotoxin injections by covering the eyes by means of a bandage in order to determine whether signals derived from visual stimuli are anyhow involved in the picrotoxin-induced behavioural response.

In a separate group of cats (n=7) the tip of the guide cannulas were implanted into the deeper layers of the colliculus superior (A 1.5, L 3.5, H 2.5) in order to determine whether picrotoxin injected into the colliculus, now being destroyed as a consequence of the implantation, was still effective.

At the end of the experiments the cats were deeply anaesthetized and perfused intracardially with a 4% formaldehyde solution. the dissected brains were sectioned (frozen slices, 40 μm) and stained (cresyl violet) in order to estimate the exact location of the tip of the injection needle. Fisher's exact probability test was used for statistical analysis.

4.2.3 RESULTS

Unilateral injection of picrotoxin into the colliculus superior (A 1.5-3.0, L 3.0-4.5, H 2.5-3.5; see Figure 4.2.1A) resulted in a characteristic behavioural response. The

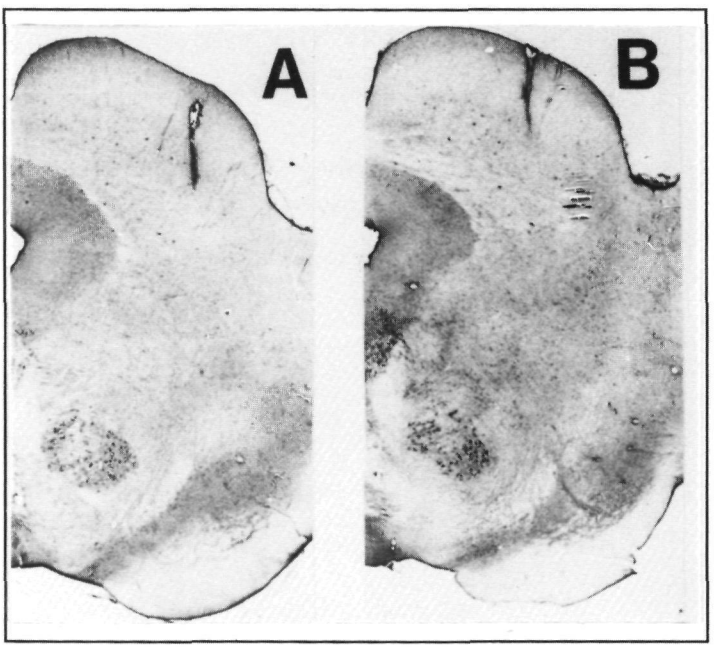

Figure 4.2.1 *Representative picture of cat brain with injection sites into the deeper layers (A) and into the superficial layers (B) of the colliculus superior.*

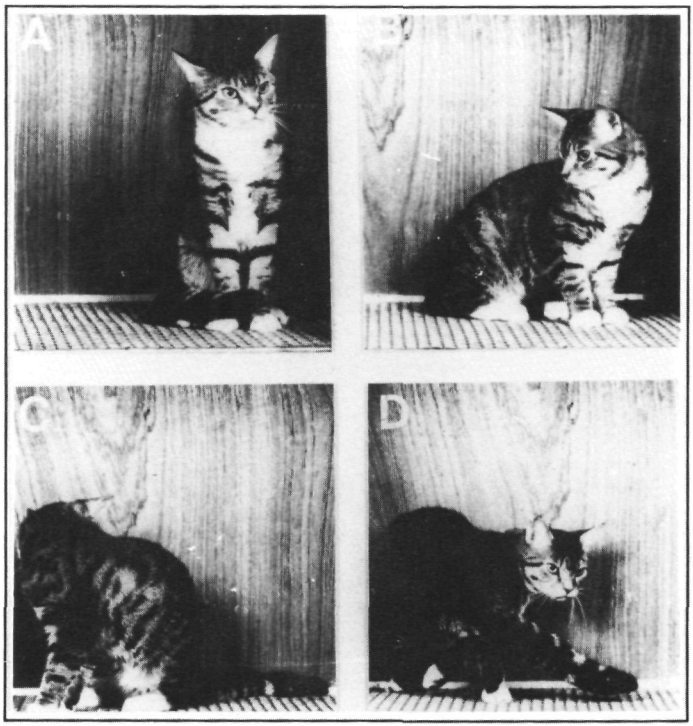

Figure 4.2.2 *The behavioural response to a maximal effective unilateral injection of 200 ng picrotoxin could be divided into different phases: A, the cat started with retroflexions of the contralateral ear, which are denoted as 'contralateral ear movements'. B, contralateral head movements. C, contralateral torso movements (front part of the body, including the forelimbs). D, contralateral body movements (maximal effect). See also text.*

behavioural response to picrotoxin (200 ng) is described in detail in order to illustrate its characteristic features. Within the first 3 min after the intracerebral injections the cat became markedly hypo-active. After 2-5 min the cat started to retroflex its contralateral ear, without displaying any other movement (see Figure 4.2.2A). These movements were labelled as 'contralateral ear movements'. After another 1-5 min the ear movements were followed by brisk, short, contralateral head turning movements

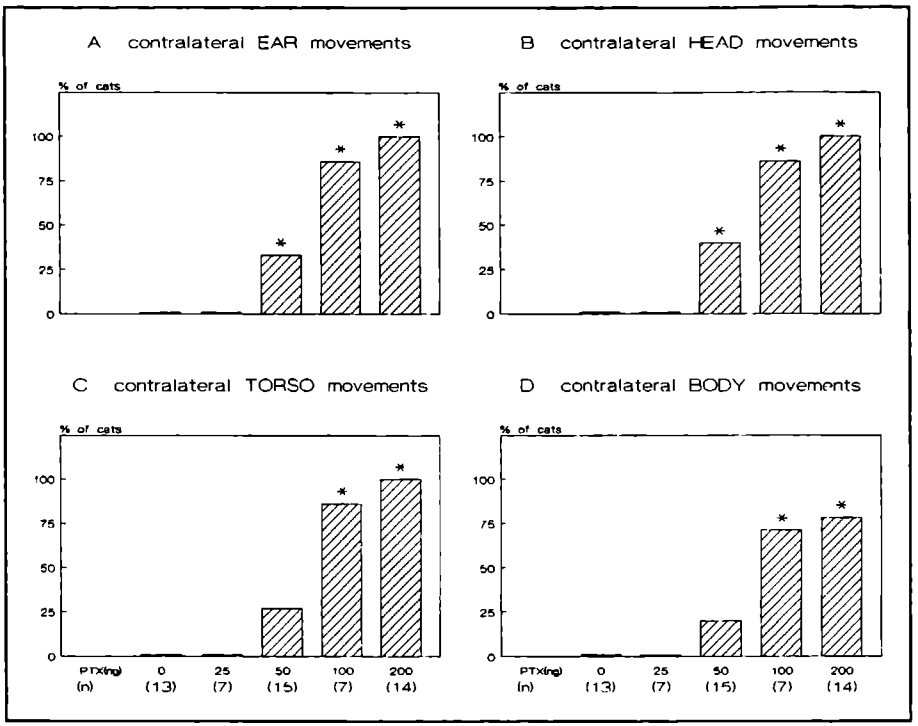

*Figure 4.2.3 Percentage of cats showing contralateral ear movements (A), head movements (B), torso movements (C) and body movements (D) after different doses of picrotoxin, 0-30 min after the unilateral injection. *p<0.05, drug vs. solvent.*

(see Figure 4.2.2B) in such a way that each subsequent contralateral head movement started from the position in which the preceding head movement had ended. This head movement was repeated several times. Finally, as the head position reached a deviation from the body axis of about 90°, the head was moved back to the neutral position, allowing the start of a new sequence of contralateral ear movements followed by contralateral head movements. These head movements were labelled as 'contralateral head movements'. As time progressed, deviations from the body axis increased resulting in the involvement of the front part of the body, including the forelimbs (see Figure

Table 4.2.1 Locus-specificity of picrotoxin-induced contralateral movements. percentage of affected cats.

The numbers in parentheses refer to the number of tested cats.

PICROTOXIN(ng/0.5 μl)	COLLICULUS SUPERIOR		
	Deeper layers	Deeper layers, lesioned	Superficial layers
50-100	62 (n=26)	0 (n=7)*	-
200-500	100 (n=19)	-	50 (n= 4)**

*p<0.05, colliculus superior, deeper layers vs. colliculus superior, deeper layers lesioned.
**p<0.05, colliculus superior, deeper layers vs. colliculus superior, superficial layers.

4.2.2C). Contralateral movements in which the front part of the body was involved were denoted as 'contralateral torso movements'. Fifteen to 20 min after the picrotoxin injection not only the torso but the whole body of the cat became involved in the contralateral movements and some of the cats displayed tardy head-to-tail circling movements (see Figure 4.2.2D). These movements were labelled as 'contralateral body movements'. On the whole, picrotoxin (200 ng) remained effective for 60-90 min. Sequences of forced, contralateral movements, as described above, were frequently interrupted by non-forced, compensating head, torso and sometimes even body movements directed to the body axis. Furthermore the animals were capable of avoiding objects by stepping over or moving away during the time in which picrotoxin was effective; they were able to fixate visual stimuli in a normal way, thereby interrupting the forced contralateral movements for a short moment.

As shown in Figure 4.2.3 the behavioural response to picrotoxin appeared to be dose-dependent. Solvent (0.5 μl), viz. distilled water, as well as picrotoxin, 25 ng dissolved in 0.5 μl, remained ineffective. In contrast, picrotoxin, 50 ng, elicited contralateral ear (33%, n=15; Figure 4.2.3A), head (40%, n=15; Figure 4.2.3B), torso

(27%, n=15; Figure 4.2.3C), and body movements (20%, n=15; Figure 4.2.3D), whereas a dose of 100 ng elicited contralateral ear, head, and torso movements in 86% of the tested cats (contralateral body movements: 71%, n=7). Finally, 200 ng picrotoxin resulted in contralateral ear, head and torso movements (in all tested cats, n=14). Contralateral body movements were observed in 78% of the tested animals. In general, picrotoxin injections were labelled 'effective' in case they resulted in contralateral ear, head, torso and/or body movements during 30 min post-injection time.

As shown in Table 4.2.1 picrotoxin, injected into the superficial layers of the colliculus superior (A 1.5-3.0, L 3.0-4.5, H 4.0-5.0; see Figure 4.2.1B), was significantly less effective (50%, n=4 vs 100%, n=19, picrotoxin 200-500 ng injected into the deeper layers: $p<0.05$). Moreover, picrotoxin remained ineffective when injected into the colliculus, being partly or fully destroyed (0%, n=7 vs 62%, n=16; $p<0.05$; see Table 4.2.1).

Table 4.2.2 *GABA-specificity of picrotoxin-induced contralateral movements: percentage of affected cats. PTX 200, 200 ng picrotoxin (0.5 µl), injected at t=0 min; MSC 50, 50 ng muscimol (µl), injected at t=20 min; solv (µl), solvent of muscimol, injected at t=20 min.*

The numbers in parentheses refer to the number of cats in which the respective movements could be observed during 25-60 min after PTX.

BODY PARTS INVOLVED (t=25-60min)	PTX 200 (0.5) SLV (1.0)	PTX 200 (0.5) MSC 50 (1.0)	PTX 200 (0.5) MSC 50 (0.5)
Ear (n=3)	100	0*	100
Head (n=6)	100	67	100
Torso (n=6)	100	17*	100
Body (n=3)	100	0*	100

*$p<0.05$.

In a separate series of experiments, 20 min after the injection of 200 ng picrotoxin (i.e., when the maximal response was reached), either muscimol (50 ng dissolved in 0.5 or 1.0 μl) or its solvent (1.0 μl distilled water) was injected at the same locus (in addition, the solvent was again injected contralaterally). As is shown in Table 4.2.2 muscimol significantly counteracted the picrotoxin-induced contralateral ear, torso and body movements, provided that muscimol was dissolved in a volume of 1.0 μl ($p<0.05$, muscimol vs solvent injected 20 min after picrotoxin). Contralateral head movements were fully abolished in 2 out of 6 cats during the observation time. In case muscimol was dissolved in 0.5 μl it remained ineffective (Table 4.2.2).

In a final series of experiments it was found that bandaging the eyes did not alter the behavioural response to picrotoxin (100 ng, Table 4.2.3).

Bilateral application of picrotoxin (200-500 ng) resulted in head movements to both sides (200 ng: 100%, n=7; 500 ng: 100%, n=12) and/or bending of head plus torso resulting in an anteroflexed position, thereby touching the floor with the ears (200 ng: 86%, n=7; 500 ng: 100%, n=12). In such a position some animals executed small head movements to both sides. Animals with strong ventroflexed torso moved

Table 4.2.3 Bandage effects of picrotoxin-induced contralateral movements: percentage of affected cats.

The numbers in parentheses refer to the number of tested cats.

BODY PART INVOLVED	PTX 100 (0.5)	PTX 100 (0.5) + bandage
Head	86 (n=7)	83 (n=6)**
Torso	86 (n=7)	67 (n=6)**
Body	71 (n=7)	67 (n=6)**

* Ear movements could not be assessed when the eyes were bandaged.
** n.s. ($p>0.05$), bandage present vs. bandage absent.

sometimes backwards or, even, 'somersaulted' and jumped backwards.

4.2.4 DISCUSSION

The present data show that the deeper layers of the colliculus superior plays a crucial role in the cerebral organization of behaviour. Decreasing the GABAergic activity by closing the chloride channels with help of picrotoxin elicited a characteristic and reproducible behavioural response. In a familiar and static environment, viz. absence of changes in exteroceptive stimuli which could direct the behaviour, picrotoxin resulted in the successive appearance of contralateral movements of respectively the ears, the head, the torso and, finally the whole bode. Moreover, the behavioural response to picrotoxin appeared to be dose-dependent. In case the deeper collicular layers were damaged, picrotoxin remained ineffective indicating that this part of the colliculus superior is crucial for the expression of the picrotoxin-induced phenomena. This finding is in agreement with studies performed with rats, in which it was also found that the colliculus superior is essential for the picrotoxin-induced turning phenomena (Imperato & Di Chiara, 1981; Kilpatrick, Collingridge & Starr, 1982). Furthermore, picrotoxin was significantly less effective when injected into the superficial layers of the colliculus superior. Taking together these data it can be concluded that in our experiments the picrotoxin-induced behavioural phenomena are mediated via the colliculus. Probably due to diffusion picrotoxin was effective in some of the animals in cases in which the injection was placed in the superficial layers (see Section 4.2.3, Results).

The behavioural response to picrotoxin appeared to be GABA-mediated, as it turned out that muscimol was able to counteract the picrotoxin-induced contralateral ear, torso and body movements. Although the dose of muscimol, which was 25 % of the dose of picrotoxin, should be potent enough to counteract the picrotoxin-induced effects (cf. Arnt & Scheel-Krüger, 1979; Scheel-Krüger et al., 1978), it turned out that muscimol had to be applied in a volume twice of that of picrotoxin to be effective. Obviously,

there seemed to be a certain minimum area which had to be covered by muscimol in order to counteract the picrotoxin-induced effects. Muscimol, which has a very low diffusion rate in contrast to picrotoxin, seemed unable to reach this minimum area in case it was injected in the same volume as picrotoxin.

With respect to muscimol, it is of interest to realize that doses up to 400 ng, dissolved in 0.5 μl, applied unilaterally or bilaterally never resulted in the occurrence of forced movements in the open field test (data not shown).

Bilateral injections of picrotoxin did not extinguish each other as might be expected because of the fact that unilateral injections elicited unidirectional responses. In fact, the behavioural response to bilaterally applied injections showed great similarity to the response to unilateral injections; the response differed with respect to the direction in which the movements were executed: 'ventrocaudal' vs contralateral.

The behavioural response to unilaterally applied picrotoxin was not comparable to the behavioural phenomena observed after disturbing the GABAergic activity in the substantia nigra pars reticulata (see Section 4.1; Wolfarth, Kolasiewicz & Sontag, 1981), a structure that projects directly onto the deeper layers of the colliculus superior (see Section 4.2.1, Introduction). Given the fact that activation of striatonigral GABA systems results in a decrease in GABA release in the colliculus superior (Scheel-Krüger, 1983), intracollicular picrotoxin should reflect an increased intranigral (pars reticulata) GABAergic activity. However, intracollicular picrotoxin did not evoke a behavioural response that was comparable to the response evoked by intranigral muscimol. Neither the fast contralateral head-to-tail circling and the fast contralateral head turning nor the stereotyped sniffing, that are elicited by intranigral muscimol, were observed after collicular injections of picrotoxin.

With respect to the reticular substantia nigra it was concluded that this structure, being an output station of the caudate nucleus, not just transmits its incoming signals but actually transforms them into new output signals (Section 4.1). The same holds true

for the deeper layers of the colliculus superior because of the following. First, the colliculus is considered to be an output station of the substantia nigra (see Section 4.O, General Introduction). Second, the behavioural expression of the deeper layers of the colliculus superior is dissimilar to that of the substantia nigra pars reticulata (see above). Both sets of data allow the conclusion that at the level of the colliculus input signals are again transformed into new output signals; the latter are qualitatively different from the former signals.

Traditionally, the colliculus superior is considered to be a structure that transforms multisensory information into motor commands, for instance resulting in eye movements (Dräger & Hubel, 1975; Gordon, 1973; McHaffie & Stein, 1982; Nagata & Kruger, 1979; Peck, Schlag-Rey & Schlag, 1980; Stein, Goldberg & Clamann, 1976; Wurz, 1978). In those experiments, in which we bandaged the eyes of the cats, it turned out that blindfolding did not change the response to picrotoxin indicating that the response to picrotoxin was not due to disturbances of the sensory input.

Summarizing, the present data have shown that decreasing the GABAergic activity within the deeper layers of the colliculus superior induced the execution of a specific kind of behaviour: picrotoxin, by lowering the GABAergic activity, seemed to fix a particular code forcing the animal to execute characteristic motor patterns.

CHAPTER 5

CONSEQUENCES OF A PROGRESSIVELY DYSFUNCTIONING BRAIN STRUCTURE ON THE PROGRAMMING OF MOTOR BEHAVIOUR

5.0 GENERAL INTRODUCTION

The results of the experiments described in Chapter 3 show that decreasing the caudate dopaminergic activity with the help of intracerebral injections of the dopamine antagonist haloperidol reduces the animal's ability to arbitrarily switch motor patterns, i.e., to switch motor patterns which are not directed by exteroceptive, proprioceptive, or even conditioned stimuli. However, activation of dopamine receptors with the help of locally applied apomorphine in a dose of 0.6 μg did not affect the ability to arbitrarily switch motor patterns in the treadmill paradigm. The latter notion is confirmed in the bar paradigm where it was also found that this dose of apomorphine was unable to affect switching behaviour (Section 3.3). On the other hand, systemic application of apomorphine in rats results in a 'break-down' of motor behaviour (see Section 5.1.1). It can be hypothesized that this break-down is due to the involvement of caudate output stations indirectly affected by activation of caudate dopamine receptors. In order to provide evidence in favour of this hypothesis it was first investigated whether activation of caudate dopamine receptors by relatively high doses of apomorphine produces a break-down of the motor pattern sequence in the treadmill paradigm. Next, it was investigated whether a comparable break-down can be induced by functional changes at the level of the deeper layers of the colliculus superior. In Section 5.1, data are presented that intracaudate injections of relatively high doses of

apomorphine are able to produce a break-down of a motor pattern sequence. In Section 5.2 data indicating that a comparable break-down may be elicited by intracollicular injections of picrotoxin are presented.

Summary

During the ontogeny of many mammalian species there exists a remarkable resemblance with respect to the strict order in the appearance of distinct motor patterns during development. The same sequence in motor behaviour can be observed when adult animals start to explore a novel environment. On the other hand, s.c. injections of apomorphine result in a reversed 'ontogenetic' sequence of motor patterns: a 'break-down' of motor behaviour (Szechtman et al., 1985). The present study investigated whether apomorphine, injected into the rostromedial part of the caudate nucleus, produces a 'break-down' of a motor pattern sequence. Therefore, cats were tested in a paradigm in which they executed sequences of distinct motor patterns in order to collect food pellets when walking on the belt of a treadmill. As only one of the motor patterns in the sequence is caudate-specific (see Section 3.1), disturbances at the level of the rostromedial caudate nucleus as well as disturbances at the level of other brain structures can be distinguished. In contrast to 0.6 and 2.5 μg, doses of 5.0 and 10.0 μg of apomorphine resulted in the successive break-down of motor pattern sequences whereby not only caudate- specific, but also non-caudate specific motor patterns were reduced. Moreover, this regression appeared in the reversed order compared to the order in which distinct patterns are executed during eating behaviour. The regression in motor behaviour following 5.0 μg apomorphine was induced via caudate dopamine receptors since it could be prevented by pretreatment with haloperidol. Because of the fact that 5.0 and 10.0 μg of apomorphine also affected non-caudate specific motor patterns, it is concluded that also brain structures receiving (in)directly caudate output signals are involved in the regression of the motor pattern sequence as observed in the present study. The clinical relevance of the present data is discussed.

5.1.1 INTRODUCTION

Dopamine stimulating agents such as apomorphine and amphetamine are known to induce the repetitive execution of particular sets of motor patterns after i.p. or s.c. injections (cf. Costall & Naylor, 1973; Fray et al., 1980; Redgrave et al., 1982). Often these motor patterns seem to be restricted to specific body parts such as the snout ("sniffing" movements), the tongue ("licking" movements), the mouth and jaws ("gnawing/biting" movements), the head ("checking" movements), the forelimbs (lateral and forward/backward movements) or the fore- and hindlimbs ("circling" and "locomotion" movements) (Fray et al., 1980; Jerussi & Glick, 1976; Ridley et al., 1980; Schoenfeld et al., 1975; Segal et al., 1980). Within the brain of mammals there are several neural substrates such as the striatum, the nucleus accumbens, the olfactory tubercle, the septum and the frontal cortex, which contain dopamine receptors (Bannon & Roth, 1983; Fallon & Moore, 1978; Moore & Bloom, 1978; Poitras & Parent 1978; Ungerstedt, 1971). Until now, it remains unclear in which way distinct responses as described above are determined by the involvement of different dopamine containing structures (cf. O'Neill & Filenz, 1985).

According to detailed observations of Szechtman et al. (1980, 1985) the apomorphine-induced locomotor behaviour actually consists of several phases when rats are tested in an open field (in which exteroceptive stimuli are static and, accordingly, do not elicit changes in the ongoing behaviour). Moreover, there exists a strict order in which various body parts become successively excluded from the initial hyperactive stage. The first phase is characterized by exploration activity including vertically directed motor patterns. The second phase is characterized by straightforward progression, i.e. predominant longitudinally directed "locomotion" motor patterns: In this phase vertically directed motor patterns are no longer executed. The third phase is characterized by lateral movements predominantly executed by the head and forelimbs; the hindlimbs are no longer involved in the execution of the motor behaviour. During the final phase, only very small lateral head movements are executed, i.e. even the forelimbs are no longer involved in the execution of the motor behaviour. Comparable kinematic

processes as well as opposite versions are found in the transition in respectively out of relative arrest in a variety of mammalian species (Golani & Moran, 1983). For example, the apomorphine-induced sequence is precisely the mirror image of the sequence appearing in rats which start to explore a novel environment (Golani & Moran, 1983). The finding that the latter sequence also occurs during the ontogeny of the rat (Golani et al., 1981) has given rise to the suggestion that different neural structures become successively involved in the execution of the behaviour during the process of development (Szechtman et al., 1980, 1985). In fact, this principle of built-up of sequences of motor patterns is characteristic for the ontogeny of motor behaviour in vertebrates in general (Eilam, 1985; Golani et al., 1979; Golani & Moran, 1983). The above-mentioned data about the exploratory behaviour of rats indicate that the ability to (in)activate successively different neural substrates still occurs in adult individuals. This notion is underlined by the finding that rats with electrolytic hypothalamic lesions, f.i., also show the 'ontogenetic' sequence of motor patterns during the recovery from their lesion. Since the latter technique is known to damage the nigrostriatal dopamine fibres (Marshall et al., 1974) these data suggest that at least neostriatal dopamine receptors are involved in the 'ontogenetic' sequence of motor patterns during the recovery process. In an attempt to provide evidence in favour of the hypothesis that apomorphine-induced break-down of motor pattern sequences can be induced by stimulation of striatal dopamine receptors the present study investigated whether intracaudate administered apomorphine indeed produces a break-down of sequences of motor patterns which are normally built-up in the reversed order. For reasons mentioned below the cat was chosen as experimental animal. The effects of intra-caudate injections of apomorphine was analyzed in cats switching motor patterns in order to collect food pellets when walking on a treadmill (Section 3.1). This paradigm was chosen because of two reasons: First, the animals were challenged to execute an ordered sequence of at least six different motor patterns. Therefore, this paradigm allowed the detection of apomorphine-induced changes in a well defined sequence of distinct motor patterns in a qualitative manner. Second, previous studies have shown that one out of the six motor patterns, which cats normally display on the treadmill, disappear after an experimentally-induced inhibition of caudate (rostromedial part)

dopamine receptors, i.e. executing gait transitions without continuously fixating any external stimulus. Since only the latter motor pattern is caudate-specific (Section 3.1), the chosen paradigm allowed the analysis of apomorphine-induced effects in terms of changes that are either due to disturbances in the involvement of the caudate nucleus itself or due to changes in the involvement of other brain structures. Data will be shown that apomorphine -dopamine-specific and dose-dependent- is able to produce a break-down of the ordered sequence of motor patterns in a highly characteristic manner. It is concluded that apomorphine is able to induce a regression in sequences of motor patterns by interaction with caudate dopamine receptors.

5.1.2 EXPERIMENTAL PROCEDURES

Animals

Male cats were trained to walk on a treadmill and to collect food pellets while the belt was running (speed 1.0-1,25 km/hr) as previously described (Section 3.1). Behind the front panel of the roofed treadmill (120 x 20 x 65 cm) a remote controlled food dispenser was attached. In order to collect a food pellet (specially shaped pellets, Hope Farms) the cat had to bend its head through the opening in the front panel (see Figure 3.1.1). The food dispenser was constructed in such a way that the animal, when walking, was unable to note the successive deliveries of single food pellets, thus preventing the use of exteroceptive stimuli to direct the eating behaviour. After the training phase, the cats (weighting 3-4.5 kg) were anaesthetized (sodium pentobarbitone 45 mg/kg i.p.) and stereotaxically equipped with stainless steel cannulas (outer diameter 0.8 mm, outer diameter of inner cannula extending 1 mm below the tip of the guide cannula: 0.55 mm) into the rostromedial part of the caudate nucleus (A 15.0, L 5.0, H 5.0 according to the atlas of Snider and Niemer, 1964). Two weeks after the implantation the experiments were started. Each cat was used for maximally five experiments, spaced by at least one week. Naive cats (n=17) received either solvent (n=11) or apomorphine 5.0 μg (n=6). The apomorphine-treated cats also participated in the following experiments (see experimental paradigm): apomorphine 2.5 μg (n=6, second

treatment), apomorphine 10.0 μg (n=5, third treatment) and the combination of haloperidol (12.5 μg) and apomorphine (5.0 μg, n=5, fourth treatment). On the other hand, the solvent-treated cats also received apomorphine 0.6 μg (n=10, second treatment). Some of the latter animals (n=5) were in addition used to extend the apomorphine 5.0 μg group. After the final experiments, the animals were deeply anaesthetized with pentobarbital and intracardially perfused with a 4% formaldehyde solution. After removal of the brains the target sites were verified according to previously described procedures (Section 3.1.2).

Experimental paradigm

At the beginning of the experiment (t= 0 min) the cat, which was deprived of food for 24 hrs, was placed on the treadmill. At t= 15, 35 and 55 min the belt was started, so each experiment consisted of three test periods each lasting 5 min. Bilateral injections (volume 5.0 μl) were manually administered with help of a 5 μl Hamilton syringe (outer diameter of needle: 0.4 mm, protruding 1.5-2.0 mm below the tip of the guide cannula) to the conscious, hand-fixed animal five minutes before the start of the second test period. Previous studies, in which open field behaviour was analyzed, have shown that the effect of intracaudate apomorphine remains constant and maximally effective during 5 to 10 min after the injection (Cools, Struyker Boudier & Van Rossum, 1976). The first test period served as a control ('PRE') whereas during the second and the third test period (respectively 'POST1' and 'POST2') drug effects could be analyzed. Freshly prepared solutions of apomorphine hydrochloride (Brocades) were used in each experiment (solvent was distilled water). In the present study, apomorphine was injected bilaterally in doses of 0, 0.6, 2,5, 5.0 and 10.0 μg. Previous experiments (Cools, Struyker Boudier & Van Rossum, 1976) have shown that doses up to 5.0 μg are dopamine- and locus-specific when injected into the rostromedial part of the feline caudate nucleus whereas a dose of 15.0 μg results in addition in behavioural effects characteristic for another caudate subregion (the anterodorsal part of the nucleus). In a separate series of experiments, the dopamine specificity of the observed effects was established by injecting 5 min prior to apomorphine the dopaminergic

antagonist haloperidol (12.5 μg, Serenase, Janssen Pharmaceutica). The motor behaviour on the treadmill was recorded on video tape with help of a closed video-circuit.

Analysis

During eating behaviour cats repeatedly executed complete sequences consisting of distinct motor patterns in the following order (Figure 5.1.1):

1. Walking in the middle of the treadmill, i.e. following the speed of the belt (walking normally: WLKnm).
2. Accelerating gait in order to approach the front panel (ACC).
3. Changing the interlimb coordination by decreasing the forelimb steplength and increasing the hindlimb steplength, i.e. executing gait transitions (TRN).
4. Collecting a food pellet by bending the head through the opening in the front panel of the treadmill enclosure, i.e. food collecting attempts (FCA).

During these sequences animals sometimes repeated a particular pattern before they switched to the next motor pattern, underlining the relative independency of the display of the distinct motor patterns. As soon as a sequence terminated with FCA, it was considered to be 'complete'. In case FCA or FCA and one or more additional motor patterns were missing, the resulting sequence was labelled as 'incomplete'.

Apart from certain walking patterns (see below), all walking patterns that were displayed during the PRE test period were labelled as 'normal' (WLKnm). Walking patterns were labelled as 'abnormal' in case the cat continuously touched the back panel with the tail and/or the hindlimbs (WLKab, see Figure 5.1.1); this motor pattern hardly occurred during the PRE test period.

Although cats displayed two types of gait accelerations, i.e. those accompanied continuously by fixation of exteroceptive stimuli (ACCed) and those devoid of continuous fixation of exteroceptive stimuli (ACCnd), the latter were hardly executed and, accordingly, not further analyzed (see Section 3.1). In contrast, the former, in which the animal continuously fixated visually a particular part of the front panel, were

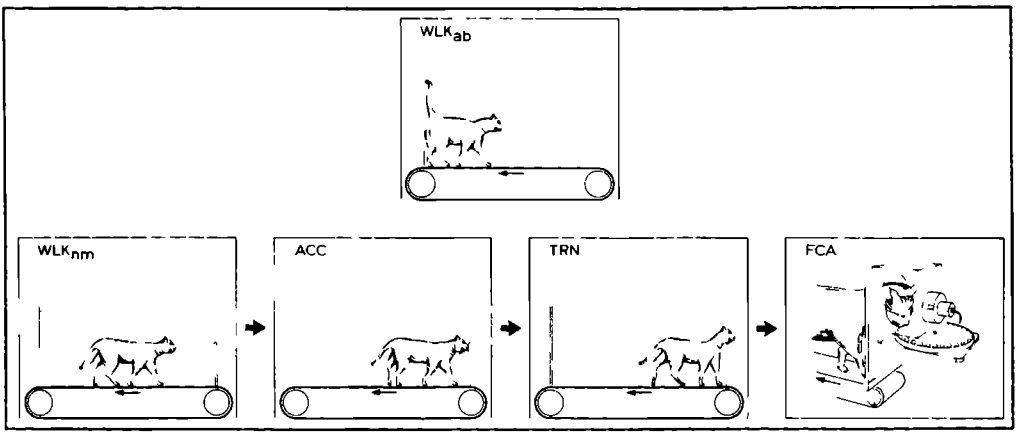

Figure 5.1.1 Sequence of motor patterns during eating behaviour of cats on the treadmill. WLKab, abnormal walking; WLKnm, normal walking; ACC, gait acceleration; TRN, gait transition; FCA, food collecting attempt.

fully analyzed; they were labelled as 'exteroceptively directed gait accelerations'. As previously described (Section 3.1), cats also displayed two types of gait transitions. This motor pattern was labelled as 'exteroceptively directed gait transition' (TRNed) in case the cat continuously fixated visually and/or tactually (with forelimbs and/or whiskers) the front panel or the belt of the treadmill. On the other hand, the motor pattern was labelled as 'non-exteroceptively directed gait transition' (TRNnd) in case the cat not continuously fixated the front panel or the belt. Previously it has been found that blocking the caudate dopamine receptors by haloperidol selectively decreases the number of TRNnd. Accordingly, only changes in the number of TRNnd reflect changes in the degree of involvement of the caudate nucleus in the execution of the motor behaviour.

During each test period, the absolute number of these motor patterns was determined. Walking was scored in 3 seconds bins in order to allow a frequency analysis. A minimal duration of 3 s for walking was chosen in view of the observation

that the maximal duration of ACC, TRN and FCA was about 3 seconds (2.2 ± 1.1 s, N=786, which is total of ACC+TRN+FCA during all PRE test periods). Drug-induced changes are illustrated as changes in number of motor patterns expressed as percentages of total of motor patterns during that observation period. Furthermore, it was previously found that intra- and interindividual variability could be reduced by expressing post-injection values as a percentage of pre-injection values. Because of the fact that certain subclasses of the above mentioned motor patterns are not executed in the PRE test period, the following motor pattern ratio was determined: The ratio of the difference (post-score minus pre-score: numerator) and sum (post-score plus pre-score: nominator). Ratio's were compared using Mann Whitney U-test (two tailed).

Figure 5.1.2 Distribution of injection sites within the rostromedial part of the caudate nucleus of cats treated with 5.0 µg apomorphine (only one side is shown; cross-sections according to Snider and Niemer, 1964).

5.1.3 RESULTS

All injections were within the target area, coordinates: A 14.0-15.5, L 5.5-6.5, H 5.5-7.0 (see Figure 5.1.2).

A representative illustration of the absolute number of distinct motor patterns per minute during POST1 following solvent treatment is shown in Figure 5.1.3 (A, left panel). This figure shows the normal distribution of different motor patterns during a POST1 test period (which in fact was comparable to that of the PRE test periods, data not shown; see also Table 5.1.1). The absolute number of distinct types of sequences is depicted in the same figure (Figure 5.1.3 A, right panel), illustrating that complete

Table 5.1.1 Absolute number of distinct motor patterns (median + range) in PRE test period (before administration of intracaudate injections.

n= number of animals; H, haloperidol 12.5 μg. For abbreviations see section 5.1.2.

μg (n)	PRE TEST PERIOD					
	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
0	0	10	14	13	10	38
(11)	0-3	0-28	3-23	2-20	0-26	9-60
0.6	0	7	10	7	9	35
(9)	0-1	3-14	4-18	5-25	1-24	21-68
2.5	0	4	22	1	5	30
(6)	0-6	1-11	1-58	0-6	0-26	21-50
5.0	0	14	21	2	7	20
(11)	0-12	0-21	6-34	0-21	0-15	8-39
10.0	0	8	15	2	2	13
(5)	0-7	3-24	12-32	0-18	1-18	9-42
5.0+H	1	5	15	8	17	48
(5)	0-7	0-42	9-36	0-18	0-40	22-56

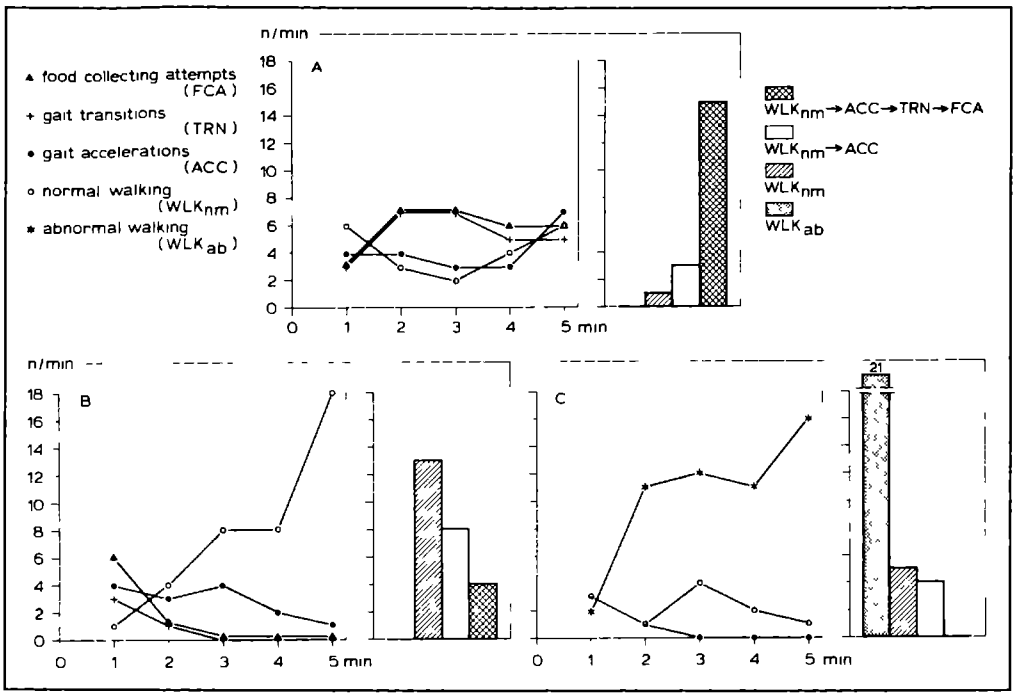

Figure 5.1.3 Representative examples of individual scores per minute of different motor patterns during POST1 following solvent (A) or apomorphine 5.0 μg (B, C, left panels) as well as of absolute number of complete and incomplete sequences during POST1 (A-C, right panels; all panels: scale of the ordinate ranges from 0 to 18 as is depicted on the left side of the figure).

sequences are mainly executed following solvent treatment. The lowest doses of apomorphine, i.e. 0.6 and 2.5 μg, did not induce any change in this respect. These doses of apomorphine neither altered the relative frequency of any particular motor pattern (see Figure 5.1.4) nor altered any ratio during any post-injection test period (Table 5.1.2 and 5.1.3): like solvent-treated cats, they repeatedly executed sequences of motor patterns, starting with normal walking (WLKnm) and followed by respectively accelerating the gait (ACCed), executing gait transitions (TRNed as well as TRNnd) and collecting food pellets (FCA, see also Figure 5.1.1). Accordingly, apomorphine 0.6

or 2.5 μg did not affect the ability to execute normal sequences of motor patterns during eating behaviour on the treadmill.

Profound changes were found after injecting higher doses of apomorphine, i.e. 5.0 and 10.0 μg. Apomorphine 5.0 μg changed in a very dramatic way the ability to execute sequences of motor patterns during eating behaviour on the treadmill: more and more incomplete sequences appeared. In fact, the motor pattern sequence was broken down in a strict order: in subsequently executed sequences, distinct motor patterns disappeared in the following order: food collecting attempts, gait transitions, gait accelerations and, finally, normal walking patterns. In several cases, the display of normal walking patterns was replaced by the display of abnormal walking patterns. In other words 5.0 μg apomorphine resulted in a progressive break-down of the motor pattern sequence in the reversed order during the POST1 test period. The strength of the apomorphine-induced break-down in motor pattern sequence differed between individuals: in some animals, 5.0 μg of apomorphine completely prevented the display of food collecting attempts (FCA) during POST1. Ultimately occurring sequences in these animals were almost completely confined to abnormal walking patterns (WLKab,

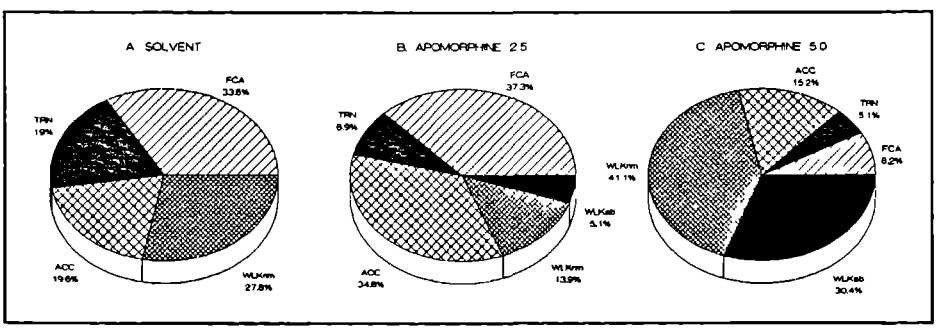

Figure 5.1.4 *Percentage of the mean frequency of the distinct motor patterns shown during POST1 test periods, i.e. 5-10 min after administration of solvent (A), apomorphine 2.5 μg (B) and 5.0 μg (C). Turning clock-wise, the distinct motor patterns are ordered according to their appearance in the sequence.*

Table 5.1.2 Median ratio of difference (numerator) and sum (nominator: POST1-PRE / POST1+PRE) of percentages of distinct motor patterns 5-10 min after injection of apomorphine (O, 0.6, 2.5, 5.0, 10.0 μg) and apomorphine (5.0 μg) 5 min after injection of haloperidol (H, 12.5 μg).

n= number of animals. (for abbreviations see section 5.1.2).

μg (n)	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
0 (11)	0	0.50	0.02	-0.19	-0.12	-0.19
0.6 (9)	0	0.31	-0.06	-0.13	-0.04	-0.14
2.5 (6)	0.09	0.11	0.10	0.08	-0.51	-0.11
5.0 (11)	0.56*	0.40	-0.21*	-0.04	-0.51*	-0.82**
10.0 (5)	0.94**	0.38	-0.81*	-1.00*	-1.00**	-0.85*
5.0+H (5)	0.02+	-0.10	0.20++	0.01	-0.04++	0.06++

*p<0.05, **p<0.02: apomorphine vs. solvent; +p<0.05, ++p<0.02: apomorphine (5.0 μg) + Haloperidol (H, 12.5 μg) vs apomorphine (5.0 μg).

n=3/11). In two of these cases, abnormal walking patterns disappeared for a short period. Instead, these animals showed disturbances in maintaining a correct posture, i.e. they started swinging with head and limbs and even tried to lie down on the moving belt of the treadmill (data not shown). In other animals, this dose of apomorphine only eliminated FCA and TRN (n=2/11). In the remaining animals (n=6/11), 5.0 μg apomorphine resulted in a break-down of the sequence until only normal (n=4/11) or abnormal (n=2/11) walking patterns remained. Representative illustrations of the apomorphine-induced effects are shown in Figure 5.1.3 (B and C). Changes in the absolute number of motor patterns per minute during POST1 reflecting the successive disappearance of FCA, TRN and ACC (in this order) and the appearance of WLKnm

Table 5.1.3 Median ratio of difference (numerator) and sum (nominator: POST2-PRE / POST2+PRE) of percentages of distinct motor patterns 25-30 min after injection of apomorphine (O, 0.6, 2.5, 5.0, 10.0 μg) and apomorphine (5.0 μg) 5 min after injection of haloperidol (H, 12.5 μg).

n= number of animals. For abbreviations see section 5.1.2.

μg (n)	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
0 (11)	0	0.32	0.13	0	0	-0.16
0.6 (9)	0	0.25	-0.26	-0.02	-0.17	-0.11
2.5 (6)	0	0.04	0.11	0.11	-0.14	-0.08
5.0 (11)	0	0.30	-0.17	0	0	-0.22
10.0 (5)	0	0.19	-0.12	-0.39	-0.49	-0.10
5.0+H (5)	0.18	0	-0.12	0.04	-0.10	-0.10

are shown in Figure 5.1.3 B (left panel). This break- down of motor pattern sequences is also reflected in the decreased number of complete sequences and the increased number of incomplete sequences (right panel). A dose of 5.0 μg of apomorphine completely prevented the execution of FCA and TRN in the case illustrated in Figure 5.1.3 C. As time progresses, ACC are decreased whereas WLKab is increased. The break-down is also illustrated by the absence of complete sequences, the decreased number of sequences consisting of WLKnm followed by ACC and the increased number of sequences solely consisting of WLKab (Figure 5.1.3 C, right panel). Thus, apomorphine 5.0 μg resulted in all tested animals (n=11) in a break-down of motor pattern sequences during eating behaviour in such a way that motor patterns 'early' in the sequence (i.e. normal walking patterns and accelerations) disappeared later than motor patterns which are executed 'late' in the sequence (i.e. transitions and food

collecting). However, the start as well as the end of the break-down differed between individuals. The overall effect of various doses of apomorphine is illustrated by changes in the relative number of distinct motor patterns during POST1 (see Figure 5.1.4). Table 5.1.2 shows that apomorphine 5.0 μg significantly reduced the ratio of food collecting attempts (FCA), non-exteroceptively directed gait transitions (TRNnd) and exteroceptively directed gait accelerations (ACCed) whereas the ratio of abnormal walking patterns (WLKab) was significantly increased.

In POST1, a dose of 10.0 μg apomorphine resulted in a break- down of the motor pattern sequence which was comparable to the effect of 5.0 μg (see Figure 5.1.4). The ratio of food collecting attempts (FCA), non-exteroceptively directed gait transitions (TRNnd) and gait accelerations (ACCed) was significantly decreased whereas the ratio of abnormal walking patterns (WLKab) was significantly increased. Apart from these effects, 10.0 μg of apomorphine also resulted in a significantly reduced ratio of exteroceptively directed gait transitions (TRNed) in POST1 (Table 5.1.2).

Normal motor pattern sequences reappeared in POST2 after 5.0 and 10.0 μg of apomorphine. The animals were able to execute sequences of motor patterns during eating behaviour, including normal walking, gait accelerations, gait transitions, and food collecting attempts (see Figure 5.1.5, Table 5.1.3).

The observed regression following 5.0 μg apomorphine was prevented by pretreatment of 12,5 μg haloperidol (Figure 5.1.6) indicating that this effect was induced by a selective interaction of apomorphine with dopamine receptors. Changes in ratio's following apomorphine were significantly reduced by the additional haloperidol injection (Table 5.1.2). Finally, the combined treatment significantly increased the ratio of exteroceptively directed gait transitions (TRNed) compared to solvent ($p<0.05$).

5.1.4 DISCUSSION

Previously, it has been shown that apomorphine 0.6 μg is able to counteract haloperidol-specific changes in motor patterns during eating behaviour of cats on the treadmill (Section 3.1). Moreover, this dose of apomorphine is able to induce caudate and dopamine-specific effects in open field behaviour (Cools, Struyker Boudier & Van Rossum, 1976). The present observations reveal that this dose of apomorphine is ineffective in changing the animal's ability to execute sequences of motor patterns during eating behaviour on the treadmill. The same holds true for 2.5 μg apomorphine. Taking together these data, it can be concluded that stimulating dopamine receptors of the rostromedial caudate nucleus with help of apomorphine in doses of 0.6 and 2.5 μg does not disturb the execution of normal motor pattern sequences during eating behaviour of cats on the treadmill.

In contrast, 5.0 μg apomorphine dramatically deteriorated the ability to execute normal sequences. Moreover, there appeared to be a strict order in which distinct motor patterns were affected following apomorphine treatment. At first the break-down of the sequence was manifested in the disappearance of the final motor patterns, i.e. food collecting attempts and gait transitions. During the next phase also gait accelerations were reduced. Finally, even abnormal walking patterns replaced normal walking patterns in part of the animals. This regression in motor pattern sequences was probably not due to interaction of apomorphine with non-dopamine receptors nor due to leakage of the drug outside the target area because of the following reasons: first, this regression following 5.0 μg apomorphine could be prevented by the additional injection of haloperidol suggesting that apomorphine induced its effect via dopamine receptors. Second, this dose of apomorphine is known to produce locus- as well as dopamine-specific effects in open field behaviour (for ref.: Cools, Struyker Boudier & Van Rossum, 1976) suggesting that these apomorphine induced effects are due to interaction with dopamine receptors within the target area. From these data it can be concluded that the break-down of the motor pattern sequence following 5.0 μg apomorphine, as is observed in the present study, resulted from a selective interaction

with caudate dopamine receptors.

Furthermore, the observed regression as described above is not likely to be due to a change in apomorphine efficacy at the level of the dopamine receptors because of the following reason: previous experiments, in which open field behaviour of cats following unilateral intracaudate injections were analyzed, have revealed that the effect of apomorphine increases during the first minutes following injection, remains maximal between 5 and 10 min, and gradually disappears during 10 to 15 min after application (for ref.: Cools, Struyker Boudier & Van Rossum, 1976) suggesting that during 5 to 10 min post injection the effective interaction of apomorphine at the level of the caudate nucleus dopamine receptors remains invariant. This implies that during the process of regression as observed in the present study the efficacy of apomorphine at the level of the caudate dopamine receptors also remained invariant.

Previously, it has been shown that intracaudate haloperidol decreases the number of non-exteroceptively directed gait transitions and increases the number of exteroceptively directed gait transitions. In contrast to the former effect, the latter turned out not to be dopamine-specific since pretreatment with apomorphine did not reduce this haloperidol-induced increase (Section 3.1). The present study shows that even a dose of 5.0 μg apomorphine was not able to reduce this haloperidol-induced increase, indicating that this effect was only triggered by, but not specific for, blockade of the caudate dopamine receptors. Moreover, in the present study only the highest dose of apomorphine used (10.0 μg) induced a significant decrease of the ratio of non-exteroceptively directed gait transitions. At present, it cannot be excluded that this decrease was due to leakage of apomorphine outside the target area.

As described previously, the rostromedial caudate nucleus plays a circumscribed role in the organism's ability to programme arbitrarily (non-stimulus directed) behaviour. A dysfunctioning caudate nucleus can be manifested not only at the level of patterning arbitrarily motor behaviour (Cools, 1980) but also at the level of programming arbitrarily social behaviour (Van den Bercken and Cools, 1982) and even at the level

of programming arbitrarily cognitive strategies in patients suffering from Parkinson's disease (Cools et al., 1984). The present data show that caudate-specific motor patterns, i.e. TRNnd, disappeared 'early' during the regression, whereas non-caudate specific motor patterns, i.e. ACCed and WLKnm, disappeared 'late'. Furthermore, intracaudate apomorphine dose-dependently and dopamine-specific resulted in a progressive regression of the motor pattern sequence. This observation suggests that in the first stage of the regression the caudate nucleus was still involved in the programming of the motor behaviour as can be judged from the presence of TRNnd. Since apomorphine is known to be effective at that time (Cools, Struyker Boudier & Van Rossum, 1976), stimulation of caudate dopamine receptors *per se* did not disturb the ability to program motor behaviour arbitrarily (see also Section 3.1). In the next stage of the regression the caudate nucleus was no longer involved as can be judged from the absence of TRNnd; the presence of ACCed and WLKnm at this stage of the regression indicates that other brain structures, involved in the programming of these motor patterns, were still functioning in a normal way. As time progresses, these structures too were no longer involved in the programming of motor behaviour as can be judged from the disappearance of ACCed and the appearance of WLKab. These data show that a relative hyperstimulation of caudate dopamine receptors has direct consequences for extrastriatal structures that direct the programming of ACCed and WLKnm. Since the apomorphine-induced decrease of ACCed and increase of WLKab was inhibited by haloperidol, it can be concluded that these extrastriatal structures receive directly or indirectly information from the caudate nucleus, implying that the structures directing ACCed and WLKnm are simply output stations of the caudate nucleus. This reasoning does not hold true for TRNed because of afore-mentioned arguments (see above).

The observation that the caudate-specific motor pattern TRNnd disappeared at a certain stage in the regression process is difficult to explain: it cannot be ascribed to an aspecific effect of an overdose of apomorphine, since haloperidol was still able to counteract it. Still, it is possible to understand this phenomenon by recalling the fact that ACCed and WLKnm were still present at the time during which TRNnd started

to disappear. Given the notion that the effective dose of apomorphine changed the caudate output and, accordingly, the activity of brain structures directing ACCed and WLKnm, it is not unlikely that the behavioural expression of the caudate nucleus was replaced by the behavioural expression of lower order structures which actually produced a functional 'shut- down' of the behaviour characteristic of their hierarchically higher order structures (for details: Cools, 1985).

The finding that the process of regression as is shown in the present study involved several stages each of them characterized by its own final motor pattern (respectively FCA, TRN, ACC, WLKnm and WLKab) suggest that this functional 'shut-down' was repeated during final stages of the regression process at successively lower order output stations of the caudate nucleus. Thus, the regression in motor behaviour may be due to a subsequent elimination of motor programming functions of the caudate nucleus and that of its output stations. This notion is in line with the suggestion of Szechtman and colleagues (1980, 1985) that different neural systems are involved in apomorphine-induced regression in motor behaviour.

Anyhow, the present data show that the feline caudate nucleus is a principal target site for apomorphine-induced behavioural regression. Future studies are needed to show that the rodent neostriatum plays a comparable role in i.p. injected apomorphine-induced regression processes.

The present study may have important clinical implications. Patients, suffering from Parkinson's Disease often receive L-Dopa. Several studies indicate that although this therapy reduces the classic symptoms such as hypokinesia and rigidity, often poor performance is still present in cognitive as well as in motor tests, appealing to the patient's ability to program arbitrarily behaviour (cf. Bowen et al., 1975; Bowen, 1976; Cools et al., 1984; Flowers & Robertson, 1985); in spite of the L-dopa application the patient's caudate nucleus apparently is not functioning in a normal way. Furthermore, during L-Dopa treatment about half of the parkinsonian patients develop so-called "On-Off"-phenomena (Lewitt & Chase, 1983), which probably are not due to chronic

treatment or progression in state of illness (Lang et al., 1982). Typically, "Off"-phenomena seem to occur after peak plasma levels of L-Dopa have been reached (Fahn, 1974). During an "Off" period, some patients do not improve following i.v. administration of L-Dopa or lisuride (Hardie et al., 1982) suggesting that this effect is not due to insufficient dopamine receptor stimulation. Considering these clinical data, the present study implies that these "Off"-phases might reflect 'regression' processes due to the subsequent exclusion of the caudate nucleus as well as of other brain structures from motor programming as a result of the (over)activation of striatal dopamine receptors.

Summary

Intracaudate injections of relatively high doses of apomorphine produce a regression in motor behaviour of cats collecting food pellets in a treadmill design (see Section 5.1). It has been hypothesized that this regression is partly due to functional disturbances in brain regions receiving (in)directly striatal output signals. In view of this hypothesis, it was investigated whether experimentally-induced changes in GABAergic activity within the deeper layers of the colliculus superior, which is a second order output station of the caudate nucleus, are also able to elicit a regression in motor behaviour. Therefore, motor behaviour of cats was tested in the treadmill paradigm before and after intracollicular injections of the GABA antagonist picrotoxin. Picrotoxin produced dose-dependently a regression in motor behaviour which was comparable to that elicited by intrastriatally injected apomorphine. The noted effects were GABA-specific since muscimol attenuated the picrotoxin-induced regression. The present data are discussed in view of a model for a hierarchical organization of the brain.

5.2.1 INTRODUCTION

Cats repeatedly execute sequences of distinct motor patterns in order to collect food pellets during walking on a treadmill (see Section 3.1). Such sequences consist of the following motor patterns: "normal walking", whereby the cat follows the speed of the belt of the treadmill (1); "gait accelerations", i.e. increases in walking speed in order to approach the front panel of the treadmill enclosure behind which a food dispenser is mounted (2); "gait transitions", i.e. changes in interlimb coordination by decreasing the steplength of the forelimbs and increasing that of the hindlimbs (3); and, finally, "food collecting attempts", whereby the cat bends its head through the opening in the opaque front panel in order to collect a food pellet (4). In Section 3.1, it has been

shown that caudate nucleus injections of the dopaminergic antagonist haloperidol only reduce the number of one particular subclass of gait transitions, i.e. so-called 'non-exteroceptively directed gait transitions'; the number of the remaining motor patterns in the sequence is not reduced. Haloperidol treated cats are still able to collect food pellets. Intracaudate injections of the dopaminergic agonist apomorphine have been found to prevent the haloperidol induced reduction. Apparently, non-exteroceptively directed gait transitions, but not other motor patterns, are selectively mediated by caudate dopamine receptors. In contrast to the rather low dose of apomorphine, viz. 0.6 μg, that is able to counteract the haloperidol-induced reduction, relatively high doses of apomorphine, viz. 5-10 μg, disrupt not only non-exteroceptively directed gait transitions, but also other motor patterns (see Section 5.1). In fact, the motor behaviour on the treadmill is broken down in a very particular way: the order in which the distinct motor patterns disappear in subsequent sequences is opposite to that in which they typically appear in intact sequences. Thus, apomorphine affects the distinct components of the sequence according to the rule 'last in, first out'. Since the apomorphine (5-10 μg)-induced 'regression' in motor behaviour as described above is inhibited by intracaudate injections of haloperidol despite the fact that at least some of the involved motor patterns are not caudate-specific (see above), it has been suggested that this regression is due to the distortion of information sent by the caudate nucleus to striatal output stations. Accordingly, we investigated whether functional changes at the level of an output station of the caudate nucleus also produce such a regression in the motor pattern sequence.

One of the major output stations of the caudate nucleus is the substantia nigra, pars reticulata (Graybiel & Ragsdale, 1979; Royce & Laine, 1984; see Section 2.3). The reticular pars reticulata gives rise to projections towards distinct thalamic nuclei, the mesencephalic reticular formation and the deeper layers of the colliculus superior (Beckstead & Frankfurter, 1982; Behan, Lin & Hall, 1987; Edwards et al., 1979; Graybiel, 1978; Hopkins & Niessen, 1976; Illing & Graybiel, 1985; May & Hall, 1984; Warton et al., 1983). The colliculus superior is divided into deeper and superficial layers according to the terminology of Kanaseki and Sprague (1974). The deeper layers

of the colliculus superior serve as an important output station funnelling striatally-derived signals as shown by studies on tonic EMG-activity (Ellenbroek et al., 1985), stereotyped movements (Dean, Redgrave & Eastwood, 1982; Imperato & Di Chiara, 1981) and turning behaviour (Kilpatrick, Collingridge & Starr, 1982; Morelli et al., 1981; Reavill et al., 1984).

Electrophysiological (Chevalier et al., 1981; Karabelas & Moschovakis, 1985), biochemical (Araki, McGeer & McGeer, 1984; Vincent, Hattori & McGeer, 1978) and behavioural (Gelissen & Cools, 1986; 1987) studies have shown that one of the neurotransmitters of the caudatonigral and the nigrocollicular pathway is the inhibitory amino acid gamma-aminobutyric acid (GABA; for rev. see Scheel-Krüger, 1983; 1986). Activation of striatal dopamine receptors is reported to decrease the release of GABA in the deeper layers of the colliculus superior (Gale & Casu, 1981; Scheel-Krüger, 1983).

In the present investigation, the GABAergic antagonist picrotoxin was injected into the deeper layers of the colliculus superior of cats tested in the treadmill design (see Section 3.1 and 5.1). Picrotoxin was chosen in view of the fact that this agent is known to elicit dose-dependent and GABA-specific effects in a dose range from 50 to 200 ng/ 0.5 μl. Moreover, these doses of picrotoxin produce locus-specific effects since they are neither effective when injected into the upper layers of the colliculus superior nor effective when injected into lesioned deeper layers of the colliculus superior (see Section 4.2). Given these considerations, the collicular injection of 25-50 ng picrotoxin would appear to be a valid tool to mimic the biochemical consequences of caudate injections of apomorphine at the level of the deeper layers of the colliculus superior (Gale & Casu, 1981; Scheel-Krüger, 1983). In the present study, the GABA-specificity of the behavioural effects induced by 50 ng picrotoxin was studied with the help of the GABAergic agonist muscimol.

5.2.2 EXPERIMENTAL PROCEDURES

Animals and apparatus

Adult male cats (3.0-4.5 kg) were selected from a breeding colony of the University of Nijmegen. They were housed in iron cages (1.9 x 1.2 x 1.6 m) in groups of 4 to 7 animals. Except during training and experiments (see below) food (Hope Farms) and water were present ad libitum. The apparatus was the same as has been described in Section 3.1 and 5.1. For an extensive description of training procedures and apparatus the reader is referred to Section 3.1. In short, the cats were trained to walk on the motor-driven belt (speed 1.0-1.25 km/hr) of a roofed treadmill (120 x 20 x 65 cm; see Figure 3.1.1). A remote-controlled food dispenser was attached at the outer side of the opaque front panel. The cat was able to collect food pellets (specially shaped, Hope Farms) by bending its head through an opening (10 x 12 cm) in the front panel. The food dispenser was constructed in such a manner that the cat, while walking, was unable to detect the delivery of a food pellet.

Surgical and histological procedures

After the training phase, the cats were stereotaxically equipped with stainless steel cannulas under sodium pentobarbitone anaesthesia (40-45 mg/kg, i.p.). In order to avoid damage to the tectal tissue, the tip of the cannula (outer diameter of the guide cannula: 0.8 mm; diameter of the inner cannula that extended one mm below the tip of the guide cannula: 0.55 mm) was directed at a point 4 mm above the injection locus: coordinates A 1.5, L 3.5, H 6.5 (Snider & Niemer, 1964). Two weeks after the implantation, the treadmill experiments were started. After the final experiment, the cats were deeply anaesthetized with sodium pentobarbitone and intracardially perfused with a 4% formaldehyde solution. Subsequently, the brains were removed and cross sections (30 μm) were cut with help of a cryostat microtome (-20 oC). The slices were mounted onto slides and stained with cresyl violet to estimate the precise location of the injection spots.

Experimental paradigm

The animals were deprived of food for a period of 24 hrs before the start of an experiment. At the beginning of each experiment (t= 0 min), the cat was placed on the static belt of the treadmill. An experiment consisted of three test periods which started at t= 15, 35 and 55 min. Each test period lasted 5 min and was recorded on video-tape. At the beginning of a test period the belt was activated; at the end of the test the belt was turned off. All solutions were bilaterally injected with help of a Hamilton syringe (diameter of the needle with sharpened tip: 0.4 mm; see Section 4.2). Each cat participated in three experiments that were spaced by at least one week. Seven cats received 25 and 50 ng picrotoxin (Serva; dissolved in 0.5 μl distilled water) at t= 25 min during the first and the second experiment, respectively; these cats received distilled water (0.5 μl; control for the picrotoxin injections) at the same time during the third experiment. Seven other cats received 0.5 μl distilled water at t= 25 min (control for the picrotoxin injection) and 1.0 μl distilled water at t= 30 min (control for the muscimol injection; see below) during the first experiment; 50 ng picrotoxin at t= 25 min and 25 ng of the GABAergic agonist muscimol (Serva; dissolved in 1.0 μl distilled water) at t= 30 min during the second experiment; and 0.5 μl distilled water at t= 25 min and 25 ng muscimol at t= 30 min during the third experiment that served as a control for the second experiment. The first test period (PRE) served as a control (see below). Drug-induced changes in motor behaviour were analyzed during the second and the third test period (POST1 and POST2, respectively). Doses and volume of picrotoxin and muscimol, the time-schedule as well as the locus-specificity of the collicular injections were based on the results of the open field tests described in Section 4.2.

Analysis of motor behaviour

The behaviour on the treadmill was analyzed in the same way as has been reported in Section 5.1. Untreated cats repeatedly execute complete sequences of the following motor patterns.

1. Walking in the middle of the treadmill, whereby the cat follows the speed of the belt (walking normally: WLKnm).

2. Accelerating gait in order to approach the front panel (ACC).
3. Changing the interlimb coordination by decreasing the forelimb steplength and increasing the hindlimb steplength, i.e. executing gait transitions (TRN).
4. Attempting to collect a food pellet by bending the head through the opening in the front panel of the treadmill enclosure, i.e. food collecting attempts (FCA).
Although the motor patterns were executed in a particular sequence (see above), each pattern was executed in a relatively independent way. The independent nature of the motor patterns was demonstrated by the observation that sometimes the cats executed a particular pattern several times before they switched to the next pattern in the sequence. Walking patterns as described above were labelled as 'normal' since they were also present during PRE test periods (WLKnm). During 'abnormal' walking, that hardly occurred during PRE test periods, the cat continuously touched the back panel of the treadmill enclosure with its tail and/or hindlimbs (WLKab). A motor pattern sequence was considered to be 'complete' if it ended with FCA; it was considered to be 'incomplete' if FCA or one or more other motor patterns were absent.

As described in Section 3.1 and 5.1, cats could display two types of ACC: those accompanied by the continuous visual fixation of a particular part of the front panel, i.e. 'exteroceptively directed gait accelerations' (ACCed) and those not accompanied by continuous fixation, i.e. 'non-exteroceptively directed gait accelerations' (ACCnd). Since the latter patterns were rarely executed during any test period, only ACCed's were analyzed. Furthermore, cats displayed two types of TRN: those accompanied by the continuous tactile (with forelimbs and/or whiskers) and/or visual fixation of the belt and front panel of the treadmill, i.e. 'exteroceptively directed gait transitions' (TRNed) and those not accompanied by continuous fixation, i.e. 'non-exteroceptively directed gait transitions' (TRNnd).

As described in Section 5.1, striatal injections of 5-10 μg apomorphine have been found to produce a behavioural regression which is illustrated by the occurrence of time-dependent changes in the number of distinct motor patterns. Given the known order in which the distinct components of the sequence appear (see above), the above-

mentioned parameters were used to provide direct information about drug-induced changes in the sequence under study. In addition, a frequency analysis of distinct motor patterns was used to study drug-induced changes in the ability to execute specific patterns. Therefore, the absolute number of each motor pattern was determined per test period. WLK was scored in 3 s bins in order to allow a frequency analysis (see Section 5.1). Drug effects were expressed as changes in the percentage of each motor pattern of total of patterns during the POST1 and POST2 test period. The ratio of the POST-score minus the PRE-score (numerator) and the POST-score plus the PRE-score (nominator) was calculated in order to reduce the inter- and intra-individual variability. Ratio's were compared using the Wilcoxon matched pairs signed ranks test (two tailed) or the Mann Whitney U-test (two tailed).

5.2.2 RESULTS

Histology

Verification of the injection loci revealed that all injections were correctly placed in the deeper layers of the colliculus superior: coordinates (Snider & Niemer, 1964) A 1.0-1.5, L 3.5-4.0,
H 2.0-3.0 (see Figure 4.2.1A).

Motor behaviour during the PRE test period

The absolute number of the distinct motor patterns (median + range) during PRE test periods of all experiments is shown in Table 5.2.1. There were no significant differences between any of the experiments with respect to the number of distinct patterns during PRE test periods ($p>0.05$).

Motor behaviour during the POST1 test period

Injection of solvent did not produce abnormal walking behaviour in any of the cats tested. A representative example of the absolute number per minute of the distinct motor patterns during POST1 after solvent is shown in Figure 5.2.1 (A, left panel). The

Table 5.2.1 Median + range of absolute number of distinct motor patterns in PRE test periods (before administration of distilled water; SOLV), picrotoxin 25 and 50 ng/ 0.5 µl (PT25 and PT50, respectively), and muscimol 25 ng/ 1.0 µl (MC25). WLKab, abnormal walking; WLKnm, normal walking; ACCed, exteroceptively directed gait accelerations; TRNed, exteroceptively directed gait transitions; TRNnd, non-exteroceptively directed gait transitions; FCA, food collecting attempts (see Materials and Methods).

Seven cats participated in three experiments: PT25, PT50 and SOLV1, respectively. Seven other cats participated in the remaining experiments: SOLV2, PT50+MC25 and MC25, respectively.

DRUG (ng)	PRE					
	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
SOLV[1]	0	6	16	1	10	24
	(0-3)	(2-47)	(5-35)	(0-32)	(1-27)	(15-46)
PT25	0	6	27	1	8	22
	(0-1)	(0-16)	(12-35)	(1-39)	(0-34)	(16-55)
PT50	0	15	18	3	11	22
	(0-0)	(4-41)	(12-44)	(0-18)	(0-41)	(11-34)
PT50	0	0	35	3	22	45
+MC25	(0-2)	(0-5)	(22-48)	(0-13)	(0-56)	(32-67)
MC25	0	0	26	2	18	43
	(0-0)	(0-5)	(13-59)	(0-15)	(0-54)	(39-72)
SOLV[2]	0	1	31	2	21	40
	(0-1)	(0-4)	(16-45)	(0-11)	(0-26)	(22-50)

[1]0.5 µl injected bilaterally 5 min after the end of the Pre test period.
[2]0.5 µl injected bilaterally 5 min after the end of the Pre test period, and 1.0 µl injected bilaterally 5 min after the first injections.

absolute number of complete and incomplete sequences is depicted in the same figure (5.2.1 A, right panel). The display of distinct motor patterns as well as the execution of sequences was comparable to those during PRE test periods in all experiments.

In contrast to solvent, 25 ng picrotoxin significantly affected the execution of FCA and WLKab during POST1. The ratio of FCA was significantly decreased, whereas that of WLKab was significantly increased (Table 5.2.2). Although the ratio of the remaining

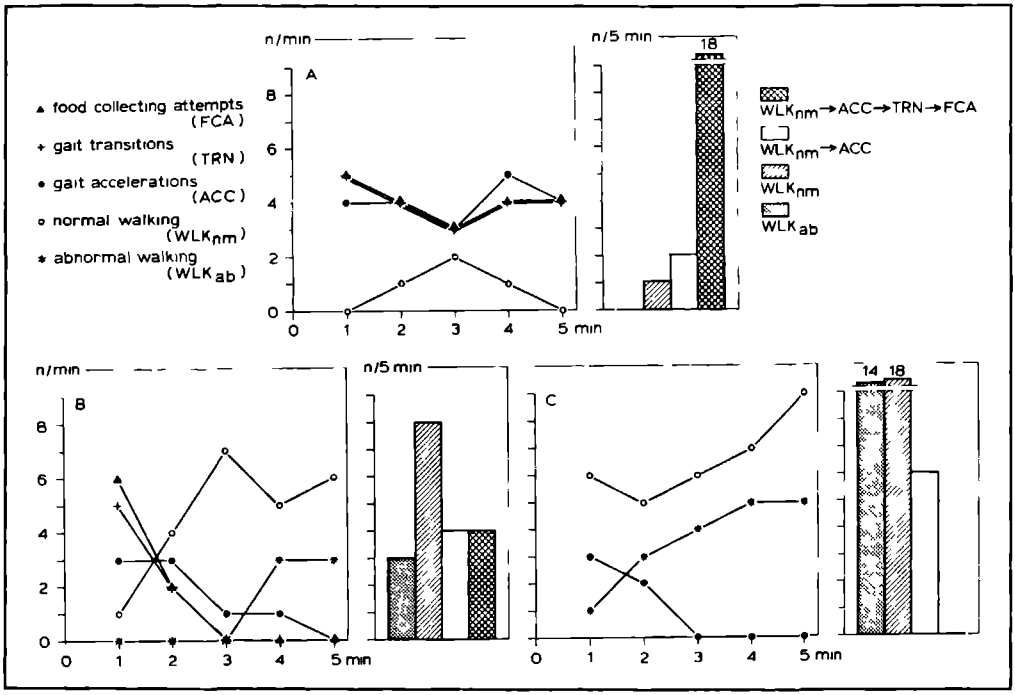

Figure 5.2.1 Representative examples of individual scores per minute of different motor patterns during the POST1 test period, i.e. 10-15 min following the bilateral 0.5 µl solvent (A) or 50 ng/0.5 µl picrotoxin (B, C; left panels) as well as of absolute number of complete and incomplete sequences during POST1 (A-C; right panels).

motor patterns, i.e. WLKnm, ACCed, TRNed and TRNnd, was not significantly changed, normal eating behaviour was clearly affected. In 4 out of 7 cats, FCA was strongly reduced, but not totally inhibited. In one other cat, this motor pattern was no longer present during POST1: this animal executed incomplete sequences that terminated with TRN during the whole POST1 test period. The remaining 2 cats displayed a regression of complete sequences which was comparable to that found after 50 ng picrotoxin (see below). WLKab was increased in 4 out of 7 cats; however, only one of these animals displayed a regression in motor behaviour. Figure 5.2.2 shows the

Table 5.2.2 Median ratio of difference (numerator) and sum (nominator. POST1-PRE / POST1+PRE) of percentages of distinct motor patterns during the POST1 test period after distilled water (0 5 µl; SOLV1), picrotoxin 25 and 50 ng/ 0.5 µl (PT25 and PT50, respectively), picrotoxin 50 ng/0 5 µl 5 min before injection of muscimol 25 ng/1 0 µl (PT50 + MC25), 0.5 µl distilled water 5 min before 1 0 µl distilled water (SOLV2) and 0 5 µl distilled water 5 min before muscimol 25 ng/ 1.0 µl (MC25)

Note that zero means no change, a positive value represents an increase and a negative value represents a decrease in the relative frequency of a pattern during POST1, compared to PRE. Each group consisted of 7 cats. The remaining abbreviations are explained in the legend of Table 5.2.1.

DRUG	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
SOLV1	0	-0.19	0.15	0	-0.10	-0.04
PT25	1.0*	0.16	-0.06	0	-0.19	-0.19**
PT50	1.0***	0.50**	-0.61*	-1.0*	-0.74**	-1.0*
PT50+MC25	0+++	0++	-0.05	0.14+	0+++	0+
MC25	0	0	0.03	0	-0.1	-0.02
SOLV2	0	0	-0.02	0	0	0.02

*p<0.05, **p<0.02, ***p<0.002: PT25, PT50, MC25 and SOLV2 vs SOLV1.
+p<0.05, ++p<0.02, +++p<0.002: PT50 + MC25 vs PT50.

relative frequency of distinct motor patterns.

A higher dose of picrotoxin, i.e. 50 ng, induced in 4 out of 7 cats a regression in the motor pattern sequence in the following way: initially, these animals executed complete sequences during POST1, but soon after the start of the test period more and more incomplete sequences appeared. The order in which motor patterns disappeared was relatively fixed; at first FCA, TRNnd and TRNed were no longer executed while the cats still displayed incomplete sequences that terminated with ACCed. During the next phase, also the latter pattern disappeared, while the frequency of WLKnm was

increased. During the final part of POST1, sequences consisted of WLKnm and/or WLKab. Two other cats did not execute complete sequences at all during POST1: they started with incomplete sequences that ended with ACCed; during POST1, ACCed disappeared while the animal executed WLKnm (one cat) or WLKab (one cat) during the remaining part of POST1. The 7th animal just executed a reduced number of complete sequences throughout POST1 (5, POST1 vs 11, PRE). In addition, this cat also displayed WLKab. Representative examples of the absolute number per minute of distinct motor patterns during POST1 are shown in Figure 5.2.1 (B and C, left panel). In the same illustration, the absolute number of complete and incomplete sequences are shown (Figure 5.2.1, B and C, right panel). Figure 5.2.2 shows the percentages of the mean frequencies of the distinct motor patterns. This illustration reveals that picrotoxin produced a shift from a predominance of FCA, TRN, ACC and Wlknm in controls to a predominance of WLKnm in cats treated with 50 ng picrotoxin. The highest dose of picrotoxin significantly reduced the ratio of ACCed, TRNed, TRNnd and FCA whereas it significantly increased that of WLKnm and WLKab (Table 5.2.2).

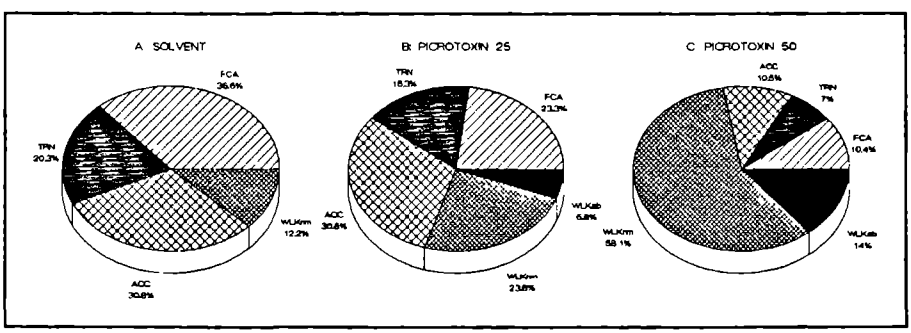

Figure 5.2.2 Percentage of the mean frequency of the distinct motor patterns shown during POST1 test periods, i.e. 10-15 min after the bilateral solvent 0.5 µl (A), picrotoxin 25 ng/0.5 µl (B) and 50 ng/0.5 µl (C); turning clock-wise, the distinct motor patterns are ordered according to their appearance in the sequence.

Motor behaviour during the POST2 test period

During POST2, picrotoxin was effective too, since some cats still showed a regression in the motor pattern sequence after 25 and 50 ng (3 and 4 animals, respectively). The relative distribution of the distinct motor patterns during POST2 was comparable to that during POST1 (data not shown). In POST2, the ratio of FCA was significantly changed after 25 ng picrotoxin, whereas that of WLKab, TRNed, TRNnd and FCA was significantly affected following 50 ng picrotoxin (Table 5.2.3).

Table 5.2.3 Median motor pattern ratio of difference (numerator) and sum (nominator POST2-PRE / POST2+PRE) of percentages of distinct motor patterns during the POST2 test period after distilled water (0.5 µl; SOLV1), picrotoxin 25 and 50 ng/ 0.5 µl (PT25 and PT50, respectively), picrotoxin 50 ng/0.5 µl 5 min before injection of muscimol 25 ng/1.0 µl (PT50 + MC25), 0.5 µl distilled water 5 min before 1.0 µl distilled water (SOLV2) and 0.5 µl distilled water 5 min before muscimol 25 ng/ 1.0 µl (MC25)

Note that zero means no change, a positive value represents an increase and a negative value represents a decrease in the relative frequency of a pattern during POST2, compared to PRE. Each group consisted of 7 cats. The remaining abbreviations are explained in the legend of Table 5.2.1.

DRUG	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
SOLV1	0	0.35	0.06	0	-0.11	-0.02
PT25	0	0.18	-0.03	0	-0.16	-0.45**
PT50	1.0**	0.54	-0.08	-1.0**	-0.42*	-0.27*
PT50+MC25	0+++	0	-0.04	0+	0.12+	0.18++
MC25	0	0	-0.08	0	0	0.04
SOLV2	0	0	-0.04	0	0	-0.03

*p<0.05, **p<0.02: PT25, PT50, MC25 and SOLV2 vs SOLV1.
+p<0.05, ++p<0.02, +++p<0.002: PT50 + MC25 vs PT50.

Additional observations

Apart from the above-mentioned regression effects, 5 out of 7 cats displayed abnormal limb movements following 50 ng picrotoxin. This effect was most pronounced during ACCed and WLKnm; it was characterized by the increase of the swing-phase of one hindlimb, as a result of an increased lifting and a short interruption of the movement just before touch-down. Sometimes, this effect was also present in the forelimb ipsilateral to the affected hindlimb. During POST2, no abnormal limb movements were observed. As a final remark, neither incorrect FCA's, i.e. head movements directed towards a food pellet without collecting a pellet, nor 'forced' head movements were observed in any post-injection test period.

GABA-specificity of picrotoxin-induced effects

The regression of motor pattern sequences during POST1 and POST2 did not occur in case 25 ng muscimol was injected after the application of 50 ng picrotoxin. The effect of picrotoxin on the ratio of WLKab, WLKnm, TRNed, TRNnd and FCA was significantly attenuated by muscimol (Table 5.2.2, 5.2.3). In contrast, the effect of picrotoxin on ACCed was not counteracted by muscimol. Finally, 25 ng muscimol itself did not induce changes in the execution of motor patterns during POST1 or POST2.

5.2.4 DISCUSSION

General

The present study shows that injection of 50 ng picrotoxin into the deeper layers of the colliculus superior produced a characteristic regression in the motor pattern sequence of cats. The order in which the distinct motor patterns subsequently disappeared was opposite to that in which they typically appeared in intact sequences. Given the known GABA- and locus-specificity of the treatment used (see Section 4.2), this finding shows that the picrotoxin-induced effects were due to an inhibition of collicular GABAergic activity. Apart from the decrease in ACCed, all effects were counteracted by muscimol. The finding that intracollicular injections of 25 ng muscimol

alone did not alter the behaviour in the present design not only excludes the possibility that the muscimol-induced attenuation of the picrotoxin-induced effects was due to a functional rather than a pharmacological antagonism, but also indicates that this dose of muscimol was too low in order to significantly stimulate the GABAergic receptors in otherwise untreated cats. Whether this implies that the baseline activity at the level of the GABAergic receptors was rather high during the performance of the treadmill behaviour remains to be established.

The finding that the picrotoxin-induced reduction in the number of ACCed was not counteracted by muscimol opens the possibility that this effect was only triggered, but not mediated, by the picrotoxin-induced attenuation of the GABAergic activity in the deeper layers of the colliculus superior. In experiments described in Section 3.1, in which haloperidol was injected into the caudate nucleus, a similar phenomenon has been observed: haloperidol produces both a dopamine-specific effect, viz. a decrease in TRNnd, and a dopamine-triggered change, viz. an increase in TRNed. In a follow-up study, it has been found that caudate nucleus injections of high doses of apomorphine similarly affect TRNed (see Section 5.1), providing additional evidence that the drug-induced change in TRNed is at best triggered by, but not specific for, experimentally-induced alterations in caudate nucleus dopaminergic activity. A comparable phenomenon might underlie the picrotoxin-induced decrease in the number of ACCed.

Anyhow, the overall response to picrotoxin was comparable to that elicited by intracaudate injections of 10 μg apomorphine: like apomorphine, picrotoxin induced a regression in motor behaviour resulting in significant reductions in the number of FCA, TRNnd, TRNed and ACCed, and a significant increase in the number of WLKab (see Section 5.1). The picrotoxin-induced increase in the number of WLKnm, however, was not found in the apomorphine experiments. The latter effect might be due to the difference in the ratio's of WLKnm in the corresponding control experiments (ratio, picrotoxin experiments: -0.19; ratio, apomorphine experiments: +0.50), suggesting that there was a so-called ceiling effect in the apomorphine experiments. In this context, it

is useful to remark that the noted differences between the controls of the two series of experiments might be due to the fact that the apomorphine-treated cats were equipped with caudate cannulas in contrast to the picrotoxin-treated animals in the present study that were equipped with collicular cannulas. Anyhow, the above-mentioned data show that the response to 50 ng picrotoxin applied to the deeper layers of the colliculus superior is highly comparable to the response to 10 μg apomorphine injected into the caudate nucleus. This finding together with the notion that intracollicular injections of picrotoxin are able to mimic the biochemical consequences of striatally-applied apomorphine at the level of the deeper layers of the colliculus superior (see Section 5.2.1: Introduction) suggest that collicular disturbances were also involved in the regression process following intracaudate administration of apomorphine.

Specific effects of picrotoxin

Twenty-five ng picrotoxin resulted in significant changes in the number of WLKab and FCA: the former increased, whereas the latter decreased. Since only deprived cats are displaying FCA in the treadmill set-up (see Section 3.1), it is theoretically possible that picrotoxin just altered the internal state inherent in food deprivation and, as a result, attenuated the number of FCA. However, most cats did collect food pellets during the first and second post injection test period, indicating that they were still hungry at that time. Moreover, the treated animals executed as many ACC and TRN as solvent-treated cats. Since these motor patterns enabled the cats to approach the food pellets, it appears that they were still motivated. Therefore, it is unlikely that the reduction in FCA was just due to drug-induced changes in food motivation. However, future research in which an independent measure for food motivation is used, is required to verify this explanation.

Previously, collicular injections of picrotoxin (50-200 ng) have been reported to induce forced head movements in cats tested in an open field (see Section 4.2). In the present study, such movements were not seen in any of the tests. Apparently, these forced movements do not occur in case picrotoxin-treated cats are forced to perform

particular tasks (cf. Gelissen & Cools, 1986). Anyhow, the absence of these forced movements exclude the possibility that such movements could have hindered the cats to display FCA.

Recently, it has been found that the deeper layers of the colliculus superior are involved in the execution of so-called 'non-externally guided targeting movements', i.e. goal-directed movements that are elicited but not continuously guided by exteroceptive stimuli (Cools, 1986; Gelissen & Cools, 1986). Since picrotoxin has been found to enhance the display of such movements (Gelissen & Cools, 1986), the picrotoxin-induced decrease in FCA found in the present study cannot be ascribed to drug-induced changes in this particular function of the deeper layers of the colliculus superior.

The fact that the behavioural regression observed in the present study was not seen in previously performed colliculus superior experiments at our laboratory is not difficult to understand. The experimental paradigms used in the previous studies were fully inappropriate to allow the cat to display any form of behavioural regression. In contrast, the paradigm used in the present study was purposely chosen to investigate this particular phenomenon. Anyhow, these data show that the nature of the experimental set-up and, accordingly, the task determines whether a behavioural deficit becomes manifest or not.

The caudato-nigro-collicular feedforward loop

Previously, it was found that striatal injections of haloperidol significantly reduce the number of a particular motor pattern without affecting the ability to execute complete sequences during the treadmill task (see Section 3.1). In a subsequent study it has been found that intracaudate injections of 5.0 μg apomorphine produce a regression in motor behaviour without changing the number of certain motor patterns such as normal walking during the treadmill task (see Section 5.1). The same holds true for the effects elicited by collicular injections of 25 ng picrotoxin (present study). All these data together indicate that the picrotoxin-induced regression in motor behaviour was not

simply the consequence of the order in which distinct motor patterns had to be executed during the treadmill task.

Some of the mentioned picrotoxin-induced effects, namely the increase in the number of WLKab and the decrease in the number of FCA, have previously been observed in treadmill experiments, in which the substantia nigra pars reticulata was manipulated (Heim et al., 1986). So, it has been found that decreasing the nigral GABAergic activity results in (a) abnormal body positions and postures in cats being free to move and (b) abnormal limb movements in cats being challenged to walk (see Section 4.1; see also Gelissen & Cools, 1987; Heim et al., 1986; Wolfarth, Kolasiewicz & Sontag, 1981). Moreover, a decrease in nigral GABAergic activity has been found to suppress FCA (Heim et al., 1986). As mentioned, collicular injections of picrotoxin too elicited such effects. Since the picrotoxin treatment used in the present study was selective and specific for the deeper layers of the colliculus superior (see Section 4.2), it can also be excluded that the picrotoxin-induced changes were due to diffusion of picrotoxin to the substantia nigra. Thus, the effects elicited by 25 ng picrotoxin were neither due to distortion of the information sent by the substantia nigra to the colliculus superior nor due to leakage of collicularly injected picrotoxin to the substantia nigra. Nevertheless, the picrotoxin treatment of the colliculus superior produced effects which were similar to those elicited by picrotoxin treatment of the substantia nigra. In fact, these data show that intracollicularly injected picrotoxin anyhow produced a 'shut-off' of the substantia nigra pars reticulata, viz. its first order input station. Whether or not this phenomenon was mediated by direct or indirect colliculonigral connections remains open for future research (cf. Harting, 1977; Martin, 1969).

The functional shut-off of the substantia nigra pars reticulata produced by 50 ng picrotoxin was even greater than that elicited by 25 ng: for, 50 ng picrotoxin resulted not only in effects seen after 25 ng, viz. a decrease in the number of FCA and an increase in the number of WLKab, but also in clearcut abnormal limb movements, viz. behavioural consequences of a stronger dysfunctioning nigra (Gelissen & Cools, 1987; Heim et al., 1986). As mentioned in the Results section, 50 ng picrotoxin also reduced

the number of TRNnd in a GABA-specific manner. Given the known function of the caudate nucleus in directing the display of the latter motor pattern (see Section 3.1), this finding shows that 50 ng picrotoxin even produced a functional shut-off of the caudate nucleus. The present data may reveal a basic principle of cerebral organization: particular changes in a hierarchically lower brain structure can produce a functional shut-off of hierarchically higher order brain structures.

Apart from the above-mentioned effects, 50 ng picrotoxin produced changes in TRNed, ACCed and WLKnm, viz. effects which were not yet elicited by 25 ng picrotoxin. The finding that collicular injections of picrotoxin inhibited the display of ACCed is in agreement with previously reported data showing that such treatment reduces the display of externally guided targeting movements (Gelissen & Cools, 1986). Anyhow, as long as the brain structures mediating these effects are unknown it is not relevant to discuss these effects in more detail.

Conclusions

The present study shows that picrotoxin injected into the deeper layers of the colliculus superior produced a regression of motor behaviour which was comparable to that elicited by striatal injections of 10 μg apomorphine. Since collicular injections of picrotoxin or caudate injections of apomorphine both produce similar GABAergic changes at the level of the deeper layers of the colliculus superior (Gale & Casu, 1981; Scheel-Krüger, 1983), it is likely that a common mechanism at the level of the colliculus is involved. Further studies examining the ability of collicular injections of muscimol to block the regression in motor behaviour elicited by intracaudate injections of apomorphine would clarify this point. Anyhow, the present data are in line with the hypothesis that experimentally-induced changes at the level of the caudate nucleus can produce behavioural deficits inherent in dysfunctioning output stations. It is evident that this notion has far reaching consequences for the interpretation of the consequences of the progressive pathology of psychomotor diseases such as Parkinson's disease. For instance, it would imply that classical parkinsonian symptoms such as hypokinesia, rigidity, etc. become only manifest when the caudate pathology has resulted in a

malfunctioning of one or more striatal output stations.

Furthermore, the present data suggest that collicular injections of a low dose of picrotoxin (25 ng) produced a functional shut-off of the substantia nigra pars reticulata, and that the injections of a higher dose of picrotoxin (50 ng) produced, in addition, a functional shut-off of the caudate nucleus. Overall, the latter data might reveal a basic principle of the cerebral organization: changes in a hierarchically lower order brain structure are able to produce a functional shut-off of hierarchically higher order brain structures. The mechanisms underlying this principle remain to be investigated.

CHAPTER 6

PROGRESSIVE PATHOLOGY IN THE CAUDATO-NIGRO-COLLICULAR PATHWAY

6.0 GENERAL INTRODUCTION

As shown in Chapter 5, a relatively high dose of apomorphine produces a functional shut-off of the caudate nucleus (Section 5.1). A comparable phenomenon could be observed following manipulation of the second order caudate output station, the deeper layers of the colliculus superior (Section 5.2) suggesting that caudate output stations might be involved in the regression process. However, the shut-off was present only when the animal had to execute a particular sequence of different motor patterns. In this chapter, two experiments are presented in which it was investigated whether acute neuropathological changes at the level of the caudate nucleus are able to produce functional changes at the level of caudate output stations. Open field behaviour of cats was analyzed immediately after the intracaudate application of the neuro-excitatory compound kainic acid or after the unilateral occlusion of the middle cerebral artery. In Section 6.1 it will be shown that intracaudate injections of kainic acid produce a sequence of behavioural changes characteristic of functional changes at the level of the rostromedial caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the colliculus superior. In addition, metabolic activity was increased at the level of these three brain regions. In Section 6.2, data will be shown that unilateral occlusion of the middle cerebral artery also produced functional and metabolic changes at the level of the three afore-mentioned brain regions: However, the latter changes were diametrically opposite to those following kainic acid.

Summary.

The acute behavioural and metabolic consequences of functional changes following unilateral intracaudate kainic acid at the level of the feline rostromedial caudate nucleus, the substantia nigra, pars reticulata and the deeper layers of the colliculus superior were investigated. The present study became possible since it was previously found that unilateral changes in neurotransmission processes in these structures all result in behavioural alterations that can be distinguished from each other. During the first 17 min after kainic acid, all animals displayed contralateral forced staccato head turning; these movements are characteristic for an activation of dopamine receptors and/or inhibition of GABA receptors in the rostromedial caudate nucleus. Between 15 and 50 min, all animals displayed fast, uninterrupted contralateral forced head, torso or body turning; these movements are characteristic for an activation of nigral GABA receptors. From about 48 min, all animals displayed sequences of short contralateral forced ear, head, torso and body turnings; these movements are characteristic for an inhibition of collicular GABA receptors. Furthermore, most cats displayed ipsilateral orofacial dyskinetic movements during the whole 180 min observation period.

Metabolism was analyzed in three cats that received [^{14}C]-2-D-deoxyglucose immediately before, 5 min after or 70 min after kainic acid. Metabolism was increased in the ipsilateral caudate nucleus; this effect was most pronounced in the cat that received deoxyglucose immediately before kainic acid. Metabolic activity was increased in the ipsilateral substantia nigra, pars reticulata; this effect was most pronounced in the cat treated with deoxyglucose 5 min after kainic acid. Metabolism was increased in the ipsilateral deeper layers of the colliculus superior in the cat that received deoxyglucose 70 min after kainic acid. The present behavioural and metabolic data suggest that kainic acid produces an increasing pathology resulting successively in functional changes in the caudate nucleus, its output station the substantia nigra, pars reticulata and the nigral output station the deeper layers of the colliculus superior. It

is suggested that the successive appearance of the latter effects is inherent in the hierarchical order of the brain structures under study. The occurrence of orofacial dyskinetic movements during the whole observation period suggest that the former movements were not mediated via the striato-nigro-collicular pathway. Finally, apomorphine injected in the ipsilateral caudate nucleus one week after kainic acid, was significantly less effective compared to apomorphine injected one week before kainic acid. The clinical implications of the present data is discussed.

6.1.1 INTRODUCTION

Motor symptoms of central disorders like Parkinson's Disease and Huntington's Chorea are generally considered to have their origin in disturbances of neuronal activity at the level of the caudate nucleus and the putamen. This view is based in part on morphological studies indicating that degeneration of striatal input and/or intrinsic neurons occurs in patients suffering from these diseases; furthermore, clinical studies have supported this idea since, for instance in Parkinson's Disease, restoration of reduced striatal dopamine levels has been found to reduce at least partly the motor disturbances. Other disorders in which changes in striatal neurotransmission processes may be involved are, for instance, Gilles de la Tourette syndrome and tardive dyskinesia. Considering the variety of clinical symptoms associated with all disorders mentioned above (Ansell, 1981; Dakof & Mendelsohn, 1986; Devinsky, 1983; Hefter et al., 1987; Lasker et al., 1987; Martin, 1984; Schneider, Diamond & Markham, 1987), the question arises whether these manifestations are primary consequences of hyper- and/or hypofunctioning striatal systems, or whether part of these symptoms are due to secondary, functional alterations at the level of brain structures receiving abnormal, striatally-derived information (cf. Lasker et al., 1987).

Animal studies have shown that, for instance, an increased tonic EMG-activity that results in muscle rigidity resembling parkinsonian rigidity, can be elicited from the rat

neostriatum, the substantia nigra pars reticulata, the deeper layers of the colliculus superior and the ventromedial nucleus of the thalamus (Ellenbroek et al., 1985; Klockgether et al., 1986). These data stress two important issues: First, the ability to induce tonic EMG activity is not specific for one of the nuclei mentioned above; accordingly, the occurrence of such a phenomenon does not allow definite conclusions with respect to the possible involvement of one or more of the structures mentioned above. Second, functional disturbances at the level of more than one brain structure can only be differentiated in case unique, structure specific parameters can be analyzed in an independent way. Anyhow, these considerations underline the necessity to investigate the way in which output stations of structures, primarily affected by irreversible, neuropathological processes, start to dysfunction as a result of abnormal input signals.

The main output stations of the caudate nucleus are the globus pallidus and the substantia nigra pars reticulata; one of the neurotransmitters of these pathways is gamma-amino-butyric acid (GABA; Gale & Casu, 1981; Graybiel & Ragsdale, 1979; Royce & Laine, 1984). Striatally-derived information is passed further down by the reticular part of the substantia nigra towards the deeper layers of the colliculus superior, the midbrain reticular formation and several nuclei of the thalamus; again GABA is one of the neurotransmitters of these pathways (Beckstead, Domesick & Nauta, 1979; Beckstead & Frankfurter, 1982; Edwards et al., 1979; Gale & Casu, 1981; Graybiel, 1978; Graybiel & Ragsdale, 1979; May & Hall, 1986). GABA is an important neurotransmitter of the striatal output pathways as is stressed by the findings that changes in striatal dopaminergic and/or GABAergic activity are known to result in alterations in GABAergic activity at the level of the substantia nigra (pars reticulata) as well as the deeper layers of the colliculus superior (Gale & Casu, 1981; Scheel-Krüger, 1983). In previous behaviour studies, indirect evidence has been presented that changes in striatal dopamine activity may have functional consequences for extrastriatal structures such as the substantia nigra pars reticulata and the deeper layers of the colliculus superior (Chapter 5.1; see also Gelissen & Cools, 1988).

In order to provide evidence that neuropathological changes at the level of the caudate nucleus indeed result in functional alterations at the level of the Substantia nigra pars reticulata and the Deeper layers of the colliculus superior, the present study was performed. The present study became possible since it was previously found that unilateral changes in neurotransmission processes in these structures all result in behavioural alterations that can be distinguished from each other (Cools, Struyker Boudier & Van Rossum, 1976; see Chapter 4). Therefore, the acute behavioural effect following intracaudate injection of the neuro-excitant kainic acid (Coyle, 1983; Foster & Fagg, 1984; Olney, 1981) was investigated. Furthermore, the kainate-induced behavioural effects inherent in disturbances at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the colliculus superior were correlated to metabolic changes occurring in these structures. For the latter purpose, it was decided to use only a limited number of cats (n=3), since the effects of intracaudate experimentally induced changes on local metabolic activity in the caudate nucleus, the substantia nigra and the colliculus superior have been previously studied by others (Aiko et al., 1988; Kimura, McGeer & McGeer, 1980; McCulloch et al., 1982; Wooten & Collins, 1980). Finally, the chronic effect of kainic acid on striatal functions was investigated by comparing the behavioural effect of intracaudate apomorphine one week before and after kainic acid.

6.1.2 EXPERIMENTAL PROCEDURES

Surgical procedures

Under sodium pentobarbitone anaesthesia (45 mg/kg i.p.), 10 adult male cats (weighing between 3.5 and 4.8 kg) were stereotaxically equipped with double-barrelled, stainless steel cannulas (outer diameter 0.8 mm; outer diameter, inner cannula which extended 1 mm below the tip of the outer cannula: 0.55 mm) into the rostromedial part of the caudate nucleus (coordinates [Snider & Niemer, 1964]: A 15.0, L 5.0 and H 5.0).

Behavioural experiments

One week after the implantation, each cat was habituated to the sound-tight observation cage (90 x 60 x 60 cm) during two 1 hour sessions on separate days. The observation cage had a plexiglass front allowing video recording with help of a closed video circuit. In addition, the animals were habituated to the injection procedures. Intracaudate injections were always performed on the right side. Immediately before the injection, the inner cannula was removed. Drug solutions were injected with help of a 5.0 μl Hamilton syringe in the conscious, hand-fixed animal. The solution was injected in about 20 s; after that the injection needle (diameter: 0.4 mm) was left in place for another 10 s. After replacing the inner cannula, the cat was placed in the observation cage.

Each cat underwent the following experiments, which started two weeks after the implantation:

Experiment 1: To investigate the behavioural effect of stimulating dopamine receptors in the intact rostromedial part of the caudate nucleus, 0.6 μg/5.0 μl apomorphine was injected. In previous studies (Cools, Struyker Boudier & Van Rossum, 1976) it was found that this dose induces a characteristic and dopamine specific effect (see below) that is maximal 10-15 min after the injection and disappears during the following 5 min. Before the injection, each cat (n =10) was readapted to the observation cage for 15 min. Subsequently, apomorphine was injected as described above; after that the cat was placed in the observation cage. During the following 15 min, the behaviour was recorded on video-tape.

Experiment 2a: To investigate the behavioural effect of direct excitation of striatal neurons, 1.0 μg/5.0 μl kainic acid was injected one week after experiment 1. Before the injection, each cat (n =7) was readapted to the observation cage for 15 min. Subsequently, kainic acid was injected as described above; after that the cat was placed into the observation cage. During the following 180 min, the behaviour was recorded on video-tape. The dose of kainic acid as well as the length of the observation period were chosen on the basis of pilot studies.

Experiment 2b: To confirm the previously reported effects of intrastriatally injected

kainic acid on metabolic activity, three cats received, in addition to intracaudate kainic acid, an i.v. injection of 175 μCi [^{14}C]-2-D-deoxyglucose in the following order. Cat 1: deoxyglucose was injected immediately before kainic acid; the animal was sacrificed 45 min after kainic acid; cat 2: deoxyglucose was injected 5 min after kainic acid; the animal was sacrificed 50 min after kainic acid; cat 3: deoxyglucose was injected 70 min after kainic acid; the animal was sacrificed 115 min after kainic acid. The i.v. injection lasted about 3 minutes. After the kainic acid injection, each animal was placed in the observation cage. Until the cat was sacrificed, the behaviour was recorded on video-tape. The intervals between the injections were chosen on the basis of pilot studies.
Experiment 3: To investigate the behavioural effect of stimulating dopamine receptors in the rostromedial part of the caudate nucleus after the kainate-induced local lesioning, each cat (n =7) received an intracaudate injection of 0.6 μg/5.0 μl apomorphine one week after experiment 2a. The procedure was identical to that of experiment 1.

Dependent variables.

1. Forced Turning, type 1 (FT1): Unilateral forced head turning movements that are interrupted at variable intervals. These movements start when the head is in line with the body. They are considered to be completed when the cat executes a smooth, non-forced head movement back to the starting position. According to previous studies (Cools, Struyker Boudier, 1976) these movements are characteristic for unilaterally induced changes at the level of the Caudate nucleus. An unilateral increase in the dopaminergic activity or decrease in the GABAergic activity in the rostromedial part of the Caudate nucleus results in contralateral staccato head turning. In the present study, these movements are labelled as contralateral FT1 movements.

2. Forced Turning, type 2 (FT2): Unilateral head, torso or body turning. Unlike FT1 movements, the cat continuously executes unilateral, uninterrupted and fast forced head turning movements which start when the head is in line with the body. After finishing such a turning movement, the animal executes a smooth, non-forced head movement back to the starting position. In case of torso-turning movements, the head remains

fixed in an unilateral position. In case of body-turning movements, the head as well as the torso remain fixed in an unilateral position. According to previous studies (see Chapter 3.1), a unilateral increase in GABAergic activity within the Substantia nigra pars reticulata results in contralateral head, torso or body turning. In the present study, these movements are labelled as contralateral FT2 movements.

3. Forced Turning, type 3 (FT3): Sequences of successive unilateral ear, head, torso and body turning. These movements are initiated when the head is in line with the body. Typically, the turning movement starts with retroflexion of the ear, that is followed by several, short unilateral forced head turning movements with a relatively fixed amplitude in such a way that each subsequent movement starts from the position in which the preceding head movement has ended. Sometimes, when the head reaches a deviation of more than 90 degrees from the body axis, the cat continues to turn unilaterally with short movements whereby the torso (including the forelimbs) becomes involved in the movement. Finally, in case the cat continues to turn also the remainder of the body becomes involved. According to a previous study (see Chapter 3.2), an unilateral decrease in GABA-ergic activity within the Deeper layers of the colliculus superior results in contralateral ear, head, torso and body turning. In the present report, these movements are labelled as contralateral FT3 movements.

4. Oro-Facial Dyskinetic (OFD) movements: Small, sometimes repetitive, contractions of individual muscles or small groups of muscles in the face region, that often are followed by tongue protrusions. According to previous studies (Cools, 1980), these movements can be elicited by stimulating dopamine receptors in the anterodorsal part of the caudate nucleus. Moreover, these movements can also be evoked from pallidal regions that receive projections from the latter part of the Caudate nucleus (Cools et al., 1989).

The number of each type of movement during 5 min blocks was determined until 90 min following kainic acid and until 15 min following apomorphine. With respect to parameter 1 to 3, the number of cats during each time-block was determined in case at least ten of these movements were executed; in case different types of movement were executed during one time block, each of them was separately taken into account.

Analysis of metabolic activity.

The uptake of labelled deoxyglucose was considered to reflect local metabolic activity. Based upon the original report of Sokoloff and coworkers (Sokoloff et al., 1977), the cats were sacrificed 45 min after the deoxyglucose application (175 μCi [^{14}C]-2-D-deoxyglucose per cat, i.e. 50 μCi/kg) with an overdose of pentobarbital. The skull was dissected and quick-frozen in isopentane at -30 °C. Cross sections (20 μm) were cut from the embedded skull with help of a LKB microtome. Slices were collected with an intersection distance of 0.5 mm at the level of the caudate nucleus, and 0.2 mm at the level of the substantia nigra pars reticulata and the deeper layers of the superior colliculus. The sections were picked up on tape, freeze-dried at -20 °C, and exposed to X-ray film (Betamax, Amersham) together with standard ^{14}C microscales calibrated for brain tissue (Amersham) for 14 days. In this study, our aim was to detect kainic acid-induced unilateral changes in metabolic activity at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus. Furthermore, all behavioural parameters used in the present study represent unilateral changes in neurotransmission processes. Therefore, analysis of metabolic activity was limited to the comparison of optical density between both sides at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus. Differences in metabolism were evaluated by visual inspection of the autoradiograms. Since this method was insufficient for detecting metabolic changes at the level of the Deeper layers of the colliculus superior, this area was analyzed by quantitative computer-assisted densitometry (Viper system, Gesotec). The colliculus superior was divided into superficial and deeper layers according to the terminology of Kanaseki and Sprague (1974). In pilot studies it was found that collicular characteristic movements occurred about 50 min after kainic acid; therefore, metabolic activity in the deeper layers of the superior colliculus was computed in one cat that was sacrificed 50 min after kainic acid and that did not execute these movements (cat 2) and in one cat that received deoxyglucose 70 min after kainic acid and that did display this characteristic behaviour (cat 3). In each autoradiogram, optical densities in both colliculi (only the deeper layers) were analyzed using the calibrated ^{14}C-labelled polymer layers,

in order to determine tissue equivalent levels of activity in nCi/g. Mean value of deoxyglucose uptake was determined in ten consecutive autoradiograms of each cat. Deoxyglucose uptake at the level of the ipsilateral colliculus was expressed as a percentage of that at the level of the contralateral colliculus.

Histology.

The animals that did not receive deoxyglucose were deeply anaesthetized with pentobarbital and transcardially perfused with a 4% formaldehyde solution immediately after the final experiment. The brains were removed and cross-sections were cut with help of a cryostat (30 μm slices) or, after embedding the brain in paraffine, with help of a microtome (15 μm slices). The slices were subsequently stained with cresyl violet for analysis of injection locus and extent of kainic acid-induced intracaudate lesioning.

Drugs.

Apomorphine hydrochloride (Brocades) was dissolved in distilled water. Kainic acid (Serva) was dissolved in 0.15 M sodium-phosphate buffer, pH 7.4. A bolus of [^{14}C]-2-D-deoxyglucose (Amersham), dissolved in 1 ml physiological saline, was injected intravenously. All drug solutions were freshly prepared immediately before each experiment.

Statistics.

The effect of apomorphine before and after kainic acid was compared with help of the Fisher exact probability test (two tailed). In cat number two and three, differences in metabolic activity between the ipsilateral and contralateral Deeper layers colliculus superior were evaluated by comparing the deoxyglucose uptake in ten autoradiograms with help of the Wilcoxon matched-pairs signed-ranks test (two tailed).

6.1.3 RESULTS

Histology

Histological verification revealed that all injections were placed in the target area, with coordinates (Snider & Niemer, 1964) A 14.0-15.0, L 4.5-6.5 and H 4.5-5.5. A representative example of an injection site as well as the extent of the kainic acid-induced lesion is shown in Figure 6.1.1.

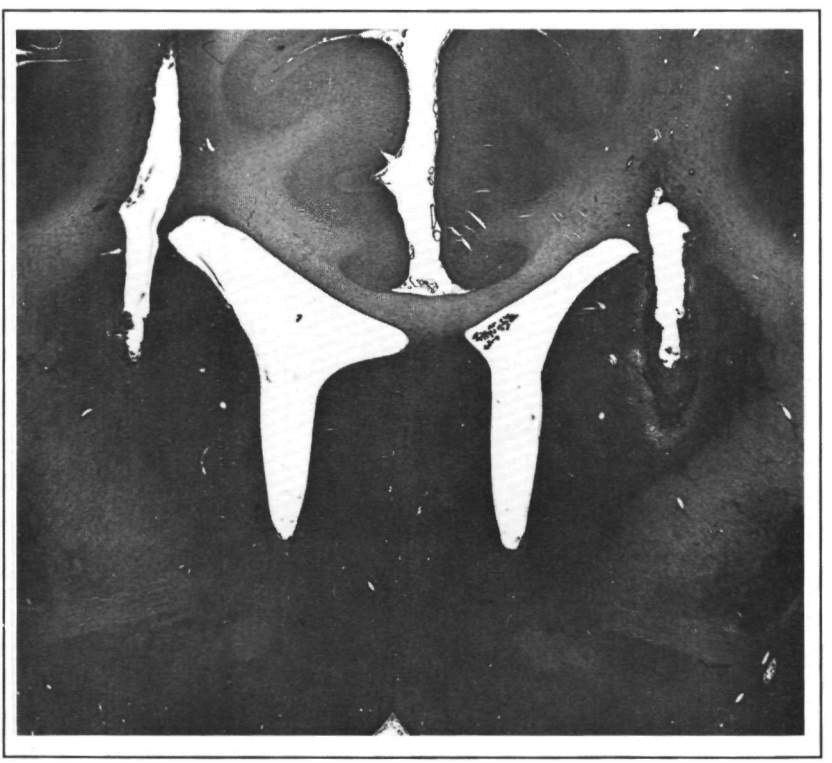

Figure 6.1.1 *Representative picture of a cross-section showing cannula tracks and injection site of kainic acid in the rostromedial part of the right caudate nucleus. Staining: cresyl-violet; note the extent of the lesion in the right caudate nucleus.*

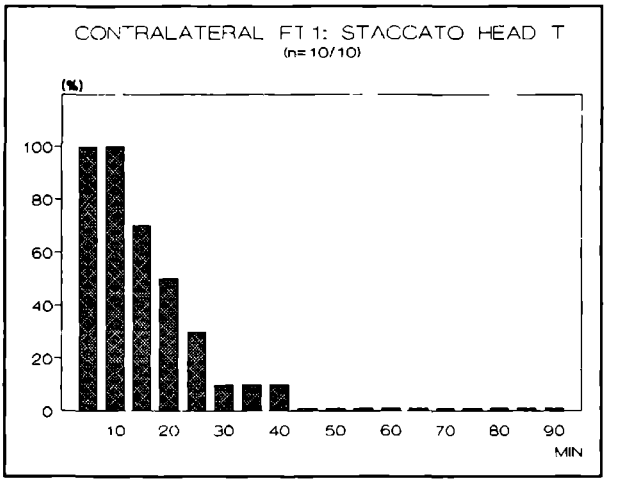

Figure 6.1.2 Percentage of animals showing contralateral forced staccato head turning (FT1 movements) during 5 min time-blocks from 0 to 90 min after the unilateral intracaudate injection of kainic acid. n= number of animals of total of animals tested displaying FT1 movements.

Behavioural observations

Three cats received in addition to kainic acid an i.v. injection of [^{14}C]-2-D-deoxyglucose (see Section 6.1.2). The display of the kainate induced movements (see below) was interrupted only for a short period by this injection in cats 2 and 3 (see Section 6.1.2: experiment 2b); the behaviour of all deoxyglucose-treated animals did not differ qualitatively from that of the other cats, therefore they were included in the behavioural analysis described below.

Unilateral injection of 1.0 μg kainic acid resulted in a very characteristic sequence of behavioural events, although there were individual differences with respect to the length and intensity of the various phases. Almost immediately after the injection, the animals started to display contralateral FT1 movements (mean starting-time ±SEM: 0.5 ±1.0 min). This behaviour lasted about 17 min (mean ending-time±SEM: 17.7±7.6 min). During this period, the total number of FT1 movements was (mean ±SEM): 66.0 ±20.1. The percentage of cats displaying FT1 movements during each time-block is shown in Figure 6.1.2.

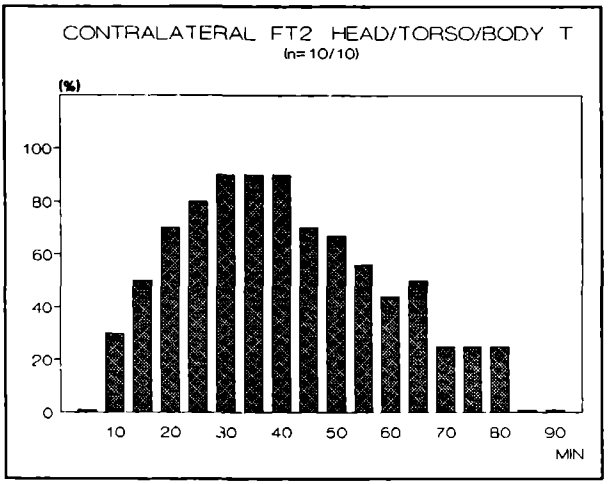

Figure 6.1.3 Percentage of animals showing fast, uninterrupted contralateral forced head, torso or body turning (FT2 movements) during 5 min time-blocks from 0 to 90 min after the unilateral intracaudate injection of kainic acid. n=number of animals of total of animals tested displaying FT2 movements.

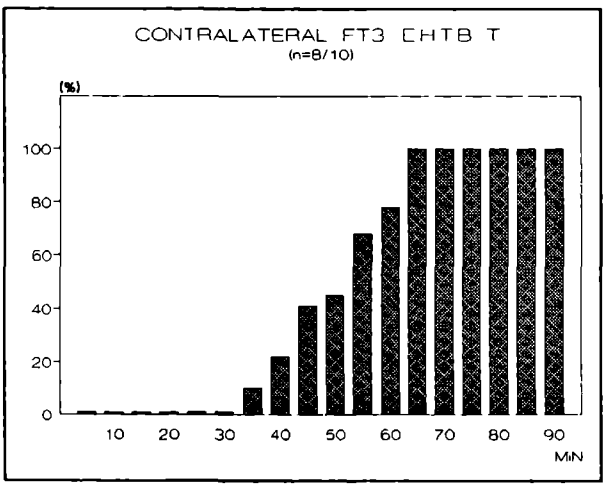

Figure 6.1.4 Percentage of animals showing sequences of short contralateral forced ear, head, torso and body turning (FT3 movements) during 5 min time-blocks from 0 to 90 min after the unilateral injection of kainic acid n= number of animals of total of animals tested displaying FT3 movements. Two cats, that were sacrificed 45 and 50 min after kainic acid, did not display this particular behaviour.

During the next phase, all cats displayed contralateral FT2 movements (mean starting-time ±SEM: 15.1±7.0 min). This behaviour disappeared about 50 min after

the kainic acid injection (mean ending-time ±SEM: 50.2±12.8 min). During this period, the cats displayed on the average more than 200 FT2 movements (mean number± SEM: 209.8±132.2). The percentage of cats displaying FT2 movements during each time-block is shown in Figure 6.1.3.

During the final phase, which started about 48 min after the injection of kainic acid eight cats showed contralateral FT3 movements (mean starting time ±SEM: 47.8 ±11.7 min). At the end of the observation period, i.e. 180 min after the injection of kainic acid, most cats still displayed these movements. Since the behaviour of the cats did not change for the remainder of the observation period, the data until 90 min after kainic acid are presented. Until 90 minutes after the injection, the cats displayed on the average more than 200 FT3 movements (mean number ±SEM: 206.9 ±98.7). The percentage of cats displaying FT3 movements during each time-block is shown in Figure 6.1.4.

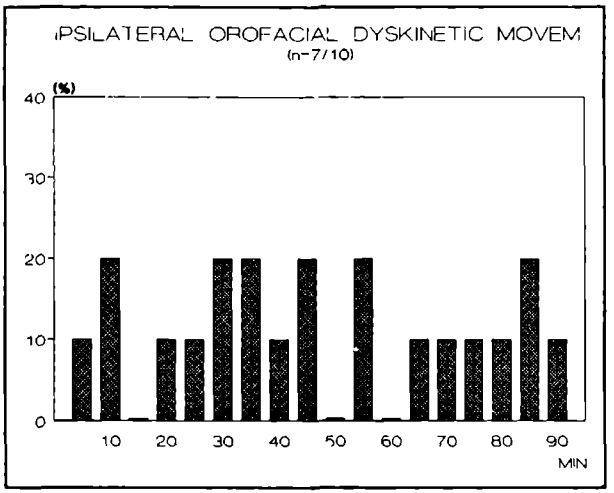

Figure 6.1.5 Percentage of animals showing ipsilateral orofacial dyskinetic movements during 5 min time-blocks from 0 to 90 min after the unilateral intracaudate injection of kainic acid. n= number of animals of total of animals tested displaying these movements

TABLE 6.1.1 Percentage of animals showing contralateral forced staccato head turning (FT1 movements) during 5 min time-blocks from 0 to 15 min after the unilateral intracaudate injection of 0.6 μg apomorphine one week before (PRE-KA; n=10) and one week after (POST-KA; n=7) kainic acid.

TIME-BLOCK (min)	PRE-KA (%)	POST-KA (%)
0 - 5	70	29
6 - 10	90	14*
11 - 15	90	14*

*$p<0.05$ (Fisher exact probability test, two tailed).

OFD movements were always observed ipsilateral to the injected side. Seven out often tested cats displayed these movements. The frequency of OFD movements is relatively low (maximal 2 spells during one time block whereby during each spell one or more OFD movements may occur). The percentage of cats displaying OFD movements during each time-block is shown in Figure 6.1.5.

Apomorphine 0.6 μg, injected one week before kainic acid elicited contralateral FT1 movements during 15 min after the intracerebral application. In contrast, one week after kainic acid, apomorphine elicited these movements in significantly less cats during the second and third time-block (Table 6.1.1). As a final remark, no abnormal movements were observed in any of the 15 min adaptation periods preceding the experiments.

Metabolic activity.

Evaluation of the deoxyglucose uptake revealed that at the level of the caudate nucleus, metabolism was enhanced on the ipsilateral side compared to the contralateral side. This difference was present until the most posterior parts of the head of the caudate nucleus. The enhancement was present in all three tested cats, but the increase

was most pronounced in the cat that had received deoxyglucose immediately before kainic acid. However, in the centre of the injection locus of the animals that had received deoxyglucose 5 and 70 min after kainic acid, metabolism was decreased. In these animals, an area of reduced metabolism was surrounded by an area with enhanced metabolic activity (compared to the contralateral side). The latter decrease

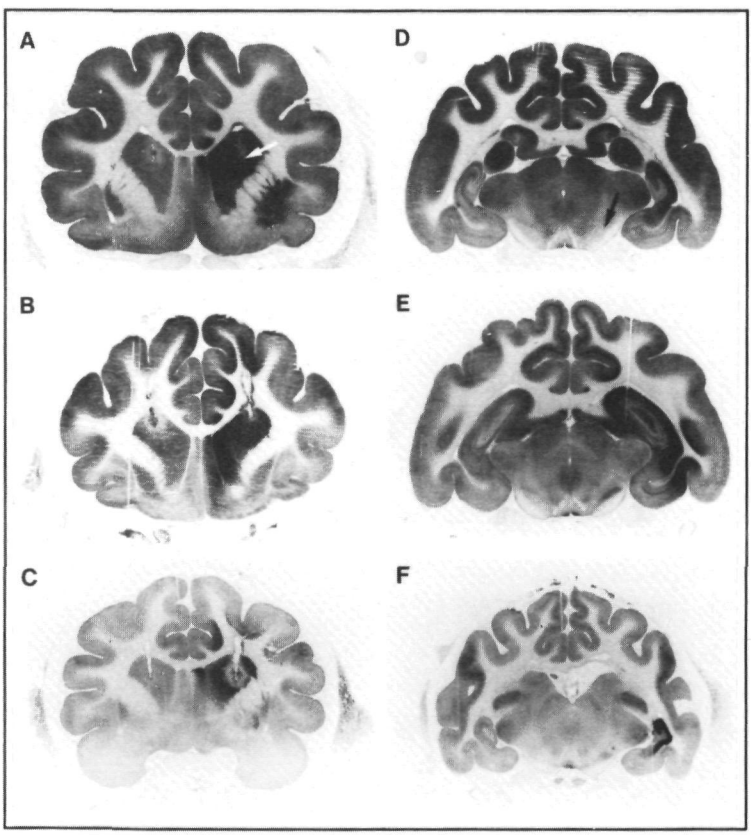

Figure 6.1.6 *Representative pictures of autoradiograms following i.v. application of [^{14}C]-2-D-deoxyglucose one min before (cat 1), five min (cat 2) and seventy min (cat 3) after kainic acid. Autoradiogram at the level of the injection site in the caudate nucleus (arrow) of cat 1 (A), cat 2 (B) and cat 3 (C); at the level of the substantia nigra pars reticulata (arrow) of cat 1 (D), cat 2 (E) and cat 3 (F).*

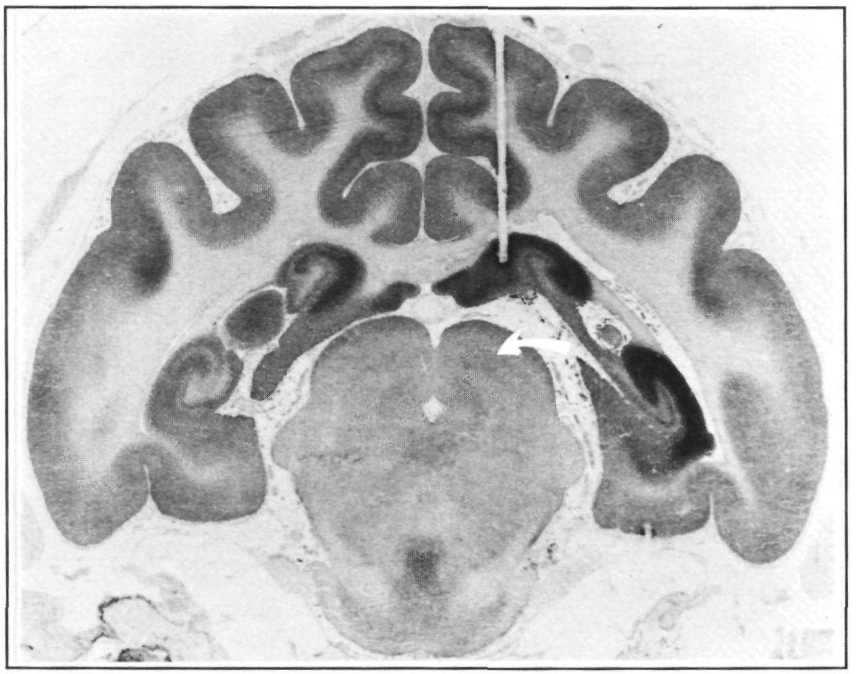

Figure 6.1.7 *Representative picture of an autoradiogram at the level of the deeper layers of the superior colliculus (arrow) of cat 3. Note that metabolic activity was only slightly increased in the ipsilateral (right) colliculus compared to the contralateral (left) colliculus.*

was most pronounced in the cat injected 70 min after kainic acid (Figure 6.1.6). Moreover, metabolic activity was also relatively enhanced at more anterior levels of the caudate nucleus, although the latter increase was less pronounced compared to that in the posterior parts (data not shown).

Metabolism was also relatively increased in the ipsilateral substantia nigra pars reticulata in all three cats; the asymmetry at this level was not present at all levels in cat 1, that received deoxyglucose immediately before kainic acid, (Figure 6.1.6). Moreover, in the latter animal, metabolic activity appeared to be the same in both colliculi.

Results of quantitative densitometry in cat 2 revealed no significant difference between both sides: density of the ipsilateral colliculus (deeper layers) was 104.0±10.8% (mean ±SEM) of that of the contralateral colliculus ($p>0.05$). In contrast, in cat 3 metabolism in the ipsilateral colliculus (deeper layers) was 114.6±6.8% of the activity in the contralateral colliculus (Figure 6.1.7). Comparing both sides, the difference appeared to be significant: $p<0.01$. As a final remark, asymmetric changes in metabolism were present at the level of the putamen and the hippocampus in all three animals (Figure 6.1.6).

6.1.4 DISCUSSION

The acute behavioural effect of an unilateral intracaudate injection of kainic acid could be divided into three phases that were characterized by FT1, FT2 and FT3 movements respectively. The final phase lasted at least several hours.

Caudate nucleus and effects of kainic acid.

In the present study, intracaudate kainic acid elicited contralateral FT1 movements in all tested cats during the first phase after the injection. Previously, it has been found that, in contrast to experimentally induced alterations in nigral or collicular GABAergic neurotransmission, an increase in dopaminergic activity or a decrease in GABAergic activity in the rostromedial part of the caudate nucleus results in contralateral FT1 movements (see Section 6.1.2). Accordingly, the initial, excitatory (Coyle, 1983; Foster & Fagg, 1984; Olney, 1981) action of kainic acid may be equivalent to an activation of dopamine receptors and/or an inhibition of GABA receptors with respect to the ability to evoke contralateral FT1 movements. Activation of dopamine receptors with help of i.v. injection of apomorphine is known to produce an increase in striatal metabolism (McCulloch et al., 1982). On the other hand, activation of GABA receptors with help of a local injection of muscimol is reported to produce a decreased metabolic activity at this level (Kelly & McCulloch, 1984). Accordingly, these data suggest that an increase in dopaminergic activity and/or a decrease in GABAergic activity may be

equivalent to an increase in metabolic activity at the level of the caudate nucleus. Furthermore, intrastriatal kainic acid is known to produce acutely an activation of local metabolism (Wooten & Collins, 1980), viz. an effect that was also found in the present study as illustrated by the relative increase in local metabolic activity in the ipsilateral caudate. On the basis of these data, it can be suggested that kainic acid induced contralateral FT1 movements as a result of its excitatory action on striatal neurons.

Substantia nigra and effects of kainic acid.

In the present study, intracaudate kainic acid elicited contralateral FT2 movements in all tested cats during the second phase after the injection. Previously, it has been reported that, in contrast to experimentally induced changes in striatal dopaminergic or GABAergic activity and experimentally induced alterations in collicular GABAergic neurotransmission, stimulation of nigral GABA receptors results in contralateral FT2 movements (see Section 6.1.2). Activation of caudate dopamine receptors is known to result in an increased activity of the striato-nigral GABAergic pathway (Gale & Casu, 1981; Scheel-Krüger, 1983). In view of the finding that excitation induced by kainic acid may be functionally equivalent to activation of dopamine receptors (see above), the present data suggest that intracaudate kainic acid induced contralateral FT2 movements by stimulation of nigral GABA receptors as a result of the activation of the striato-nigral GABAergic pathway. According to others, excitation of striatal neurons by electrical stimulation (Aiko et al., 1988) or by local injection of kainic acid (Kimura, McGeer & McGeer, 1980) enhances metabolic activity in the substantia nigra pars reticulata, whereas activation of dopamine receptors by i.v. injection of apomorphine is also reported to produce an increase in glucose metabolism in this brain region (McCulloch et al., 1982). The present study confirmed this as illustrated by the finding that kainic acid relatively increased metabolic activity in the ipsilateral reticular substantia nigra compared to its contralateral counterpart. In view of the finding that striatal excitation induced by kainic acid may be functionally equivalent to activation of dopamine receptors (see above), the present data suggest that intracaudate kainic acid induced an increase in metabolic activity at the level of the ipsilateral substantia nigra pars reticulata as a result of the activation of the GABAergic striato-nigral

pathway. Taking together, the present data indicate that intracaudate kainic acid produced indirectly a stimulation of GABA receptors of the ipsilateral nigra resulting in contralateral FT2 movements.

Colliculus superior and effects of kainic acid.

Except for two cats (cat 1 and 2) that participated in experiment 2b, and had to be sacrificed before the start of the third phase, intracaudate kainic acid elicited contralateral FT3 movements in all tested cats during the third phase after the injection. Previously, it has been found that, in contrast to experimentally induced changes in striatal dopaminergic or GABAergic activity and experimentally induced alterations in nigral GABAergic neurotransmission, deactivation of collicular GABAergic receptors results in contralateral FT3 movements (see Section 6.1.2). Activation of striatal dopamine receptors is known to result in a decreased activity of the GABAergic nigro-collicular pathway (Gale & Casu, 1981; Scheel-Krüger, 1983). In view of the finding that striatal excitation induced by kainic acid may be functionally equivalent to an increase in striatal dopaminergic activity (see above), the present data suggest that kainic acid induced contralateral FT3 movements as a result of an indirectly induced deactivation of ipsilateral collicular GABA receptors by decreasing the activity of the nigro-collicular GABAergic pathway. Furthermore, analysis of the autoradiograms revealed a relatively enhanced metabolism in the deeper layers of the ipsilateral superior colliculus of the cat that received deoxyglucose 70 min after kainic acid. In other words, in this animal the display of contralateral FT3 movements appeared to be accompanied by a relative activation of local metabolism at the level of the ipsilateral colliculus. In contrast, metabolism was not changed at the level of the deeper layers of the ipsilateral superior colliculus compared to the contralateral colliculus of the cat that was sacrificed 50 min after kainic acid, and that did not display FT3 movements. These data suggest that metabolic changes are present only in cases where functional changes in the colliculus are reflected in behavioural alterations. Finally, these data fit in with those of others (McCulloch et al., 1982), who found that activation of dopamine receptors by i.v. injection of apomorphine results in an increased metabolic activity in the deeper layers of the superior colliculus.

GABAergic input and changes in metabolic activity.

Increases in glucose metabolism may result from an enhanced activity in presynaptic terminals and/or in excitatory postsynaptic processes (for ref., see Aiko et al., 1988). Moreover, even inhibitory postsynaptic processes may result in an enhanced metabolism (Aiko et al., 1988). Considering the present observation that at the level of the substantia nigra pars reticulata activation of the GABAergic input appeared to be accompanied by a relative increase of metabolism, whereas at the level of the deeper layers of the superior colliculus an inhibition of the GABAergic input appeared to be accompanied by a relative increase of metabolism, it remains to be investigated to which degree the observed metabolic changes were due to alterations in GABAergic input and/or GABAergic interneurons (cf.Lu et al., 1985).

Kainic acid-induced increasing pathology.

In general, the behavioural effects, as described above, suggest that intracaudate kainic acid induced acutely an increasing pathology that resulted successively in functional changes at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus. Moreover, these alterations were also reflected in changes in local metabolic activity.

Although metabolic changes at the level of the caudate and the substantia nigra were found in all three animals tested, the behavioural effects, characteristic for functional changes at these levels disappeared in a particular sequence. These data suggest that once a functional disturbance at a particular brain level becomes manifest in behavioural alterations, behavioural changes due to dysfunctioning hierarchically higher-ordered brain structures are no longer present. It remains to be investigated whether the latter phenomenon is due to a 'shut-down' of output-signals and/or simply due to a competition of different behaviours. Finally, the same phenomena may have hindered the occurrence of behavioural alterations inherent in metabolic asymmetric changes at the level of the putamen and the hippocampus.

Orofacial dyskinetic movements.

In the present study, kainic acid was able to induce ipsilateral OFD movements. In previous studies, neither changes in the rostromedial caudate dopaminergic or GABAergic activity nor alterations in nigral or collicular GABAergic neurotransmission resulted in the occurrence of orofacial dyskinetic movements as observed in the present study. Accordingly, these data may imply that such movements were manifestations of striatal neuropathological processes that were not mediated via the caudato-nigro-collicular pathway. This notion fits in with the observation that OFD movements occurred during all three behavioural phases. As it has been found that OFD movements can be elicited by activation of dopamine receptors in the anterodorsal part of the caudate nucleus (Cools, 1980), as well as from pallidal regions receiving projections from the latter part of the Caudate nucleus (Cools et al., 1989), it is suggested that these movements are indeed mediated via another output pathway, that starts to dysfunction as a result of striatally injected kainic acid.

Kainic acid-induced lesion.

Previously, it has been reported that kainic acid produces neuronal lesioning, at least partly as a result of its neuro-excitatory action (Coyle, 1983; Foster & Fagg, 1984; Matyja, 1986; McGeer, McGeer & Singh, 1978; Olney, 1981; Schwarcz & Coyle, 1977; Wuerthele et al., 1978). As a result, metabolic activity in the rat neostriatum was decreased 7-10 days following local injection of kainic acid (Kimura, McGeer & McGeer, 1980; Wuerthele et al., 1978). In the present study, metabolic activity appeared to be diminished in the centre of the injection locus especially in the animal that received deoxyglucose 70 min after kainic acid. Furthermore, one week after kainic acid, intracaudate apomorphine was no longer able to elicit FT1 movements, suggesting local neuronal damage. Indeed evidence has been presented indicating the loss of cholinergic and GABAergic striatal neurons following intrastriatal kainic acid injection (Beal et al., 1986; Hruska & Silbergeld, 1979; Schwarcz & Coyle, 1977).

Clinical implications.

Summarizing, the present study suggests that kainic acid injected into the feline

caudate nucleus produces an increasing pathology resulting successively in functional changes at the level of the caudate, its output station the substantia nigra pars reticulata, and at the level of a nigral output station, the deeper layers of the superior colliculus. The present data may imply that at least part of the symptoms of central disorders like Parkinson's Disease and Huntington's Chorea, are derived from dysfunctioning extrastriatal structures as a result of a disturbed striatal input.

Summary

Behaviour and limb placing ability were analyzed acutely and subacutely (up to 21 days) following unilateral occlusion of the middle cerebral artery (MCA) in cats. Immediately following occlusion, all tested cats started to display a sequence of different behaviours, characteristic for (1) an ipsilateral inhibition of dopaminergic activity in the caudate nucleus; (2) an inhibition of GABAergic activity in the reticular substantia nigra; (3) a stimulation of GABA receptors in the deeper layers of the colliculus superior (starting-time of these phases: about 4, 12 and 25 min, respectively). The latter behaviour was also present subacutely. In addition, unilateral orofacial dyskinetic movements were observed acutely as well as subacutely. Contralateral limb placing was deficient in all cats 60 min postocclusion; it was at least partly restored subacutely. Twenty-one days after the occlusion, [^{14}C]-2-D-deoxyglucose uptake was relatively reduced in the ipsilateral caudate nucleus (especially in its posterior part), the ipsilateral substantia nigra pars reticulata and the deeper layers of the ipsilateral superior colliculus. The anterior caudate nucleus appeared to be less affected than the posterior caudate. Metabolism was relatively reduced in the sensorimotor cortex only in part of the tested cats. The data show that unilateral MCA occlusion produces consistent functional changes in all structures studied apart from the sensorimotor cortex, viz. the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus.

6.2.1 INTRODUCTION

The caudate nucleus sends neuronal information to several output stations, such as the substantia nigra, pars reticulata, and the globus pallidus (Graybiel & Ragsdale, 1979; Royce & Laine, 1984). The substantia nigra pars reticulata in turn, sends its output signals towards the deeper layers of the colliculus superior, the midbrain

reticular formation and several thalamic nuclei (Beckstead, Domesick & Nauta, 1979; Beckstead & Frankfurter, 1982; Edwards, Ginsburgh & Henkel, 1979; Graybiel, 1978; May & Hall, 1986; see Section 2.3). The inhibitory amino acid gamma-aminobutyric acid (GABA) is one of the neurotransmitters of the caudato-nigral as well as the nigrocollicular pathway (Chevalier et al., 1981; Kim et al., 1971; Yoshida & Precht, 1971; for review, see Gale & Casu, 1981; Scheel-Krüger, 1983). An experimentally induced decrease in striatal dopaminergic activity induces an inhibition of the caudatonigral GABAergic pathway; the resulting decreased release of GABA at the level of the nigral pars reticulata disinhibits in turn the nigrocollicular GABAergic fibres (Gale & Casu, 1981; Scheel-Krüger, 1983). In case the caudate nucleus produces abnormal output signals as a result of pathological disturbances in striatal neuronal functions it might be expected that structures directly innervated by the caudate, such as the substantia nigra pars reticulata, also start to dysfunction as a result of the abnormal input signals. In turn, the substantia nigra pars reticulata may then start to produce abnormal output signals resulting in dysfunctioning nigral output stations, such as the deeper layers of the colliculus superior (see Chapter 5; see also Gelissen & Cools, 1988). Therefore, it might be expected that at least part of the symptoms, inherent in striatal neuropathological processes characteristic for disorders like Parkinson's Disease or Huntington's Chorea, are actually due to functional disturbances of brain regions receiving (in)directly caudate output signals (cf. Lasker et al., 1987).

Recently, it has been found that an experimentally induced neuropathological change at the level of the caudate nucleus resulted acutely in functional alterations at the level of the caudate, the substantia nigra pars reticulata and the deeper layers of the superior colliculus (see Section 6.1). Unilateral injections of the neuro-excitant kainic acid (Coyle, 1983; Foster & Fagg, 1984; Olney, 1971) into the rostromedial part of the caudate nucleus in cats resulted successively in behavioural changes characteristic for (a) an activation of striatal dopamine receptors, (b) an activation of nigral GABA receptors and (c) an inhibition of collicular GABA receptors (starting ± 1, 15 and 48 min after the kainic acid injection, respectively). Furthermore, the observed behavioural changes were accompanied by a relative increase in metabolic activity at the level of

the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus as shown by the drug-induced changes in [^{14}C]-2-D-deoxyglucose uptake. On the basis of these data, it has been concluded that striatally injected kainic acid produced a progressive pathology resulting successively in functional changes in the rostromedial caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus (Section 6.1).

In order to investigate whether the above-mentioned progressive pathology is actually due to the distorted information sent by the caudate nucleus to its output stations rather than due to typical features of the neurotoxin used, it was decided to study whether a completely different manipulation with striatal tissue results also in behavioural and metabolic alterations inherent in disturbances at the level of caudate output stations such as the substantia nigra, and at the level of nigral output stations, such as the colliculus superior. Because of the following reasons we focused our attention on the effects of focal ischemia induced by unilateral occlusion of the middle cerebral artery (MCA) in cats:

1. MCA occlusion produces neuronal lesioning in a limited number of structures such as the dorsolateral neostriatum in rats (Shibuya, Arita & Yamamoto, 1987; Shigeno et al., 1985; Tyson et al., 1984). In the feline caudate nucleus MCA occlusion results in neuronal lesioning especially in the posterior part, i.e. the part containing the rostromedial subregion that controls the striatal output signals directed to the substantia nigra pars reticularis (Berkelbach van der Sprenkel & Tulleken, 1988); the anterodorsal region, i.e. a caudate subregion that is located rostral from the anterior commissure and that projects to output stations other than the substantia nigra is less affected (J.W. Berkelbach van der Sprenkel, H.J. Groenewegen and C.A.F. Tulleken, subm.).
2. The ischemic insult resulting from MCA occlusion never includes the substantia nigra and the superior colliculus, allowing the possibility to study behavioural and metabolic consequences of remote functional alterations due to abnormal output signals derived from structures located within the ischemic region (cf. Kogure et al., 1974; Kuhl et al., 1980; Metter et al., 1985; Pozzilli et al., 1987; Pulsinelli, Levi & Duffy, 1982; Shigeno

et al., 1985).

3. Evidence is available that metabolic activity at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus is affected following MCA occlusion (Nakayama et al., 1987; Pulsinelli, Levy & Duffy, 1982; Shibuya, Arita & Yamamoto; 1987; Shigeno et al., 1985).

4. Experimentally induced changes at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus all result in behavioural changes that can be distinguished from each other (Cools, Struyker Boudier, 1976; see Chapter 4). Accordingly, functional changes at the level of these structures following MCA occlusion can be differentiated.

In the present study, the acute and subacute (up to 21 days) behavioural alterations after unilateral MCA occlusion were analyzed in cats. In addition, the behavioural changes induced by MCA occlusion inherent in alterations at the level of the caudate, the substantia nigra and the superior colliculus were correlated to chronic metabolic changes in these structures 21 days after occlusion. Apart from striatal lesioning as described above, MCA occlusion is also known to damage -to a variable extent- parts of the frontal cortex. Since frontal cortex lesions as well as extensive lesions of the caudate nucleus may result in deficient limb placing abilities (Armstrong, 1986; Bard, 1933; Villablanca et al., 1976), it was investigated whether the occlusion also affected the latter ability.

6.2.2 EXPERIMENTAL PROCEDURES

Five female cats weighing 3-4 kg were used in the present study. Each animal underwent occlusion of the right MCA.

Implantation of occluding device

The occluding device was implanted in fluothane (2%) anaesthetized cats as previously described (Berkelbach van der Sprenkel & Tulleken, 1988). The optic

foramen was approached subperiostally, leaving the orbital contents intact. A burrhole was made on the laterosuperior side of the optic canal. After opening the dura and preparation of the MCA from the arachnoid, a 5-0 silk thread was put around the MCA and led to the vertex through a silicon tube. The ligature was placed proximal to the lenticulostriatal arteries.

Occlusion procedure

After at least two weeks during which the animals were habituated to the behavioural tests (see below), the MCA was occluded while the animal was conscious. After local anaesthesia (about 2 ml xylocaine 2% intracutaneously), a small incision was made through the skin to expose the silicon tube containing the thread which, at its other end, was put around the proximal MCA. By gentle traction to the thread, the MCA was occluded. Subsequently, the thread was fixed and the incision was closed using two wound clips. About 3 min after the occlusion, open field observation started (see below). The method of MCA occlusion in conscious cats, as used in the present study, has been proven to produce consistent and reproducible infarcts at least in the posterior part of the caudate nucleus (Berkelbach van der Sprenkel & Tulleken, 1988).

Behavioural experiments

Upon complete recovery from the implantation of the occluding device (see above), placing responses, righting reflexes, food intake and reactivity to visual and acoustic stimuli were controlled. Since all operated cats showed normal responses, all animals were subsequently habituated to the observation cage (90 x 60 x 60 cm) during two one hour sessions on separate days. The observation cage had a plexiglass front allowing video recording with help of a closed videocircuit. During the habituation sessions, abnormal movements, such as those described below, were never observed. Therefore, we did not include an additional sham-operated group of cats since each animal used in the present study served as its own control.

The cats participated in the following experiments:

Experiment 1: About 3 min after the completion of the occlusion procedure (see

above) the animal was placed in the observation cage. Open-field behaviour was recorded on videotape for 60 min to detect whether caudate-, nigral- and/or collicular-specific effects appeared (see below). This recording session was followed by the limb placing tests (based on the methods described by Villablanca et al., 1976). The ability to walk on a small bar and to place the limbs correctly was measured by placing the cat on a wooden bar (200 x 5 x 5 cm) which was situated about two meters above the floor. The animal was allowed to walk on the bar towards the other end where it was rewarded with food pellets. This procedure was performed three times (bar placing). In case of misplacing the affected limb was noted. Next, the animal was gently fixated by the experimenter in such a way that only one limb was hanging freely without the cat being able to see that limb. Tactile placing was tested by slightly touching the edge of a wooden table with the dorsum of the paw. This was performed three times. At first the left forelimb was tested, next the right forelimb, then the left hindlimb and finally the right hindlimb. In case of absence of correct placing in at least two out of three attempts the affected limb was noted. In case tactile placing did not occur, proprioceptive placing was tested. Therefore the animal was fixated in the same way as described above and, subsequently, moved slowly forwards whereby the freely hanging limb continuously made contact with the edge of the table. In case placing did not occur, the limb was 'dragged' along the surface of the table whereby the limb was extremely moved in the shoulder or the hip. Proprioceptive placing was tested three times, but only in the limbs that failed to react on tactile stimulation. In case of absence of placing in at least two out of three attempts the affected limb was noted. Finally, visual placing of each forelimb was tested by moving the cat, which now was allowed to move its head freely, from a short distance towards the surface of the table, whereby one forelimb was fixated. Visual placing was tested three times before the other (right) forelimb was tested in the same way. In case of absence of placing in at least two out of three attempts the affected limb was noted.

Experiment 2a: To investigate the subacute effects of MCA occlusion, each animal was readapted to the observation cage for 15 min one day after the occlusion. Immediately following this readaptation period, open-field behaviour was recorded during 15 min for subsequent analysis. Immediately following the recording session, limb

placing was tested using the same procedure as described above (see Experiment 1).

Experiment 2b: The same as experiment 2a, but now 3 days after the occlusion.

Experiment 2c: The same as experiment 2a, but now on day 8 (n=3) or day 11 (n=2) after the occlusion.

Experiment 2d: The same as experiment 2a, but now on day 18 (n=1) or day 21 (n=2) after the occlusion.

Experiment 3: In order to investigate chronic changes in local metabolic activity following MCA occlusion in the brain regions under study all cats (n=5) received an i.v. injection of [^{14}C]-2-D-deoxyglucose 21 days after the occlusion. Therefore, the cats were lightly anaesthetized with fluothane (1.7 %). Catheters were introduced in the femoral artery and vein and tunnelled to a small cutaneous incision on the back, close to the tail. The cats were allowed to recover from the anaesthesia for four hours before they received an injection of 175 μCi [^{14}C]-2-D-deoxyglucose (Amersham; dissolved in one ml saline). During the following 45 min the cats were lying quietly in a small box. After this period the cats were sacrificed with an overdose of pentobarbital. Next, the skull was dissected and quick-frozen in isopentane (-30 °C); cross sections (20 μm) were cut on an LKB microtome and subsequently processed for densitometric analysis of labelled deoxyglucose uptake according to previously reported procedures (see Section 6.1.2). The sections (caudate nucleus and sensorimotor cortex: intersection distance 500 μm; substantia nigra and superior colliculus: intersection distance 200 μm) were exposed to X-ray film (Betamax, Amersham) together with standardized ^{14}C microscales (Amersham) for 14 days.

Dependent variables open field test

In principle, only overt behavioural effects which render superfluous double-blind analysis because of their pathological nature were taken into account.

1. FT1; Forced Turning, type 1: Abnormal, 'forced' head turning movements that start when the head is in line with the body. They are considered to be completed when the cat executes a smooth, non-forced head movement back to the starting position. Typically, these movements are interrupted at variable intervals. According to previous

studies (for ref., see Cools, Struyker Boudier & Van Rossum, 1976) ipsilateral FT1 movements as defined above are characteristic for an unilateral decrease in the dopaminergic activity or increase in the GABAergic activity in the rostromedial part of the caudate nucleus.

2. FT2; Forced Turning, type 2: Abnormal, 'forced' head, torso or body turning. In contrast to FT1 movements, unilateral FT2 head movements are uninterrupted, fast 'forced' head turning movements which start when the head is in line with the body. Such a turning movement is considered to be completed in case the animal executes a smooth, non-forced head movement back to the starting position. In case of torso (including the forelimbs) or body turning movements, the head or the head and torso, respectively, remain fixed in an unilateral position. According to previous studies (see Section 4.1; see also Wolfarth, Kolasiewicz & Sontag, 1981), contralateral FT2 movements as defined above are characteristic for an unilateral increase in GABAergic activity within the substantia nigra pars reticulata.

3. Freezing behaviour: Absence of any movement for a period of at least 30 seconds. Freezing behaviour occurs typically into the midst of a non-forced movement. According to previous studies (Section 4.1; see also Wolfarth, Kolasiewicz & Sontag, 1981), this behaviour is characteristic for a decrease in GABAergic activity within the substantia nigra pars reticulata.

4. Forced Turning, type 3 (FT3): This turning behaviour is characterized by sequences of successive, abnormal 'forced' ear, head, torso and body turning movements. These movements are initiated when the head is in line with the body. Typically, the turning movement starts with retroflexion of the ear, that is followed by several, short unilateral forced head turning movements with a relatively fixed amplitude in such a way that each subsequent movement starts from the position in which the preceding head movement has ended. In case the head reaches a deviation of more than 90 degrees from the body axis, the cat sometimes continues to turn unilaterally with short movements whereby the torso becomes involved in the movement. Finally, in case the cat continues to turn the remainder of the body becomes also involved. According to a previous study (Section 4.2) contralateral FT3 movements as defined above are characteristic for an unilateral decrease in GABAergic activity within the deeper layers

of the superior colliculus.

5. Hypo-activity: In case the cat displays less than four normal and/or abnormal movements per minute, its behaviour is labelled as a state of hypo-activity. During control sessions, cats execute more than four (normal) movements per minute in an open field test. Thus, all movements executed by the cat are taken into account. According to previous studies (Cools et al., 1984; Gelissen & Cools, 1986), an increased intracollicular GABAergic activity induces a state in which the behaviour of the animal is fully directed by changes in exteroceptive stimuli. In case the animal is placed in a familiar environment and there are no changes in exteroceptive stimuli, the behaviour is characterized by a state of hypo-activity.

6. Oro-Facial Dyskinetic (OFD) movements: Small movements in the face region as a result of (repetitive) contractions of individual muscles or small groups of muscles. Such movements are often followed by tongue protrusions. According to previous studies these movements can be elicited by stimulating dopamine receptors in the anterodorsal part of the caudate nucleus or inhibition of GABA receptors within the subcommissural part of the globus pallidus (Cools et al., 1989).

The number of FT1, FT2 and FT3 movements was determined for each cat until 60 min after the occlusion. Subsequently, the percentage of animals showing a particular behaviour during 10 min time-blocks until 60 min post-occlusion was determined and presented graphically (see Section 6.2.2, Results). In addition, the number of cats that displayed one of the above-mentioned effects during the 15 min observation period on day 1, 3, 8-11 and 18-21 post-occlusion was determined. In case different behavioural effects were displayed during one time-block or observation period they were separately taken into account.

Analysis of metabolic activity

The uptake of labelled deoxyglucose was considered to reflect local metabolic activity. In this study, our purpose was to detect whether or not chronic asymmetric changes in metabolic activity occur at the level of the rostromedial caudate nucleus, the anterodorsal part of the caudate, the substantia nigra pars reticulata and the deeper

layers of the superior colliculus as a result of the MCA occlusion. Thus, the posterior caudate nucleus, i.e., caudal to the anterior commissure and encompassing the rostromedial region, and the anterior caudate, i.e., rostral to the anterior commissure and encompassing the anterodorsal region, were analyzed separately. In addition, deoxyglucose uptake at the level of the sensorimotor cortex (gyri sigmoideus and coronalis) was determined.

The colliculus superior was divided into superficial and deeper layers according to the terminology of Kanaseki and Sprague (1974). For each region, changes in metabolic activity were analyzed using the same method as has been reported previously (Section 6.1). Uptake of [^{14}C]-2-D-deoxyglucose was analyzed with help of computer-assisted densitometry (Viper System, Gesotec) by using the calibrated ^{14}C-labelled polymer layers in order to determine tissue equivalent levels of activity in nCi/g. For each region, the mean value of deoxyglucose uptake was determined in 10 consecutive autoradiograms of each cat. Deoxyglucose uptake of the region ipsilateral to the occlusion was expressed as a percentage of that of its contralateral counterpart. In brain structures containing areas completely devoid of any deoxyglucose uptake as a result of the ischemia, only the tissue of that structure surrounding this area was used for the uptake analysis.

6.2.3 RESULTS

Open field behaviour: acute effects

Immediately following occlusion, four out of five cats showed contralateral hemiparesis as well as slipping and misplacing of the contralateral fore- and hindlimb. In addition, three of these animals showed difficulty in maintaining an upright posture during this period. Overall, these symptoms only occurred during the first 12 min of the observation period (data not shown). Moreover, after a relatively hyperactive initial stage all cats adopted a sitting or lying posture. One cat immediately lied down after it was placed in the observation cage; this animal did not show the limb or posture

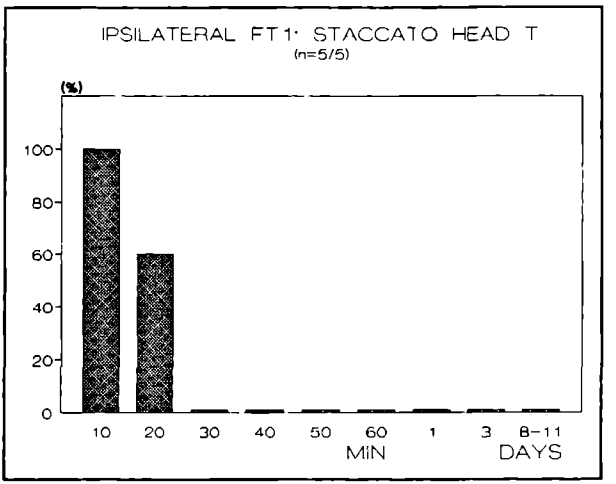

Figure 6.2.1 Percentage of animals showing ipsilateral FT1 movements in the open field test during 10 min time-blocks from 0 to 60 min, and during one 15 min time-block per day 1, 3, and 8-11 days following MCA occlusion. n= number of animals of total of animals tested displaying these movements.

abnormalities as described above.

Apart from the effects mentioned above, unilateral MCA occlusion induced a sequence of behavioural changes that was present in all occluded animals (see below). All animals showed ipsilateral FT1 movements (mean number ± SEM: 20.2 ± 6.7); the latter movements occurred only acutely following occlusion, between 1 (mean starting-time ± SEM: 3.8 ± 1.8 min) and 14 min (mean ± SEM: 10.0 ± 1.9 min; see Figure 6.2.1).

The next phase following occlusion was characterized by the display of freezing behaviour which occurred in all cats between 5 and 25 min after the start of the observation period (mean starting time ± SEM: 11.8 ± 2.6 min; mean duration of this phase ± SEM: 15.6 ± 3.0 min; see Figure 6.2.2).

During the final part of the observation period, the behaviour of all tested cats was characterized by hypo-activity. Mean start-time of this phase (±SEM) was 24.6 (±6.1)

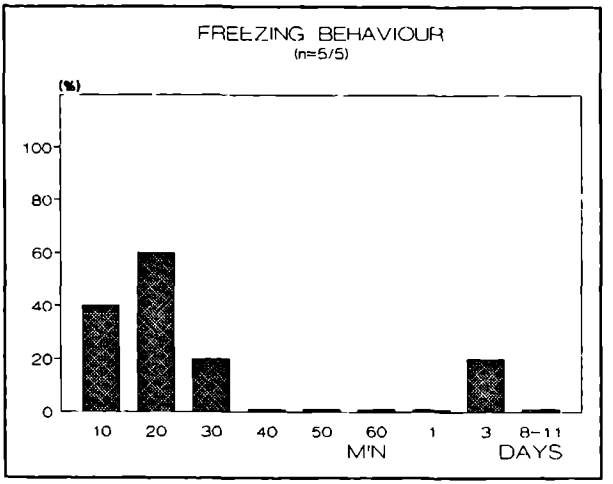

Figure 6.2.2 Percentage of animals showing freezing behaviour in the open field test during 10 min time-blocks from 0 to 60 min, and during one 15 min time-block per day 1, 3, and 8-11 days following MCA occlusion n= number of animals of total of animals tested displaying these movements.

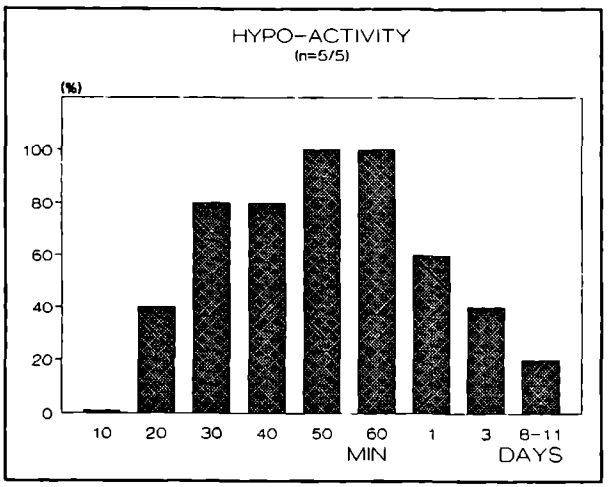

Figure 6.2.3 Percentage of animals showing hypo-activity in the open field test during 10 min time-blocks from 0 to 60 min, and during one 15 min time-block per day 1, 3, and 8-11 days following MCA occlusion. n= number of animals of total of animals tested displaying these movements.

min (see Figure 6.2.3). In general, the cats adopted a lying posture during this phase and hardly moved for the remainder of the observation time (Table 6.1.1). However,

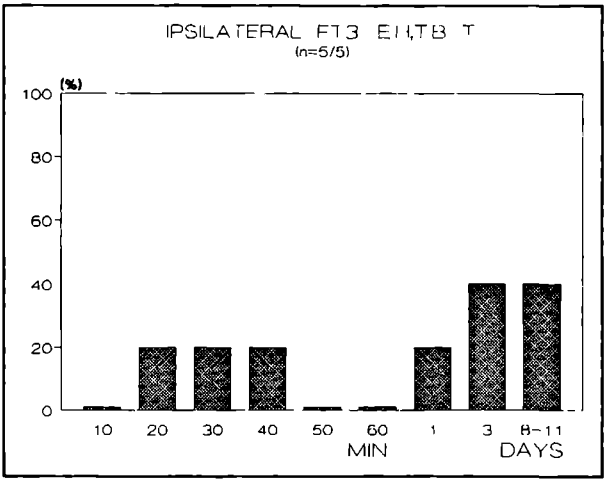

Figure 6.2.4 Percentage of animals showing ipsilateral FT3 movements in the open field test during 10 min time-blocks from 0 to 60 min, and during one 15 min time-block per day 1, 3, and 8-11 days following MCA occlusion. n= number of animals of total of animals tested displaying these movements.

when challenged, for instance by the approach of the experimenter, all cats executed ipsilateral FT3 movements (data not shown). These movements also appeared when the cat was handled, such as during the limb placing tests.

Apart from the effects described above, two cats (number 1 and 3) displayed ipsilateral FT3 movements (mean number ± SEM: 13.0 ± 0.0; Figure 6.2.4) during a short period (lasting 6 and 9 min, respectively) between the second and the final behavioural phase.

During the open-field observation, all cats displayed unilateral OFD movements. OFD movements were displayed at the contralateral as well as at the ipsilateral side during the first 60 min following the occlusion (Figure 6.2.5).

Open-field behaviour: subacute effects

One day following occlusion, three out of five cats showed hypo-activity (Figure 6.2.3, Table 6.2.1), whereas one animal displayed ipsilateral FT3 movements during the open

field test (Figure 6.2.4). The results of the remaining observations, viz. 3, 8-11 and 18-21 days post-occlusion, are depicted in Table 6.2.1 and Figures 6.2.1-6.2.4.

During the subacute observations all cats (n= 5) again displayed ipsilateral FT3 movements when handled (data not shown). As shown in Figure 6.2.5, ipsilateral dyskinetic movements were displayed subacutely by only one cat whereas contralateral OFD movements were displayed subacutely by four animals. Finally, FT2 movements were not observed during any of the open field tests.

Limb placing tests: acute effects

Unilateral MCA occlusion clearly affected limb-placing abilities (Table 6.2.2): during bar walking, all cats (n= 5) had difficulty with correctly placing the contralateral limbs on the bar. Frequently, the fore- and/or the hindlimb slipped from the bar; often, the animals remained in an awkward posture because they did not retract the affected limb

Table 6.2.1 Changes in open field behaviour elicited by focal ischemia from 50 to 60 min, and during 15 min on day 1, 3, 8-11 and 18-21 following occlusion of the right MCA.*

HYPO, hypo-activity; FR, freezing behaviour; ipsi FT3, **ipsilateral** forced turning movements, type 3 (see Section 6.2.2). O, no abnormal behaviour; -, not tested.

CAT	50-60 min	day 1	day 3	day 8-11	day 18-21
1	HYPO	0	FR	HYPO	HYPO
2	HYPO	HYPO	ipsi FT3	0	ipsi FT3
3	HYPO	ipsi FT3	ipsi FT3	ipsi FT3	ipsi FT3
4	HYPO	HYPO	HYPO	ipsi FT3	-
5	HYPO	HYPO	HYPO	0	-

*Note that changes in open field behaviour displayed between 0 and 60 min after occlusion are illustrated in Figures 6.2.1-6.2.5.

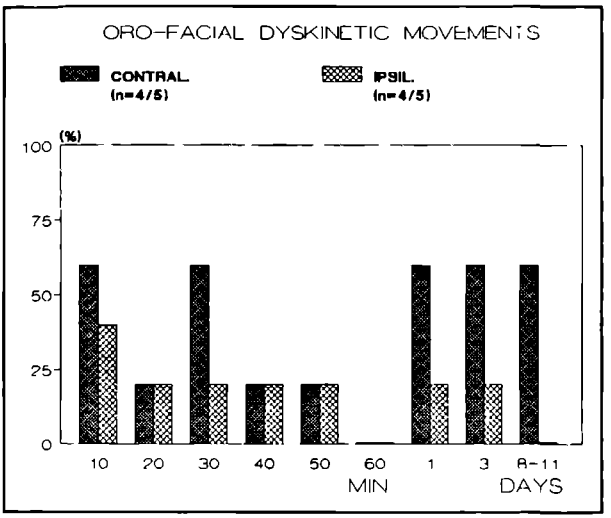

Figure 6.2.5 Percentage of animals showing OFD movements in the open field test during 10 min time-blocks from 0 to 60 min, and during one 15 min time-block per day 1, 3, and 8-11 days following MCA occlusion. n= number of animals of total of animals tested displaying these movements.

that was hanging alongside of the bar. Tactile placing too was deficient in all tested cats: they did not lift their contralateral fore- and hindlimb when the dorsum touched the edge of the table. Only one out of five cats showed intact proprioceptive placing; all other tested cats did not make any attempt to place the contralateral fore- and hindlimb after proprioceptive stimulation. On the other hand, placing reactions of the ipsilateral limbs were fully intact. Finally, visual placing was not affected; in fact, disturbed visual placing never occurred.

Limb placing tests: subacute effects

One day after the occlusion limb placing was recovered in part of the animals (Table 6.2.2). Moreover, on the bar only forelimb placing was still reduced, whereas hindlimb placing was already intact. Tactile as well as proprioceptive placing was absent in part of the animals (3 and 2 cats, respectively). The results of the remaining tests are presented in Table 6.2.2. As shown, there was a significant improvement of contralateral forelimb placing responses as well as of bar-placing of the contralateral hindlimb;

Table 6.2.2 Percentage of cats (n=5) showing intact contralateral limb placing 60 min, 1 day, 3 and 8-11 days after MCA occlusion.

	FORELIMB			HINDLIMB		
	BAR[a]	TAC[b]	PROP[c]	BAR[a]	TAC[b]	PROP[c]
60 min	0	0	20	0	0	20
Day 1	60	40	60	100	40	60
Day 3	80	80	80	100	60	60
Day 8-11	80	100	100	100	60	80
p	**	**	*	***	n.s.	n.s.

[a]bar placing; [b]tactile placing; [c]proprioceptive placing (see Section 6.2.2). *, $p < 0.05$; **, $p < 0.02$; n.s., not significant ($p > 0.05$): Cochran Q test (Siegel, 1956).

on the other hand, tactile and proprioceptive placing of the hindlimb did not improve significantly.

Metabolic effects

Unilateral MCA occlusion produced in all cats areas completely devoid of deoxyglucose uptake as a result of the ischemia in the caudate nucleus, temporal and insular regions of the cortex, the putamen, the globus pallidus, the internal capsule and the claustrum. Whereas the size of these areas in the posterior part of the caudate was comparable between different cats, their extent was rather variable in the anterior part of the caudate nucleus as well as in the other areas mentioned. The level of resolution revealed by the analysis of the autoradiograms as used in the present study was too low in order to correlate individual-specific behavioural effects with individual-specific changes in the glucose uptake. On the other hand, it was evidently sufficient to detect common effects throughout the tested population of cats. Representative autoradiograms at the level of the caudate nucleus are shown in Figure 6.2.6. As mentioned before, the posterior caudate nucleus was always severely affected after the occlusion

whereas the anterior part appeared to be less affected. Figure 6.2.7 shows a representative autoradiogram at the level of the substantia nigra pars reticulata and the deeper layers of the colliculus superior. No infarction was detected at the level at the level of the substantia nigra pars reticulata or the deeper layers of the colliculus superior. As illustrated in Figure 6.2.8, consistent relative reductions were observed in all areas studied, except for the sensorimotor cortex. At the level of the anterior caudate nucleus, uptake was significantly less at the occluded side compared to that of its contralateral counterpart in all occluded cats (Figure 6.2.8). The same holds true for

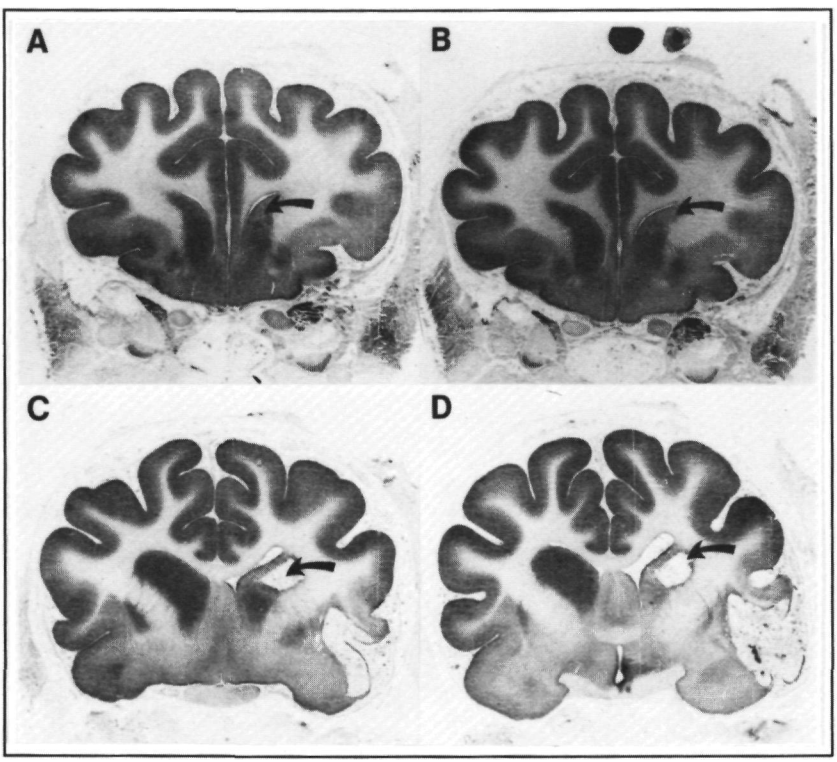

Figure 6.2.6 *Representative [^{14}C]-2-D-deoxyglucose autoradiograms at the level of the anterior (A, B) and the posterior caudate nucleus (C, D) of cat number 3.*

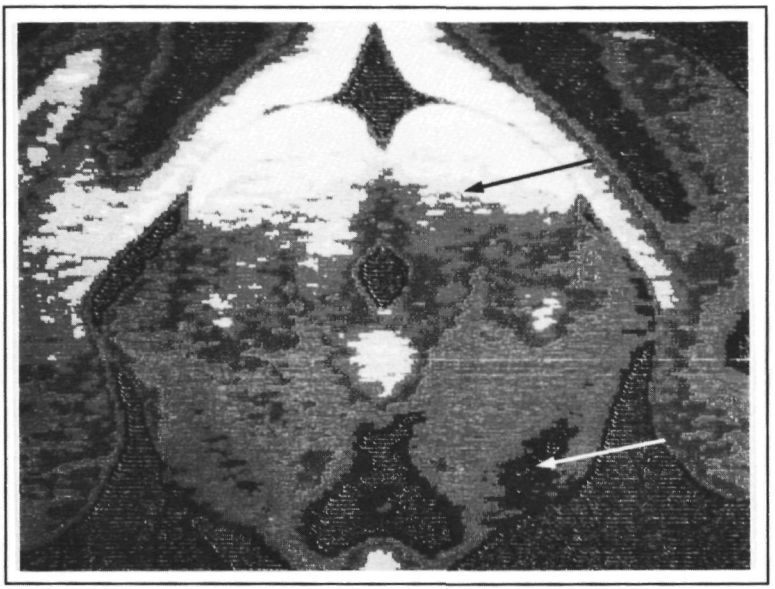

Figure 6.2.7 *Representative [^{14}C]-2-D-deoxyglucose autoradiograms at the level of the substantia nigra pars reticulata (lower arrow) and the deeper layers of the colliculus superior (upper arrow) of cat number 2. Differences in optical density are expressed in different grey tones (minimum activity: black; maximum activity: white) in order to illustrate the relative hypo-activity at the level of the substantia nigra pars reticulata and the deeper layers of the colliculus superior at the occluded (right) side compared to the non-occluded (left) side.*

the posterior caudate nucleus (Figure 6.2.8). Moreover, the relative decrease in deoxyglucose uptake in the anterior part appeared to be less than that in the posterior caudate in most cats. At the level of the substantia nigra and the colliculus superior, the uptake at the occluded side was also significantly less than that at the contralateral side in all cats (Figure 6.2.8).

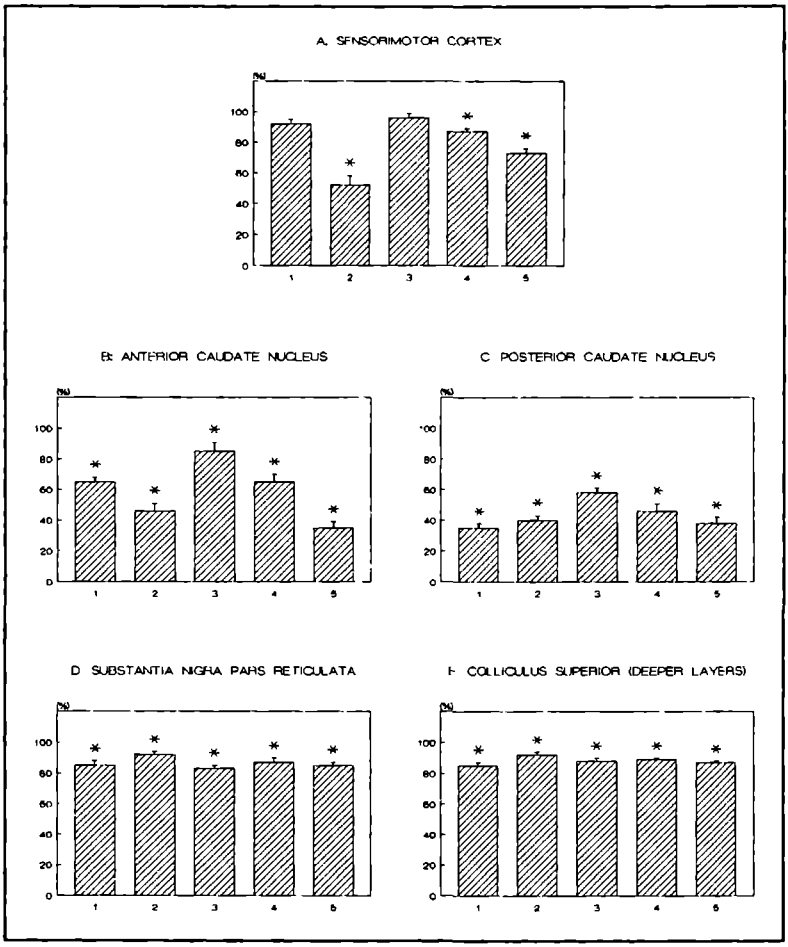

*Figure 6.2.8 Ipsilateral [^{14}C]-2-D-deoxyglucose uptake three weeks after permanent occlusion of the right MCA depicted as the percentage of the uptake in the corresponding region at the contralateral side (mean ± SEM of ten slices per region). Values of uptake in the following regions are shown: sensorimotor cortex (A), anterior caudate nucleus (B), posterior caudate nucleus (C), substantia nigra pars reticulata (D) and deeper layers of the colliculus superior (E). Values are shown per cat. *, $p<0.01$ (Wilcoxon matched-pairs signed-ranks test, two tailed; Siegel, 1956).*

6.2.4 DISCUSSION

The present study shows that permanent unilateral occlusion of the MCA in conscious cats produced a reproducible ischemic infarct in the ipsilateral posterior part of the caudate nucleus. In addition, the present data confirm the previously reported findings of others that MCA occlusion does not result in ischemic infarcts at the level of the substantia nigra and/or the colliculus superior (Kogure et al., 1974; Kuhl et al., 1980; Shigeno et al., 1985).

Focal cerebral ischemia as a result of occluding the right MCA resulted, apart from the well-known symptoms such as hemiparesis (Crowell et al., 1970; Tyson et al., 1984; Yamamoto et al., 1988), in a characteristic, reproducible sequence of behavioural events which was present in all tested animals. Moreover, these behavioural changes were not present before the actual occlusion showing that the implantation of the occluding device itself had no effect in this respect. Below, the data will be discussed in view of the consequences of unilateral MCA occlusion for the integrity of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus.

Caudate nucleus and effects of MCA occlusion

MCA occlusion induced ipsilateral FT1 movements in all cats during the first phase after the occlusion. Previously, it has been shown that inhibition of dopamine receptors and/or stimulation of GABA receptors in the rostromedial part of the caudate results in ipsilateral FT1 movements (see Section 6.2.2, Experimental procedures). Neither experimentally induced changes in the substantia nigra nor in the colliculus superior do result in such movements (see Chapter 4; see also Wolfarth, Kolasiewicz & Sontag, 1981). Accordingly, these data suggest that MCA occlusion affected striatal neuronal activity in a way comparable to inhibition of dopamine receptors and/or stimulation of GABA receptors. In this context, it is of interest to note that both stimulation of striatal GABA receptors (Kelly & McCulloch, 1984) and cerebral ischemia (Crowell et al., 1970; Osborne et al., 1987; Pulsinelli, Levy & Duffy, 1982; Shigeno et al., 1985; Symon, Pasztor & Branston, 1974; Tyson et al., 1984) can reduce local metabolic

activity in the striatum of acutely treated animals. The present data suggest that striatal metabolism was still reduced 21 days following MCA occlusion.

Substantia nigra and effects of MCA occlusion

MCA occlusion induced freezing behaviour in all cats during the second phase following the acute occlusion. Previously, it has been shown that inhibition of GABA receptors within the substantia nigra pars reticulata results in freezing behaviour (see Section 6.2.2, Experimental procedures). Neither experimentally induced changes in the caudate nor in the superior colliculus do induce such behaviour (Section 3.2; Cools, Struyker Boudier & Van Rossum, 1976). In view of the fact that MCA occlusion does not produce ischemic insults in the substantia nigra (see Section 6.2.1, Introduction), these data suggest that MCA occlusion might have indirectly decreased the release of nigral GABA. Such a decrease is indeed the consequence of inhibition of striatal dopamine receptors and/or stimulation of striatal GABA receptors (Gale & Casu, 1981; Scheel-Krüger, 1983), viz. an effect that appears to be mimicked after MCA occlusion (see above: Caudate nucleus and effects of MCA occlusion section). Given this suggestion, one would expect that MCA occlusion and stimulation of striatal GABA receptors similarly affect the metabolic activity within the substantia nigra pars reticulata. This is indeed the case: both acute MCA occlusion (Diemer & Siemkowicz, 1980; Shibuya, Arita & Yamamoto, 1987; Shigeno et al., 1985) and stimulation of striatal GABA receptors (Kelly & McCulloch, 1984)) have been found to enhance nigral metabolism. However, the present study shows that there was a consistent dysbalance in metabolism at the level of the substantia nigra pars reticulata: deoxyglucose uptake was decreased ipsilaterally and/or increased contralaterally 21 days following occlusion (cf. Nakayama et al., 1987). This finding may be explained by considering the subacute consequences of MCA occlusion for the GABAceptive cells in the pars reticulata of the substantia nigra. As discussed above, MCA occlusion might have reduced the release of nigral GABA and, accordingly, disinhibited the GABAceptive cells in the pars reticulata. As a consequence, the latter cells might become hyperactive. Since hypermetabolism can ultimately result in neuronal death (cf. Coyle et al., 1981; Foster & Fagg, 1984; Ingvar, Folbergrova & Siesjö, 1987; McGeer,

McGeer & Singh, 1978; Olney, 1971), it is not unlikely that such a secondary hypermetabolic damage to the substantia nigra pars reticulata might have caused the metabolic decrease in the substantia nigra pars reticulata found three weeks after the occlusion. In order to test the latter possibility detailed histological analyses are in progress. The finding of Tamura and colleagues, that one week after unilateral MCA occlusion a marked atrophy of the ipsilateral substantia nigra can be found in rats fits in with this suggestion (Tamura et al., 1990).

Colliculus superior and effects of MCA occlusion

During the final behavioural phase following the acute MCA occlusion, all cats displayed hypo-activity; in addition, all cats displayed unilateral FT3 movements that were triggered by changes in exteroceptive stimuli. Both the hypo-activity as well as the latter movements were also observed during the subacute tests, up to 21 days following occlusion. Previously, it has been found that stimulation of GABA receptors within the deeper layers of the superior colliculus results in a state in which the cat's behaviour is fully directed by changes in exteroceptive stimuli. In case of absence of exteroceptive stimuli the behaviour of the animal is characterized by hypo-activity (Cools et al., 1984; Gelissen & Cools, 1986). Neither experimentally induced changes in the caudate nucleus nor in the substantia nigra pars reticulata do induce hypo-activity or FT3 movements (Section 4.1; see also Cools, Struyker Boudier & Van Rossum, 1976; Wolfarth, Kolasiewicz & Sontag, 1981). In view of the fact that MCA occlusion does not produce ischemic insults in the colliculus superior (see Section 6.2.1, Introduction), these data suggest that MCA occlusion might have indirectly enhanced the release of GABA in this region. Such an enhancement is indeed the consequence of a reduced GABAergic activity at the level of the substantia nigra pars reticulata (Gale & Casu, 1981; Scheel-Krüger, 1983), viz. an effect that appears to occur after MCA occlusion (see above: Substantia nigra and effects of MCA occlusion section). Since the latter is in turn the consequence of an inhibition of striatal dopamine receptors and/or stimulation of striatal GABA receptors, viz. an effect that also appears to be mimicked by MCA occlusion (see above: Caudate nucleus and effects of MCA occlusion section), one would expect that both MCA occlusion and stimulation of striatal GABA receptors

similarly affect collicular metabolism. This is indeed the case: both (transient) unilateral occlusion (Pulsinelli, Levy & Duffy, 1982) and unilateral stimulation of striatal GABA receptors (Kelly & McCulloch, 1984) have been found to reduce the metabolic activity in the ipsilateral superior colliculus. The present study suggests that the latter reduction was still present 21 days following occlusion. Since the FT3 movements were solely directed towards the occluded side, it appears that MCA occlusion actually produced a dysbalance between the left and right colliculus. Given the earlier reported finding that FT3 movements that are elicited from the deeper layers of the superior colliculus are always directed away from the side at which the GABAergic activity is inhibited (Section 4.2), the present findings suggest that the MCA occlusion produced a dysbalance marked by a relative GABAergic hyperactivity at the occluded side and/or by a relative GABAergic hypo-activity at the non-occluded side. In line with the latter suggestion is the finding that striatal lesions actually produce an increase in metabolic activity in the deeper layers of the contralateral superior colliculus (Kelly & McCulloch, 1987). In this context, however, it has to be stressed that it is not possible to ascribe the demonstrated asymmetries in metabolism to unilateral and/or bilateral changes in metabolic activity because of methodological constraints.

Orofacial dyskinetic movements

In the present study, OFD movements were elicited at both sides during 60 min post occlusion, whereas they were mainly displayed at the contralateral side subacutely. In previous studies, such movements have never been observed after experimentally induced changes at the level of the rostromedial part of the caudate nucleus, the substantia nigra pars reticulata or the deeper layers of the superior colliculus (Chapter 3; see also Cools et al, 1984; Wolfarth, Kolasiewicz & Sontag, 1981). Moreover, OFD movements were displayed during all behavioural phases acutely as well as subacutely after MCA occlusion. These data together suggest that the latter movements were not mediated by the caudato-nigro-collicular circuitry. On the other hand, ipsilateral OFD movements are elicited by unilateral activation of dopamine receptors within the anterodorsal part of the feline caudate as well as by inhibition of GABA receptors within the so-called subcommissural part of the globus pallidus, i.e. a region that

receives output signals from the anterodorsal part of the caudate nucleus (Cools et al., 1989). The present study also shows that metabolism was relatively decreased in the ipsilateral anterior caudate nucleus, i.e. the striatal part that encompasses the anterodorsal region. These data suggest that the OFD movements under discussion were induced by functional changes at the level of the anterodorsal part of the caudate and/or at the level of one of its output stations.

Limb placing deficits

Contralateral bar-placing deficits, as found in the present study, have never been observed following experimentally induced changes in dopaminergic activity at the level of the caudate nucleus, or alterations in GABAergic activity at the level of the substantia nigra pars reticulata or the deeper layers of the superior colliculus (see Sections 3.2, 3.3, 4.1; see also Gelissen & Cools, 1986; 1988). Accordingly, these data suggest that at least deficient bar-placing responses were not induced by functional disturbances in the caudato-nigro-collicular pathway. On the other hand, deficient limb placing does occur after large lesions of the frontal cortex (Armstrong, 1986; Bard, 1933; Villablanca et al., 1976) or after more than 70 % ablation of the striatum (Villablanca et al., 1976). In the present study, deficient contralateral forelimb and hindlimb placing responses were present acutely; forelimb placing as well as hindlimb bar-placing improved subacutely. In contrast to the present findings, limb placing deficits following crude caudate and cortical lesions last at least several months (for rev., see Armstrong, 1986). Following pyramidotomy, recovery of contralateral limb placing does not occur at all (Liddell & Phillips, 1944). The latter fact together with the present finding that the occlusion always affected only the contralateral limbs may imply that these effects were actually due to changes in neuronal activity in cortical regions and/or damage to extra-striatal cortical efferents. The present finding that metabolic activity appears to be relatively reduced in the ipsilateral sensorimotor cortex 21 days following the occlusion in part of the cats fits in with the latter suggestion.

Increasing pathology induced by MCA occlusion

Focal ischemia as a result of the unilateral occlusion of the MCA induced acutely

an increasing pathology resulting successively in functional changes at the level of the rostromedial part of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus. In addition, metabolism appeared to be reduced at the level of these structures 21 days after the occlusion. The present data show that MCA occlusion not only produced metabolic disturbances in structures within the ischemic region such as the caudate nucleus, but also in brain regions located remote from the insult such as the substantia nigra and the superior colliculus. The finding that behavioural effects characteristic for remote output stations of the caudate were still present subacutely, opens the perspective that at least part of the symptoms in human beings with MCA occlusion may be due to dysfunctioning output stations of the ischemic area rather than due to the ischemic area itself. Accordingly, drugs affecting these output stations might have a therapeutic value in such patients.

In Section 6.1, we have reported that unilateral intrastriatal injections of kainic acid induced acutely the successive display of the following abnormal movements: (1) contralateral FT1 movements, viz. movements that are characteristic for a stimulation of striatal dopamine receptors and/or inhibition of striatal GABA receptors; (2) contralateral FT2 movements, viz. movements that are characteristic for a stimulation of substantia nigra pars reticulata GABA receptors; and (3) contralateral FT3 movements, viz. movements that are characteristic for an ipsilateral inhibition of deeper layers of the colliculus superior GABA receptors. Comparing the behavioural changes following intrastriatal injections of kainic acid with those elicited acutely by unilateral MCA occlusion (see above), it appears that MCA occlusion produces successive functional alterations at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the superior colliculus which are diametrically opposite to those found after kainic acid. As discussed in Section 6.1, the kainic acid-induced effects could be ascribed to its neuro-excitatory effects, but not to its lesioning effects. Anyhow, these data indicate that the progressive pathology acutely induced by striatal injections of kainic acid are not due to typical features of this neurotoxin, but are indeed due to the resulted distortion of information sent by the caudate towards output stations such as the reticular substantia and the deeper layers of the superior

colliculus. The same holds true for the progressive pathology induced by MCA occlusion: However, in the latter case the distortion of information results from the occlusion-induced cell death in the caudate nucleus. In other words, the successive appearance of the behavioural and metabolic changes in the substantia nigra and the superior colliculus following striatal manipulation appears to be inherent in the hierarchical order of the brain structures under discussion (see Section 5.1, 6.1; cf. Cools et al., 1984). This notion implies that at least part of the symptoms inherent in central disorders such as Parkinson's Disease or Huntington's Chorea, may actually be due to disturbed functioning of striatal output stations.

CHAPTER 7

CAUDATE NUCLEUS AND THE PROGRAMMING OF MUSCLE ACTIVITY

Summary

The effect of intracaudate injections of the dopaminergic antagonist haloperidol on muscle activity was investigated in cats that had to jump down from different heights, i.e. 60, 90 and 120 cm. Electromyographic (EMG) activity of an elbow extensor was used as a dependent variable. The EMG patterns of each cat were averaged per jump height. Two prelanding bursts, i.e. burst1 and burst2, and one postlanding part labelled as burst3 could be distinguished in the resulting records: these bursts were separately analyzed. Haloperidol treatment produced two types of effects: First, it reduced the postlanding part of burst2 during jumps from all three jump levels. This subtle, but consistent effect was dopamine-specific and required a certain GABAergic activity at the level of the deeper layers of the colliculus superior. Second, haloperidol also produced the following changes in the EMG patterns: an earlier termination of burst2 during 90 and 120 cm jumps; a reduction in the prelanding part of burst2 during 90 cm jumps; an increase in burst3 in some of the tests. Type B effects were neither dopamine-specific nor funnelled via the striato-nigro-collicular pathway. A reduced ability to program arbitrarily behaviour is suggested to give rise to the haloperidol-induced dopamine-specific changes in EMG activity.

7.1 INTRODUCTION

In order to control landing from a fall or a jump, limb muscles are activated in a specific way. After impact, electromyographic (EMG) activity is at least in part controlled by reflex mechanisms in the limb muscles and tendons (Dietz, Noth & Schmidtbleicher, 1981; Greenwood & Hopkins, 1976; Lewis et al., 1979). However, prior to landing vestibular stimuli seem to play an important role, especially in the case of unexpected falls. After a sudden unexpected fall, for example, the onset of prelanding EMG activity starts after a fixed delay following release. However, the first part of the EMG signal is absent following labyrinthectomy. These findings suggest that especially the initial phase of the signal is controlled by vestibular information (man: Greenwood & Hopkins, 1976; Melvill-Jones & Watt, 1971; monkey: Lacour, Xerri & Hugon, 1978; cat: Watt, 1976). In the case of self-initiated falls or jumps-downs repeatedly executed from a particular height, prelanding muscle activity is fixed in relation to the moment of impact, but not to the moment of release. Accordingly, vestibular stimuli are less important in the programming of prelanding muscle contractions during landing from self-initiated falls or jumps (man: Dietz & Noth 1978; monkey: Laursen et al., 1978; cat: McKinley, Smith & Gregor, 1983). Dietz and Noth (1978) even suggested that the vestibulospinal reflex was suppressed as a result of a learning process during self-initiated falls. This suggestion is supported by the finding that during self-initiated falls from semi-randomly varied heights, the onset of prelanding muscle contractions, being defined in relation to the moment of impact, begin earlier when the fall trajectory is increased (Dietz, Noth & Schmidtbleicher, 1981). Further, conditioning processes also seem to play an important role in the control of prelanding EMG activity since the display of EMG activity does not depend on visual information as long as the height is known (Dietz & Noth, 1978; McKinley & Smith, 1983). Finally, Greenwood and Hopkins (1976) showed that prelanding EMG activity during unexpected falls is comprised of two bursts consisting of a first peak related to the moment of release and, as suggested before, likely to depend on vestibular stimuli and a second peak related to the moment of landing and most likely controlled by other mechanisms (cf. Dietz & Noth, 1978; Greenwood & Hopkins, 1976;

Lacour, Xerry & Hugon, 1978; McKinley & Smith, 1983; McKinley, Smith & Gregor, 1983; Melvill Jones & Watt, 1971). Taken together, these reports show that the first part of prelanding EMG activity during a fall or jump is controlled, at least partly, by vestibular stimuli provided the task is not conditioned, whereas the final part of the preprogrammed EMG pattern is controlled, at least partly, by central programming mechanisms (cf. Dietz & Noth, 1978; Greenwood & Hopkins, 1976; Lacour, Xerri & Hugon, 1978; McKinley, Smith & Gregor, 1983; Melvill Jones & Watt, 1971). In this context it is relevant to note that several studies on anaesthetized cats have shown that supraspinal centres such as the substantia nigra and the colliculus superior are directly involved in the control of skeletomotor (alpha) and/or fusimotor (gamma) motoneuron activity (cf. Alstermark et al. 1984a, 1984b; Schwarz, Sontag & Wand, 1984a, 1984b; Sontag et al., 1984; Wagner & Kalmring, 1968; York, 1973). Thus, the analysis of perilanding EMG activity during experimentally induced changes in brain regions may provide insight in the way in which such structures are involved in the programming of muscle activity.

Chapter 3 (Section 3.1) presented evidence that the feline caudate nucleus is selectively involved in the ability to switch motor patterns which are not dictated by exteroceptive stimuli. Additional studies have demonstrated that this programming function extends to behaviour strategies in rats (Cools, 1980), social interactions in monkeys (Van den Bercken & Cools, 1982) and motor and cognitive strategies in man (Cools et al., 1984). On the basis of these and related data it has been concluded that the caudate nucleus programmes motor behaviour in a highly specific manner: It directs the ability to program arbitrarily behaviour.

In view of these data the question arose whether the above-mentioned role of the caudate nucleus also becomes manifest at the level of muscle activity. If so, it would become possible to use task-specific changes in EMG activity as additional tools in the diagnosis of a dysfunctioning caudate nucleus in man. Because of technical reasons, it was impossible to use the motor task employed previously to delineate the above-mentioned programming function of the caudate nucleus. In the present study we

analyzed the effects of intracaudate injections of haloperidol upon EMG activity in the lateral head of the triceps brachii, a forelimb extensor muscle, of cats during landing on a platform. This model was chosen for the following reasons. First, it is a classic paradigm which is well-studied. Second, the caudate nucleus sends information to the substantia nigra pars reticulata and the deeper layers of the colliculus superior, that is structures known to be directly involved in the control of the spinal motor elements. And, thirdly, evidence is available that at least part of the perilanding EMG activity during jump-downs is neither fully dictated by vestibular stimuli nor by stimuli derived from receptors within the forelimbs.

The animals were required to jump off a platform before and after the intracaudate application of haloperidol. Jump height was varied throughout the test sessions in order to avoid conditioning of the task. In order to establish the dopamine receptor specificity of haloperidol-induced changes in EMG patterns, caudate injections of the dopamine agonist apomorphine were given 5 min after haloperidol in an additional series of experiments.

In a final series of experiments, it was investigated whether the deeper layers of the colliculus superior, a structure receiving neuronal information derived from the striatal output station the substantia nigra pars reticulata (Edwards et al., 1979; Graybiel, 1978; Graybiel & Ragsdale, 1979; Royce & Laine, 1984; see Section 2.3) were involved in haloperidol-induced changes in EMG activity. Inhibition of striatal dopaminergic receptors is known to result ultimately in an activation of collicular gamma-aminobutyric acid (GABA) receptors (Gale & Casu, 1981; Scheel-Krüger, 1983). Thus, it was decided to study the effect of intracollicular injections of the GABAergic antagonist picrotoxin on haloperidol-induced changes in EMG patterns. Data will be presented showing that intracaudate injections of haloperidol produce subtle changes in perilanding EMG activity which are, in some cases, dopamine-specific. Moreover, only the latter changes appeared to be mediated via the deeper layers of the colliculus superior.

7.2 EXPERIMENTAL PROCEDURES

Apparatus

The starting platform consisted of a wooden plank (14 x 32 cm) which could be adjusted to a height of 60-120 cm above the landing platform. The landing platform (65 x 65 cm) was situated immediately in front of the starting platform and consisted of a thin (1 mm) metal plate that covered a second, thick (6.5 mm) metal plate. In order to detect the moment of landing, the two plates of the landing platform were separated from each other using air pressure. Air pressure was adjusted in such a way that both plates were pressed together at the slightest touch, triggering an electronic signal which indicated the moment of landing. Care was taken to assure that the air pressure was held constant throughout all experiments.

Animals

Male cats (n= 20) were selected from the laboratory breeding colony of the University of Nijmegen. They were housed in iron cages (1.9 x 1.2 x 1.6 m) in groups of maximally four animals. Food (Hope Farms) and water were present ad lib. Only cooperative cats were used in the present study. During four to seven training sessions on separate days, they were habituated to the experimental set-up. A training session lasted 60 min and consisted of three tests interspaced by an interval of 15 min. Fifteen minutes before the first test and during the intertest intervals, the cat was placed in a wooden observation cage (90 x 60 x 60 cm). During each 5 min test, the cat was repeatedly placed on the starting platform to jump down onto the landing platform where it was rewarded with a few food pellets (Brekkies, Effem B.V., Etten-Leur, The Netherlands). Initially, the starting platform was mounted 60 cm above the landing platform; as soon as the animal jumped down without hesitation onto the landing platform, the height of the starting platform was increased to 90 cm and, finally, to 120 cm. During the next sessions, the height of the starting platform was varied in a fixed order to avoid conditioning of the jump distance as much as possible (cf. Dietz & Noth, 1978; McKinley & Smith, 1983). During each training test, the cat had to execute 12 jumps from varying heights in the following order: 60-90-120-90-60-120-60-

120-90-120-90-60 cm. In this way, 4 jumps from every height were executed per test. As soon as the cat was able to execute 12 correct jumps per test during one session, training was stopped.

Implantation of cannulas

The trained cats were stereotaxically equipped (under sodium pentobarbital anaesthesia, 45 mg/kg, IP) with stainless steel cannulas (outer diameter 0.8 mm). In the first group of cats (n= 8), the tip of the cannulas was placed into the rostromedial part of the caudate nucleus, with coordinates: A 14.5, L 5.0, H 5.0 (according to the atlas of Snider and Niemer, 1964; see also Chapter 3). In a second group, cats (n= 12) were equipped with caudate cannulas as well as with cannulas aimed at the deeper layers of the superior colliculus. The colliculus superior is divided into superficial and deeper layers according to the terminology of Kanaseki and Sprague (1974). In order to avoid damage to the tectal tissue, the tips of the latter cannulas were implanted just above the colliculi: A 1.5, L 3.5, H 6.5 (Snider and Niemer 1964; see Section 4.2).

Injection procedure and drugs

Drugs were bilaterally injected with the help of a 5.0 μl Hamilton syringe (diameter of the injection needle: 0.4 mm; see Chapter 3). The volume of the caudate injections was 5.0 μl. Drug solutions were injected into the deeper layers of the superior colliculus (A 1.5, L 3.5, H 2.5) using a Hamilton syringe with a sharpened tip (see Section 4.2). Vehicle (distilled water) injections into the caudate nucleus and/or the deeper layers of the superior colliculus served as a control. Doses and time schedule were based on the outcome of previous studies. The doses used were: 12.5 μg/5.0 μl haloperidol (Haldol, Janssen Pharmaceutica), 0.6 μg/5.0 μl apomorphine (Brocades) and 0.1 μg/0.5 μl picrotoxin (Serva). These concentrations were maximally effective in previous behavioural experiments (see Section 3.1 and 4.2). All solutions were freshly prepared immediately before each experiment. Each cat participated in maximally 4 experiments that were spaced one week apart. The order of experiments in which the cats participated as well as the injection time of the different drugs are shown in Table 7.1.

Implantation of EMG electrodes

During the same surgical session (see: Implantation of cannulas), the cats were also equipped with a pair of chronic EMG electrodes implanted into the lateral head of the right triceps brachii, an elbow extensor muscle of the forelimb. The electrodes were made of teflon-insulated, stainless-steel wires (AS 632 SS, Cooner Wire, Chabworth, USA). The electrodes were implanted following the method of Betts and coworkers (1976), with some modifications. After exposure of the muscle, the electrodes were subcutaneously passed from a multi-pin connector attached on the skull to the forelimb. Next, a hypodermic needle was passed through the muscle. One electrode was threaded into the tip of the needle; subsequently, the needle was removed. The same procedure was repeated for the implantation of the second electrode. The insulated ends were knotted together and, after scraping off a small portion (about 2 mm) of the insulation, the wires were retracted until both uninsulated parts were embedded in the muscle and the knotted ends were resting on the surface of the muscle. The electrodes were implanted in such a way that the distance between both uninsulated ends was 5-6 mm. In addition, a grounding electrode was implanted in the vicinity of the recording electrodes. The animals were allowed to recover for a period of at least one week before the experiments were started.

Experimental design

The experimental design was identical to the final training session. At t=0 min the experiment was started by placing the cat into the observation cage in order to adapt to the experimental surroundings. The first test (PRE-injection test) that served as a control was started at t=15 min. Drug solutions and/or solvent were injected between t=20 and t=30 min (see Table 7.1 in which the exact time schedule of the distinct treatments is presented). The second and third test, during which the drug effects could be analyzed, were started at t=35 min (POST-injection test1) and t=55 min (POST-injection test2), respectively. The cat was placed in the observation cage during the 15 min intertest intervals.

TABLE 7.1 Schematic representation of the order in which various drugs were tested and their injection times (CN-inj: bilateral injections into the caudate nucleus; CSDL-inj: bilateral injections into the deeper layers of the colliculus superior). Slv= solvent (i.e. distilled water); Hal= haloperidol 12.5 µg/5 µl; Apo= apomorphine 0.6 µg/5 µl; Ptx= picrotoxin 0.1 µg/0.5 µl.

The number of cats per experimental group is given in parentheses.

	GROUP 1			GROUP 2		
Expe-riment	CN-inj t=25 min	CN-inj t=30 min	(n)	CSDL-inj t=20 min	CN-inj t=25 min	(n)
1	Slv	Slv	(8)	Ptx	Slv	(12)
2	Hal	Slv	(8)	Ptx	Hal	(10)
3	Hal	Apo	(7)	Slv	Slv	(8)
4	Slv	Apo	(5)			

EMG recording and analysis

Immediately before a test, a multiwire cable was attached to the head contact. EMG activity was amplified and filtered (high pass filter: 80 Hz; low pass filter: 1000 Hz). The trigger signal provided by the landing platform was used to sample EMG activity from 250 ms before until 250 ms after impact with the help of a personal computer (A/D sampling rate: 4000 Hz). For each jump height, EMG records were analyzed in several steps. First, the myograms recorded during each test were rectified and averaged per cat in order to reduce the intraindividual variability. In general, the averaged records (AEMG) were characterized by two bursts of activity which started before landing, and several bursts of activity which started after impact. The bursts that started before landing were labelled as burst1 and burst2 and the bursts that started after landing were taken together and labelled as burst3. The second step in the analysis consisted of the determination of the start and the end of these bursts by visual inspection of the AEMG records on the screen using a cursor. The start of burst1 and the end of burst3 were defined as an increase of more than 200% respectively a decrease to less than 200% of average baseline activity; baseline activity was

determined during the first 50 ms of the records, i.e., between 250 and 200 ms before landing. Since we were interested in burst2 (see Section 7.1, Introduction), the start and the end of this part of the myogram was determined as described below. In view of the fact that the AEMG records did not reach a level below 200% of average baseline activity between distinct bursts we used an alternative method to determine the onset and the offset of burst2. The onset of burst2, which always started prior to landing, was determined as follows (the moment of onset of burst2 was also considered to be the moment of offset of burst1): At first, the time-point at which burst2 was maximal was determined. Starting from that point, the amplitude of the signal was traced backwards: as soon as a 'time-point of minimum activity' was reached, it was considered to be the moment of onset of burst2. A 'time-point of minimum activity' is characterized by a change of the slope from negative to positive and an amplitude less than 50 % of the peak value of burst2. The offset of burst2 which terminated always after landing, was determined in the same way (the moment of offset of burst2 was also considered to be the moment of onset of burst3): starting from the time-point at which burst2 reached its maximum, the amplitude was traced forwards until again a point of minimum activity was reached.

In a third step, the EMG activity was integrated to determine experimentally-induced changes in the amount of EMG activity. The integrated EMG (IEMG) activity of burst1, the prelanding part of burst2 (i.e., that part of burst2 which occurred immediately before the moment of landing), the postlanding part of burst2 (i.e., that part of burst2 that was present immediately after touch-down) and of burst3 were separately computed. Since none of the treatments prevented the cats from landing successfully (See Section 7.3, Results), we expected no dramatic changes in the overall perilanding EMG activity. Our interest was especially concerned with the possibility that the total amount of muscle activity might be rearranged between the distinct parts of the myograms. Therefore, we expressed the IEMG activity of the distinct bursts as a percentage of that of the overall EMG signal. In step four of the analysis, drug effects were analyzed for each part of the myogram separately using the following ratio which was calculated per cat, per jump height and per experiment: IEMG (POST-injection

test1) / IEMG (PRE-injection test). Such a ratio has previously been found to be a valid tool for correcting possible pre-drug differences. Drug effects during POST-injection test2 were evaluated in the same way by computing the ratio IEMG (POST-injection test2) / IEMG (PRE-injection test).

In case the raw EMG signal showed abnormal spikes such as artifacts due to movements of the electrodes, the experimental data of that animal were discarded.

Histology

After the final experiment, the cats were deeply anaesthetized with an overdose of pentobarbital and perfused transcardially with a 4 % formaldehyde solution. The brains were removed and slices were cut (30 μm) on a freezing microtome. After staining with cresyl violet, the exact location of the injection sites was determined.

Statistics

Drug effects were determined using the Mann Whitney U-test (two tailed), unless otherwise mentioned.

7.3 RESULTS

Histology

Verification of the injection sites revealed that all injections were correctly placed within the rostromedial part of the caudate nucleus [coordinates (according to the atlas of Snider and Niemer, 1964) found were: A 14.0-15.0; L 5.0-5.5; H 4.5-5.5; see Figures 3.2.1 and 5.1.2]. The same holds true for the injections placed in the deeper layers of the superior colliculus [coordinates (Snider & Niemer, 1964) found were: A 0.5-1.5; L 3.0-4.5; H 2.0-3.0; see Figure 4.2.1A].

General description of the EMG recordings

Figure 7.1 shows raw EMG signals of representative jumps from 60, 90 and 120 cm

recorded during a PRE-injection test. Figure 7.2 shows an example of the averaged EMG signals of four jumps before (A) and after (B) haloperidol treatment. As described in Section 7.2 (Experimental Procedures), only the latter signals were used for the analysis of the haloperidol-induced changes. As shown, the myograms contain two prelanding burst, i.e., burst1 and burst2, respectively, with burst2 continuing after

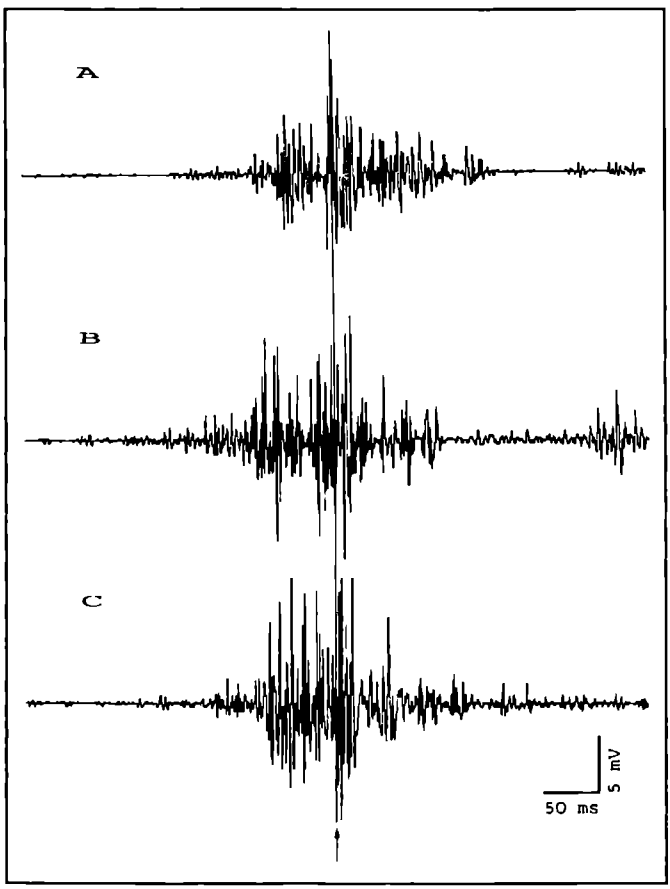

Figure 7.1 Raw EMG records of representative jumps from a height of 60 (A), 90 (B) and 120 cm (C) of cat 476 during the PRE-injection test of the solvent experiment. The moment of landing is indicated by the vertical bar (arrow).

TABLE 7.2 Mean ± SEM of onset and offset times of burst1, burst2 and burst3 (ms, with respect to the moment of landing) of averaged (n= 4) myograms per cat and per jump height during PRE tests of solvent-treated cats (n=16).

JUMP HEIGHT	onset Burst1 (ms)	onset Burst2 (ms)	offset Burst2 (ms)	offset Burst3 (ms)
60 cm	70.2 ±29.2	14.7 ±6.6	5.9 ±2.4	135.5 ±31.6
90 cm	79.6 ±21.0	16.1 ±5.4	4.8 ±3.0	120.8 ±29.2
120 cm	91.3 ±26.7	17.2 ±5.5	5.5 ±2.4	105.3 ±28.6
p*	<0.001	>0.05	>0.05	<0.001

*Friedman two way analysis of variance.

impact. After landing, butst2 is followed by several other, smaller bursts labelled together as burst3.

Table 7.2 shows the mean (± SEM) onset and offset times of burst1, burst2 and burst3 of the AEMG signals obtained during Pre-injection tests of solvent-treated cats (animals from group 1 plus group 2: n=16). There appeared to be a significant difference in onset of burst1 as well as offset of burst3. Apparently, muscle activity started earlier and terminated earlier when jump height was increased.

In addition, the IEMG activity of burst1, the prelanding part and the postlanding part of burst2 was significantly enhanced when jump height was increased (Table 7.3). In contrast, IEMG of burst3 was not affected by jump height.

None of the treatments described below prevented the cats from jumping down and landing on the platform in a normal way despite of the drug-induced changes in EMG activity (see below). During PRE-injection tests, there were no significant

differences per jump level between experimental groups with respect to onset and offset of various parts of the myograms.

Extensor EMG activity during POST-injection test1

Neither intracaudate injections of haloperidol or apomorphine nor collicular injections of picrotoxin affected the onset of burst1 and burst2 or the offset of burst3 during the POST-injection test1, i.e., 10-15 min after the intracerebral injections. The only effect noted was a more rapid offset of burst2 in haloperidol-treated cats during 90 cm jumps [median offset time, solvent (6.3 ms) vs haloperidol (4.3 ms) treated cats: $p<0.05$] as well as during 120 cm jumps [median offset time, solvent (8.3 ms) vs haloperidol (6.3 ms) treated cats: $p<0.01$]. Neither intracaudate injections of apomorphine nor intracollicular injections of picrotoxin prevented these effects.

Haloperidol did affect the integrated signals of several bursts; especially the postlanding part of burst2 was affected (Figure 7.2). As described below, the latter effect was dopamine-specific. Table 7.4 presents the median values of IEMG ratio's of

Table 7.3 Mean ± SEM of IEMG activity (mVms) of burst1, prelanding and postlanding part of burst2 and burst3 of averaged (n=4) myograms per cat and per jump height during PRE tests of solvent-treated cats (n=16).

JUMP HEIGHT	PRELANDING BURST1	PRELANDING BURST2	POSTLANDING BURST2	POSTLANDING BURST3
60 cm	30.9 ±27.3	21.5 ±17.6	9.6 ±8.1	86.5 ±53.3
90 cm	63.4 ±49.3	25.1 ±14.3	6.7 ±6.3	96.8 ±58.6
120 cm	91.9 ±69.0	30.3 ±18.6	11.1 ±6.6	97.8 ±56.2
p*	<0.001	<0.01	<0.05	>0.05

*Friedman two way analysis of variance.

burst1, the prelanding and the postlanding part of burst2, burst3 and the complete myogram for each jump level. None of the treatments affected the IEMG activity of burst1 (Table 7.4A). The prelanding IEMG of burst2 was significantly reduced by haloperidol during 90 cm, but not 60 and 120 cm jumps as shown in Table 7.4B. This effect of haloperidol was neither prevented by apomorphine nor by picrotoxin, which *per se* did not induce any significant effect. Furthermore, haloperidol significantly reduced prelanding IEMG activity of burst2 during 60 cm jumps only in picrotoxin-

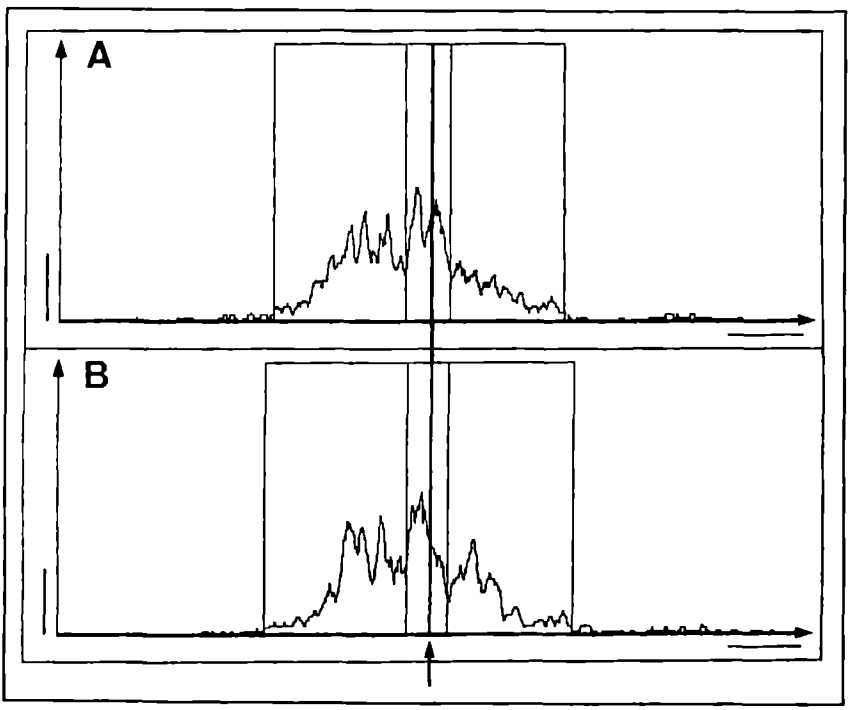

Figure 7.2 Representative examples of rectified and averaged myograms of four 120 cm jump-downs of cat 433 during the PRE-injection test (A) and during the POST-injection test1 (B), i.e. 10-15 min after CN-injections of 12.5 µg/5.0 µl haloperidol. The moment of landing is indicated by an arrow. In addition, the onset of burst1 and burst2 as well as the offset of burst2 and burst3 are indicated by thin vertical lines. Bars indicate 50 ms (abscissa) and 2.5 mV (ordinate).

Table 7.4 Median value (25-75% range) of the ratio of (IEMG activity during the POST-injection test1) / (IEMG activity during the PRE-injection test) of burst1, the prelanding part of burst2 and the postlanding part of burst2, burst3 and of the complete AEMG per jump height CN. intracaudate injections at t= 25 (Hal, haloperidol 12 5 μg/5 μl; or Slv, solvent 5 μl) and/or t= 30 min (Apo, apomorphine 0 6 μg/5 μl), CSDL/CN· intracollicular injections at t= 20 min (Ptx, picrotoxin 0 1 μg/0 5 μl; or Slv 0 5 μl) and intracaudate injections at t= 25 min (Hal)

Differences were tested per jump level. * $p<0.05$; ** $p<0.02$; *** $p<0.002$: drug vs. corresponding control experiment. $^{+}$ $p<0.05$; $^{++}$ $p<0.02$: Hal/Apo and Ptx/Hal vs Hal. $^{\circ}$ $p<0.05$; $^{\infty}$ $p<0.02$: Hal/Ptx vs Ptx.

A: BURST1

INJECTIONS	JUMP LEVEL 60 cm	90 cm	120 cm
CN:			
Slv/Slv	0.94 (0.94-1.01)	0.95 (0.91-0.97)	0.94 (0.91-1.01)
Hal/Slv	1.12 (0.85-1.17)	0.96 (0.90-0.97)	0.95 (0.93-1.00)
Hal/Apo	1.05 (0.94-1.15)	1.05 (0.94-1.06)	0.93 (0.88-1.01)
Slv/Apo	0.84 (0.82-1.01)	1.00 (0.97-1.05)	1.00 (0.96-1.04)
CSDL/CN:			
Slv/Slv	1.02 (0.81-1.17)	0.97 (0.85-1.00)	1.02 (0.94-1.11)
Ptx/Slv	0.86 (0.81-1.03)	0.97 (0.85-1.00)	1.02 (0.92-0.98)
Ptx/Hal	0.72 (0.68-0.86)	0.97 (0.82-1.01)	0.90 (0.83-0.95)

B: BURST2 PRELANDING PART

INJECTIONS	JUMP LEVEL 60 cm	90 cm	120 cm
CN:			
Slv/Slv	0.82 (0.74-1.20)	1.25 (1.13-1.25)	0.89 (0.86-0.93)
Hal/Slv	0.84 (0.70-1.12)	0.87* (0.76-0.93)	0.83 (0.73-1.14)
Hal/Apo	0.80 (0.56-0.91)	1.00 (0.92-1.09)	1.17 (1.07-1.30)
Slv/Apo	0.85 (0.80-1.00)	1.00 (0.82-1.13)	0.89 (0.88-0.92)
CSDL/CN:			
Slv/Slv	0.83 (0.71-1.18)	1.00 (1.00-1.07)	1.02 (1.00-1.08)
Ptx/Slv	1.14 (1.10-1.17)	1.13 (0.83-1.29)	0.90 (0.83-1.00)
Ptx/Hal	0.92$^{\infty}$ (0.79-1.00)	0.67$^{**\circ}$ (0.60-1.00)	1.00 (0.91-1.26)

Table 7.4 (continued).

INJECTIONS	C: BURST2 POSTLANDING PART JUMP LEVEL 60 cm	90 cm	120 cm
CN:			
Slv/Slv	1.29 (0.93-1.33)	1.17 (1.00-1.33)	1.17 (1.17-1.33)
Hal/Slv	0.50** (0.29-0.67)	0.47*** (0.40-0.50)	0.83** (0.71-0.83)
Hal/Apo	0.67 (0.42-0.83)	0.89^{++} (0.77-1.00)	1.13^{+} (0.82-1.25)
Slv/Apo	1.00 (1.00-1.10)	1.00 (0.71-1.07)	1.00 (0.88-1.20)
CSDL/CN:			
Slv/Slv	0.86 (0.67-1.00)	1.10 (1.00-1.35)	0.86 (0.71-1.00)
Ptx/Slv	0.78 (0.70-0.80)	0.83 (0.64-1.25)	0.85 (0.80-1.00)
Ptx/Hal	1.04^{+} (0.75-1.33)	1.00^{++} (0.79-1.13)	0.80 (0.60-1.09)

INJECTIONS	D: BURST3 JUMP LEVEL 60 cm	90 cm	120 cm
CN:			
Slv/Slv	1.03 (0.97-1.05)	1.02 (0.95-1.06)	1.02 (1.02-1.05)
Hal/Slv	1.07 (0.94-1.08)	1.08* (1.04-1.17)	1.06 (1.00-1.09)
Hal/Apo	1.08 (0.99-1.18)	1.02 (0.96-1.08)	1.05 (0.91-1.10)
Slv/Apo	1.08 (1.00-1.11)	1.09 (0.98-1.12)	1.06 (0.94-1.08)
CSDL/CN:			
Slv/Slv	1.06 (0.98-1.10)	0.97 (0.93-1.02)	0.99 (0.96-1.00)
Ptx/Slv	1.09 (0.93-1.19)	1.00 (0.98-1.02)	1.05 (0.94-1.06)
Ptx/Hal	1.19$^{+\circ}$ (1.16-1.21)	1.00 (0.96-1.08)	1.10 (0.98-1.15)

INJECTIONS	E: TOTAL MYOGRAM JUMP LEVEL 60 cm	90 cm	120 cm
CN:			
Slv/Slv	1.19 (1.02-1.24)	1.00 (0.90-1.01)	1.03 (0.88-1.12)
Hal/Slv	1.15 (1.05-1.16)	1.12 (0.99-1.16)	1.10 (0.97-1.13)
Hal/Apo	1.10 (1.01-1.16)	1.05 (1.02-1.13)	1.00 (0.99-1.10)
Slv/Apo	1.01 (1.00-1.08)	0.99 (0.95-1.06)	1.04 (1.02-1.08)
CSDL/CN:			
Slv/Slv	1.05 (0.99-1.13)	1.08 (1.04-1.10)	1.05 (0.99-1.08)
Ptx/Slv	1.11 (1.04-1.16)	1.02 (0.99-1.06)	1.06 (1.02-1.08)
Ptx/Hal	0.89$^{++\infty}$ (0.86-1.00)	1.01 (0.90-1.03)	0.99 (0.87-1.04)

pretreated cats. The combined treatment of picrotoxin and haloperidol significantly reduced prelanding IEMG activity of burst2 during 90 cm jumps.

Table 7.4C shows that haloperidol produced significant effects upon the IEMG activity of the postlanding part of burst2, which were consistently present during jumps of all three levels. Moreover, these changes were counteracted by additional intracaudate injections of apomorphine as well as intracollicular application of picrotoxin. Thus, haloperidol significantly reduced postlanding IEMG activity of burst2 during 60 cm jumps, 90 cm jumps and 120 cm jumps. In addition, the haloperidol-induced reduction in 90 and 120 cm jumps was significantly counteracted by apomorphine. The same holds true for 60 cm jumps, although it was not yet significant ($p>0.05$). Furthermore, the reduction following haloperidol was significantly prevented by picrotoxin injected into the deeper layers of the superior colliculus. Finally, neither apomorphine nor picrotoxin *per se* did affect the postlanding part of burst2.

Table 7.4D shows that haloperidol significantly increased the IEMG activity of burst3 during 90 cm jumps. The latter effect was neither present during 60 or 120 cm jumps nor could be prevented by apomorphine or picrotoxin. Finally, the combined treatment of intracollicular picrotoxin and intracaudate haloperidol significantly reduced the IEMG activity of burst3 only in 60 cm jumps.

Table 7.4E shows that the IEMG activity of the complete myograms are only significantly affected during 60 cm jumps after the combined treatment of picrotoxin and haloperidol.

Extensor EMG activity during POST-injection test2

Analysis of the data collected during the POST-injection test2 revealed that, apart from those shown in Table 7.5, most haloperidol-induced effects were no longer present. This table reveals that haloperidol still reduced the postlanding IEMG activity of burst2 during 90 cm jumps as well as during 120 cm jumps. Moreover, both apomorphine and picrotoxin were still able to counteract these effects.

*Table 7.5 Median value (25-75% range) of the ratio (POST-injection test2 / PRE-injection test) of IEMG activity of the postlanding part of burst2 for 90 and 120 cm jumps. * $p<0.05$; ** $p<0.02$: drug vs corresponding control experiment. + $p<0.05$; ++ $p<0.02$: Hal/Apo and Ptx/Hal vs Hal/Slv.*

For abbreviations, see legend of Table 7.4.

INJECTIONS		JUMP LEVEL	
		90 cm	120 cm
CN:	Slv/Slv	1.06 (1.00-1.31)	1.00 (1.00-1.00)
	Hal/Slv	0.44* (0.40-0.71)	0.50** (0.29-0.80)
	Hal/Apo	1.33+ (1.20-1.93)	0.75+ (0.63-1.00)
	Slv/Apo	0.93 (0.69-1.00)	1.33 (1.14-1.42)
CSDL/CN:	Slv/Slv	0.90 (0.80-1.00)	0.81 (0.75-1.00)
	Ptx/Slv	0.86 (0.83-1.17)	1.00 (1.00-1.25)
	Ptx/Hal	1.20+ (0.89-1.50)	1.27++ (1.00-1.33)

7.4 DISCUSSION

General considerations

Jump height did have a clearcut effect on forelimb extensor EMG activity prior to, and immediately after landing. The EMG signal started and terminated earlier and IEMG activity of burst1 and burst2 was enhanced when jump height was increased. Absence of a fixed relation between onset of EMG activity and moment of landing during jumps from different heights, as found in the present study, may imply that vestibular stimuli were involved in the control of at least part of the signal (see Section 7.1, Introduction).

In contrast to the present findings, it has been reported that the onset of prelanding extensor activity is related to the moment of landing, but not to the moment of take-off. On the basis of these findings, McKinley and colleagues have suggested that the timing of prelanding activity is triggered by visual information (McKinley & Smith, 1983; McKinley, Smith & Gregor, 1983). In view of the fact that in the present study

the onset of prelanding EMG activity was height-dependent, it is unlikely that visual information triggered the timing of prelanding muscle activity in our paradigm. The difference between the outcome of McKinley's experiments and that of the present study is easy to understand in view of the following. In contrast to McKinley's paradigm in which cats had to perform continuous series of 31-42 jumps of a single height, we purposely used a paradigm, in which cats were never allowed to jump successively from the same height. In other words, our paradigm actually prevented the use of visual information to trigger the prelanding EMG activity.

In the present study, the offset of burst2 appeared about 5-6 ms after impact. According to McKinley, Smith and Gregor (1983), the first postlanding burst in the cat lateral triceps muscle does not start until about 18 ms after landing from jump-downs. The latter delay is comparable to that found for the onset of stretch reflex responses of cat hindlimb extensor muscles after landing from a fall (Prochazka et al., 1977). According to Laursen and coworkers (1978), monkey extensor EMG activity following a jump-down is not of reflexive origin until at least 20 ms after impact. In human subjects, short-latency responses do not occur until 20-30 ms after landing from self-initiated falls (Dietz, Noth & Schmidtbleicher, 1981). Considering these reports, it seems unlikely that spinal stretch reflexes were involved in the postlanding part of burst2 as defined in the present study. Therefore, it is suggested that this part of the EMG signal was programmed before the cat touched the landing platform.

Haloperidol and perilanding EMG activity

Apart from the finding that intracaudate injections of the dopaminergic antagonist haloperidol did not affect EMG activity of burst1, it produced two types of effects: A. Effects which were consistently present during jumps of all three levels (1) and which could be prevented by intracaudate application of apomorphine (2) as well as by intracollicular injections of picrotoxin (3). B. Effects which were not consistently present during jumps from all three levels (1) and which were not counteracted by apomorphine (2) or picrotoxin (3).

Type B effects were apparently not dopamine-specific since the dopaminergic agonist apomorphine was unable to counteract these effects. Furthermore, type B effects were apparently not funnelled via striato-nigro-collicular fibres since inhibition of GABA at the level of the deeper layers of the colliculus superior could not interfere with the effects induced by haloperidol injections into the caudate nucleus. It can be argued that the type B effects were either behavioural consequences of an aspecific action of haloperidol within the caudate nucleus or behavioural consequences of changes occurring outside the caudate nucleus which *per se* were elicited by a specific action of haloperidol within the caudate. Since both the dose and volume of haloperidol are known to elicit dopamine- and locus-specific effects (Cools, Struyker Boudier & van Rossum, 1976), only the latter possibility remains. In fact, there is already evidence that similar injections of haloperidol are able to elicit behavioural effects in such a manner (see Section 3.1).

The postlanding part of burst2 was significantly affected by haloperidol during jumps of all three levels (type A effects). Moreover, the latter effect appeared to be selectively mediated by dopamine receptors of the caudate nucleus since an otherwise ineffective dose of apomorphine was able to prevent the haloperidol-induced decrease in at least two out of the three jump levels tested. Finally, the haloperidol-induced reduction was prevented by inhibition of collicular GABAergic activity with the help of picrotoxin, i.e., a treatment unable to affect EMG patterns when tested alone. The latter data show that the haloperidol-induced decrease apparently required a certain GABAergic activity at the level of the deeper layers of the superior colliculus. Taking these findings together it appears justified to conclude that the haloperidol-induced type A effects upon the postlanding part of burst2 were consistent, dopamine-specific and inherent in the (rostromedial) caudate nucleus which is known to funnel its information via the striato-nigro-collicular fibres downstream in the brain (cf. Cools, 1986; Heim et al., 1986).

First order and lower order output stations of the caudate nucleus have been found to control spinal motor elements in anaesthetized animals (Alstermark, Lundberg &

Sasaki, 1984a, 1984b; Schwarz, Sontag & Wand, 1984a; Sontag et al., 1984; Wagner & Kalmring, 1968; York, 1973). The present data reveal that the caudate nucleus itself exerts such a control, even in awake animals. As shown previously, decreasing caudate dopaminergic activity by means of intracaudate injections of haloperidol selectively inhibits switching to motor patterns which are not dictated by exteroceptive stimuli (see Section 3.1). As mentioned in Section 7.1 (Introduction), these effects are considered to be the manifestation of a deficient ability to arbitrarily program motor behaviour and known to be funnelled via the deeper layers of the colliculus superior (see Chapter 3 and 5). The present study shows that a similar treatment affected only the postlanding part of burst2 in a dopamine-specific manner. The present study also shows that the deeper layers of the colliculus superior play a role in the dopamine-specific changes in the perilanding muscle activity. These data together lead to the suggestion that perilanding EMG activity of the triceps brachii during self-initiated jump-downs is the manifestation of changes in the ability of the caudate nucleus to arbitrarily program motor behaviour. Given the role of exteroceptive and proprioceptive stimuli in directing a large part of the perilanding EMG activity it is not amazing that the intracaudate injections of haloperidol produced only minor changes in the recorded EMG activity. Given the present data, it makes sense to assess paradigms in which a far greater part of the EMG activity is not directed by exteroceptive and\or proprioceptive stimuli.

The present study opens the perspective that EMG activity which is not dictated by exteroceptive or proprioceptive stimuli may serve as a tool in studies on patients with basal ganglia diseases, such as patients suffering from Parkinson's Disease or Huntington's Chorea. In this respect, it is of interest to recall the report of Dietz and coworkers who detected abnormalities in 'preprogrammed' EMG activity during a simple motor task in parkinsonian patients (Dietz, Quintern & Berger, 1985) and of Noth and colleagues (Noth, Podoll & Friedemann, 1985), who found deficient long-latency responses in Huntington patients.

CHAPTER 8

SUMMARY AND CONCLUSIONS

Functional disturbances at the level of the mammalian caudate nucleus result in a variety of behavioural effects ranging from sensory neglect to motor disorders and/or cognitive disturbances. In Chapter 1, several factors were discussed which may underlie the diversity of the reported behavioural effects following experimental manipulation of the caudate nucleus. (1) The caudate nucleus may be involved in a universal programming ability which *per se* is not restricted to certain categories of observable behaviour. (2) At least part of the behavioural changes following experimentally-induced alterations at the level of the caudate nucleus may be due to functional changes at the level of other brain regions which start to dysfunction as a result of distorted neuronal information directly and indirectly received from the affected caudate. The goal of this thesis was to investigate both possibilities.

In Chapter 2 the literature concerning the heterogeneous character of the caudate nucleus was discussed. Caudate tissue can be divided into 'patches' or 'striosomes' and surrounding 'matrix' with the help of neurochemical markers. Striosomes and matrix are differentially innervated by mesencephalic dopaminergic cell groups. In addition, cortico- and thalamocaudate projections innervate both compartments differentially. Moreover, caudate cells located in patches and matrix appear to project to different subregions within the target regions, i.e. the substantia nigra pars reticulata and the globus pallidus. Apart from morphological and anatomical data, behavioural data were also reviewed showing that at least two functional subregions can be distinguished within the caudate nucleus, i.e. the rostromedial region and the anterodorsal region.

Comparing the subdivisions based on functional, behavioural studies on the one hand, and the subdivisions based on histochemical techniques and anatomical characteristics on the other hand it seems that patches predominate in the rostromedial region whereas the matrix predominates in the anterodorsal region. In the present thesis, attention was focused on the role of the rostromedial part of the caudate nucleus on the control and modulation of behaviour using the cat as an experimental model.

In Chapter 3, experiments were described in which the role of the rostromedial caudate nucleus in switching motor patterns was investigated. In Section 3.1, experiments were described in which cats were trained to walk on a specially-designed treadmill test situation. They were able to collect food pellets by switching motor patterns with or without the help of exteroceptive stimuli inherent to the treadmill. Intracaudate (rostromedial region) injections of the dopaminergic antagonist haloperidol were given in order to study the involvement of the dopaminergic caudate nucleus in switching motor patterns. Results indicate that haloperidol selectively decreased the number of so-called 'non-exteroceptively directed motor patterns'. This reduction appeared to be dopamine-specific since intracaudate application of the dopamine agonist apomorphine was able to prevent this effect in a dose-dependent way. Moreover, haloperidol did not affect the number of food collecting attempts indicating that this treatment did not influence the motivational state inherent in food deprivation. Furthermore, haloperidol did not reduce the number of so-called 'exteroceptively directed motor patterns' and this indicated that the capacity to switch patterns with the help of exteroceptive stimuli was not reduced. In addition, absence of incorrect adjustments of body postures and positions on the belt indicated that haloperidol did not affect the capacity to switch to proprioceptively directed motor patterns. Finally, haloperidol did not produce abnormal electromyographic or length signals in hindlimb muscles of cats walking on the belt of the treadmill. These data show that, as far as normal walking movements are concerned, intracaudate haloperidol did not produce limb deficits *per se*. On the basis of these observations it was concluded that haloperidol selectively reduced the animal's capacity to 'program non-stimulus directed motor behaviour'. That is, haloperidol selectively reduced the ability to arbitrarily switch

motor patterns. The data from animal and human studies together strongly suggest that the function of the caudate nucleus in arbitrarily programming behaviour is not limited to certain behavioural categories. The manner in which disturbances in this universal programming capacity are manifested in behaviour appears to depend on the constraints of the test used.

Experiments investigating the role of the glutamatergic corticocaudate pathway in switching behaviour are presented in Section 3.2. Several glutamate receptor subtypes have been described including the quisqualate receptor, the N-Methyl-D-Aspartate (NMDA) receptor and the kainate receptor. Many corticocaudate fibres are known to activate caudate quisqualate receptors. Therefore, the effect of intracaudate injections of the selective quisqualate receptor agonist dl-α-Amino-3-hydroxy-5-Methyl-isoxazole-4-Propionic Acid (AMPA) on switching behaviour was studied. Cats were tested in a 'bar paradigm' in which they had to switch to different motor patterns, for example, switching from hanging to climbing, from sitting to walking and, finally, from walking to jumping. During the test, there were no changes in exteroceptive stimuli which could have directed the behaviour of the animal under study. Intracaudate application of AMPA reduced the time required to climb on the bar (in cats which did not show abnormal limb movements) and increased the number of head movements as well as that of walking-restarts. These data suggest that AMPA enhanced the ability to switch behaviours. Apart from changes in switching behaviour, AMPA produced abnormal limb movements in some of the treated cats. AMPA-induced changes in switching behaviour could be attenuated by additional intracaudate injections of kynurenic acid, i.e., a broad spectrum excitatory amino acid receptor antagonist, but not by d-2-amino-7-phosphonoheptanoate (AP7), i.e., a specific NMDA receptor antagonist. In contrast, abnormal limb movements were prevented by pretreatment with either kynurenic acid or AP7.

In Section 3.3, experiments were presented in which the interplay of dopamine and glutamate on switching behaviour was investigated. In these studies, the effects of intracaudate injections of apomorphine on behavioural changes induced by caudate

injections of AMPA were analyzed. Results indicated that AMPA-induced increases in switching behaviour were dose-dependently prevented by apomorphine. In contrast, AMPA-induced limb deficits were not attenuated by apomorphine. The results described in Section 3.2 and 3.3 showed that quisqualate receptors were involved in switching behaviour whereas quisqualate as well as NMDA receptors were involved in AMPA-induced limb disturbances. The fact that apomorphine was able to prevent the AMPA-induced changes in switching behaviour but not the AMPA-induced limb deficits suggests that the two phenomena are mediated by different pathways. It was suggested that the AMPA-induced changes in switching behaviour are mediated by caudato-nigro-collicular fibres and that the AMPA-induced limb deficits are funnelled via the caudato-nigro-thalamo-cortical pathway.

Chapter 4 described experiments in which attention was focused on two brain regions receiving direct or indirect caudate nucleus-derived neuronal information, i.e. the substantia nigra pars reticulata and the deeper layers of the colliculus superior, respectively. Many caudatonigral as well as nigrocollicular fibres contain the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). In Section 4.1, it was investigated whether the reticular substantia nigra indeed mediates caudate output signals and whether it also serves as part of a negative feed-back system controlling the activity of the dopaminergic cells of the nigral pars compacta projecting to the caudate nucleus. The data showed that intracaudate injections of apomorphine or haloperidol had no effect on the behavioural changes produced by alterations in GABAergic activity induced at the level of the substantia nigra pars reticulata. These observations underline the function of the nigral pars reticulata as an output station of the caudate nucleus. The fact that neither the behavioural effects following intranigral injections of the GABA agonist muscimol nor the behavioural phenomena following application of the non-competitive GABA antagonist picrotoxin were altered after experimental manipulation of the caudate nucleus shows that the pars reticulata not only transmits, but actually transforms, its incoming signals into new output signals.

Section 4.2 presented data on the effects of picrotoxin injected into the deeper

layers of the feline colliculus superior. Unilateral injections of picrotoxin produced a cascade of motor patterns, starting with small contralateral ear movements followed by head movements, torso movements and, finally, whole body movements. Most of these phenomena were (1) dose-dependent, (2) locus-specific, and (3) GABA-specific. Bilateral injections of picrotoxin resulted in similar movements, but these movements were directed towards both sides and/or directed 'ventrocaudally'. The behavioural phenomena found after experimentally-induced changes in GABAergic activity at the level of the deeper layers of the colliculus superior were dissimilar to the effects observed after similar experimentally-induced changes at the level of the substantia nigra pars reticulata. These data indicate that the deeper layers of the colliculus superior, which serves as an output station of the substantia nigra pars reticulata also transforms incoming signals into new output signals.

Chapter 5 presented two studies analyzing the consequences of a progressive dysfunctioning caudate nucleus or deeper layers of the colliculus superior on switching motor patterns. It is known that systemic application of apomorphine may result in a 'break-down' of motor behaviour in rats. It can be hypothesized that this break-down is due to the involvement of caudate output stations indirectly affected by activation of caudate dopamine receptors. In order to obtain evidence of this hypothesis, it was first investigated whether activation of caudate dopamine receptors by relatively high doses of apomorphine produce a break-down of the motor pattern sequence in the treadmill paradigm (Section 5.1). Further, it was also investigated whether a comparable break-down could be induced by experimentally induced changes at the level of the deeper layers of the colliculus superior (Section 5.2). Since only one of the motor patterns in the 'treadmill' sequence is caudate-specific (see Section 3.1), disturbances at the level of the rostromedial caudate nucleus as well as disturbances at the level of other brain structures could be distinguished. Data were presented in Section 5.1 showing that relatively high doses of apomorphine resulted in the successive break-down of motor pattern sequences whereby not only caudate-specific motor patterns were reduced, but also non-caudate specific motor patterns. This regression in motor behaviour following apomorphine appeared to be induced via caudate dopamine receptors since this

phenomenon could be prevented by pretreatment with haloperidol. Because the relatively high doses of apomorphine also affected non-caudate specific motor patterns, it was concluded that other brain structures (indirectly) receiving caudate output signals were involved in the observed regression of the motor pattern sequence.

The possible involvement of caudate output stations in the regression of motor behaviour as described above was further investigated in experiments presented in Section 5.2. In this study, it was investigated whether changes in the GABAergic activity at the level of the deeper layers of the colliculus superior also result in a regression in motor behaviour. Therefore, cats were also tested in the treadmill paradigm before and after collicular injections of the GABA antagonist picrotoxin. Picrotoxin produced dose-dependent and GABA-specific regression of motor behaviour comparable to that elicited by intracaudate-injected apomorphine. This observation implies that a functional disturbance at the level of the deeper layers of the colliculus superior also produces a behavioural regression comparable to that found after a functional disturbance at the level of the rostromedial caudate nucleus. It was suggested that this regression process in motor behaviour is inherent in the hierarchical organization of the brain.

In Chapter 6, experiments were described in which it was investigated whether acute neuropathological changes at the level of the caudate nucleus are able to (1) produce behavioural changes characteristic for behavioural changes at the level of the caudate nucleus; and (2) produce changes in behaviour inherent in dysfunctioning output stations such as the substantia nigra pars reticulata and the deeper layers of the colliculus superior. Therefore, open field behaviour was analyzed immediately after the intracaudate application of the neuro-excitant kainic acid (Section 6.1) or after the unilateral occlusion of the middle cerebral artery (Section 6.2). Section 6.1 described the results from an experiment showing that intracaudate injections of kainic acid produced a sequence of behavioural changes characteristic for: (1) an activation of caudate (rostromedial part) dopamine receptors, (2) activation of nigral (pars reticulata) GABA receptors and, finally, (3) inhibition of collicular (deeper layers) GABA receptors. In addition, the above-mentioned functional changes at the level of these

regions appeared to be accompanied by an increase in metabolism as measured by the uptake of [^{14}C]-2-D-deoxyglucose uptake.

In Section 6.2, an entirely different manipulation of the caudate nucleus was used to investigate whether the progressive pathology following kainic acid indeed reflects changes characteristic of alterations in the cerebral organization or whether they are aspecific features of the neuro-excitatory compound. Therefore, the middle cerebral artery was permanently occluded and the behavioural consequences were analyzed up until 21 days after the occlusion. Data are shown that occlusion of the middle cerebral artery also produced functional and metabolic changes at the level of the caudate nucleus, the substantia nigra pars reticulata and the deeper layers of the colliculus superior. However, the latter changes were diametrically opposite to those following kainic acid. On the basis of these data, it was suggested that the successive appearance of the behavioural effects following caudate injections of kainic acid as well as unilateral occlusion of the middle cerebral artery are inherent in the hierarchical order of the brain structures under study.

Chapter 7 dealt with experiments investigating the role of the rostromedial caudate nucleus in programming muscle activity *per se*. In these studies, EMG activity of a forelimb extensor muscle was analyzed in cats during jumping and landing on a platform before and after intracaudate injections of haloperidol. Results indicate that perilanding EMG activity consisted of several bursts, i.e. two prelanding and several postlanding bursts. Haloperidol produced a significant reduction in part of the EMG activity during jumps from different heights. In addition, these haloperidol-induced EMG effects appeared to be dopamine-specific since intracaudate apomorphine was able to prevent the reduction in the majority of tested jump heights. Moreover, intracollicular injections of picrotoxin were also able to counteract this haloperidol-induced reduction indicating that this effect required a specific GABAergic activity at the level of the colliculus. Apart from the afore-mentioned reduction in part of the perilanding EMG-activity, haloperidol also produced other subtle changes. However, these effects were neither dopamine-specific nor funnelled via the colliculus superior.

Considering the fact that haloperidol reduced those aspects of the EMG signal which are not directed by exteroceptive or proprioceptive stimuli, it seems likely that the latter effect was due to a reduced ability to program arbitrarily muscle activity.

CLINICAL IMPLICATIONS

According to the data described in Section 3.1, patients suffering from a reduced dopaminergic activity in the basal ganglia should show a reduced ability to switch arbitrarily behaviours. Indeed, others have found that parkinsonian patients suffer from a deficient ability to shift set without external 'cues' compared to set-shifting with the help of exteroceptive information in eye-tracking tasks (Crawford, Henderson & Kennard, 1989; White et al., 1988), motor pattern tasks (Cools et al., 1984b; Rogers & Chan, 1988; Benecke et al., 1987; Flowers, 1976), memory tasks (Helkala et al., 1988; Brown & Marsden, 1987) and cognitive tasks (Brown, 1989; Cools et al., 1984; Lees & Smith, 1983; Flowers & Robertson, 1985; Taylor, Saint-Cyr and Lang, 1986). Furthermore, the data presented in Chapter 7 suggest that a deficient ability to switch behaviours even extends to the programming of muscle activity *per se*. Therefore, the analysis of EMG activity which is not dictated by exteroceptive or proprioceptive stimuli may serve as a tool in studies of patients with basal ganglia diseases, including patients suffering from Parkinson's Disease or Huntington's Chorea. In addition, the data presented in Section 3.1 show that the haloperidol-induced reduction in switching to non-exteroceptively directed motor patterns could be compensated for by an increase in switching to exteroceptively directed motor patterns. Accordingly, learning to use exteroceptive and/or proprioceptive stimuli for directing behaviour may be therapeutically effective in terms of compensating the reduced ability to switch arbitrarily behavioural programmes (cf. Stern, Lander & Lees, 1980).

With respect to the possible consequences of an overactivation of caudate dopamine receptors (see Section 5.1) it is relevant to note that several clinical studies have shown that L-Dopa-treated parkinsonian patients still perform poorly in cognitive as well as in motor tests which call for the patient's ability to switch arbitrarily behaviours (cf. Bowen et al., 1975; Bowen, 1976; Cools et al., 1984; Flowers & Robertson, 1985). Thus,

in spite of L-dopa treatment, the patient's caudate nucleus apparently is not functioning optimally. Furthermore, many L-Dopa-treated parkinsonian patients develop so-called 'On-Off'-phenomena (Lewitt & Chase, 1983), which probably are not due to chronic treatment or progression in state of illness (Lang et al., 1982). The occurrence of 'Off'-phenomena seem to coincide with peak plasma levels of L-Dopa (Fahn, 1974). During an 'Off' period, some patients do not improve following i.v. administration of L-Dopa or lisuride (Hardie et al., 1982) excluding the possibility that this effect is due to insufficient dopamine receptor stimulation. Considering these clinical data, the data presented in Chapter 5 imply that these 'Off'-phases might reflect 'regression' processes due to the subsequent exclusion of the caudate nucleus as well as of other brain structures from motor programming as a result of the overactivation of striatal dopamine receptors.

Finally, the data described in Chapter 6 suggest that classical parkinsonian symptoms such as hypokinesia, rigidity, etc. become only manifest when the caudate pathology has resulted in a malfunctioning of one or more striatal output stations. From this point of view, it seems to be highly relevant to develop therapeutic drugs which can restore normal functions of caudate output stations in order to relieve parkinsonian patients from these symptoms.

Functionele veranderingen binnen de nucleus caudatus kunnen leiden tot een reeks van gedragsveranderingen variërend van 'sensory neglect' tot motorische stoornissen en/of cognitieve effecten. In Hoofdstuk 1 werden een aantal factoren besproken die ten grondslag zouden kunnen liggen aan die diversiteit van mogelijke gedragsveranderingen als gevolg van experimentele manipulatie van de nucleus caudatus: (1) De nucleus caudatus zou betrokken kunnen zijn bij een 'universeel' programmeer mechanisme dat betrokken is bij verschillende categorieën van observeerbaar gedrag. (2) Een deel van de waargenomen gedragseffecten na experimentele manipulatie van de nucleus caudatus zouden kunnen voortkomen uit functionele veranderingen buiten deze kern, als gevolg van het verwerken van verstoorde neuronale signalen afkomstig van de nucleus caudatus, of als gevolg van het reorganiseren van neuronale netwerken buiten de nucleus caudatus om. Het doel van deze dissertatie was beide mogelijkheden nader te onderzoeken.

Hoofdstuk 2 behandelde literatuurgegevens betreffende het, in vele opzichten, heterogene karakter van de nucleus caudatus. Met behulp van biochemische markers kan caudatus weefsel worden verdeeld in een tweetal compartimenten, namelijk de 'patches' of 'striosomes' enerzijds en de 'matrix' anderzijds. Het is gebleken dat patches en matrix differentieel worden geïnnerveerd vanuit de mesencephale dopamine celgroepen. Hetzelfde geldt ook voor de cortico-caudatale en thalamo-caudatale projecties. Aan de andere kant blijken neuronen gelegen in de patches en de matrix ook onderscheiden projectiegebieden te hebben binnen de hersenkernen die vanuit de nucleus caudatus geïnnerveerd worden, zoals de substantia nigra pars reticulata en de globus pallidus. Met behulp van gedragsparameters kunnen ook meerdere subregionen in de nucleus caudatus worden onderscheiden, zoals het rostromediale deel en het anterodorsale gedeelte. Wanneer een vergelijking getrokken wordt tussen de functioneel, op basis van gedragsexperimenten, onderscheiden gebieden, en de op basis van histochemische technieken en anatomische kenmerken gemaakte indeling, valt het op dat in het rostromediale deel van de nucleus caudatus de patches lijken te domineren,

terwijl in het anterodorsale gedeelte met name het matrix compartiment lijkt te overheersen. In de experimenten beschreven in deze dissertatie is de aandacht met name gericht op het eerstgenoemde deel van de nucleus caudatus, het rostromediale deel.

In hoofdstuk 3 werden experimenten beschreven waarin de rol van de (rostromediale) nucleus caudatus in het wisselen van motorische patronen nader wordt onderzocht. In Sectie 3.1 werd een onderzoek beschreven waarvoor katten getraind werden te lopen op een speciaal voor dit doel ontwikkelde tredmolen. De dieren werden in staat gesteld voer pellets te verzamelen door het uitvoeren van sequenties van verschillende motorische patronen; enkele patronen werden gekenmerkt door het feit dat de dieren tijdens het uitvoeren bepaalde visuele en/of tactiele prikkels continu fixeerden; deze patronen werden continu gestuurd met behulp van exteroceptieve informatie. Andere patronen gingen juist niet gepaard met het fixeren van externe prikkels. De rol van dopamine receptoren in het wisselen van de verschillende typen patronen werd bestudeerd door het lokaal, in de nucleus caudatus, toedienen van de dopamine receptor antagonist haloperidol. Haloperidol verlaagde selectief het aantal 'niet-exteroceptief gestuurde motorische patronen'. Dit effect was dopamine-specifiek hetgeen bleek uit het feit dat de dopamine agonist apomorfine in staat was de door haloperidol geïnduceerde reductie dosis-afhankelijk te remmen. Haloperidol had geen effect op het aantal pogingen voer pellets te pakken waaruit volgde dat de dopamine antagonist geen effect had op de lichamelijke toestand inherent aan voedsel deprivatie. Bovendien bleek haloperidol het aantal 'exteroceptief gestuurde patronen' niet te verlagen, waaruit volgde dat de dopamine antagonist niet het vermogen om te wisselen naar exteroceptief gestuurde patronen had gereduceerd. Abnormale correcties van lichaamshoudingen en posities op de lopende band werden niet waargenomen hetgeen er op wees dat haloperidol geen effect had op het vermogen om motorische patronen te wisselen met behulp van proprioceptieve prikkels. Tenslotte bleek haloperidol geen veranderingen te veroorzaken in electromyografische (EMG) activiteit van een achterpootspier tijdens het lopen op de tredmolen; het farmacon veroorzaakte geen verstoringen in achterpoot bewegingen *per se*. Op basis van deze waarnemingen kon

worden geconcludeerd dat haloperidol selectief het vermogen verminderde om zogenaamd 'niet-stimulus gestuurd motorisch gedrag' te programmeren; met andere woorden haloperidol verminderde specifiek het vermogen 'eigenmachtig gestuurde' motorische patronen uit te voeren. De gegevens afkomstig van humaan en dierexperimenteel onderzoek geven aan dat deze functie van de nucleus caudatus zich niet beperkt tot bepaalde gedragscategorieën. Een verstoring op het niveau van de nucleus caudatus kan tot uiting komen in het eigenmachtig wisselen van cognitieve oplosstrategieën (Parkinson patienten), van sociale interacties (apen), van motorische strategieën (ratten) en van motorische patronen (Parkinson patienten, katten). De manier waarop een verstoorde functie van de nucleus caudatus tot uiting komt in gedragveranderingen lijkt in belangrijke mate af te hangen van de aard van de gebruikte test.

In Sectie 3.2 werden experimenten beschreven waarin de rol van de corticocaudatale glutamaterge projektie in het wisselen van motorische patronen werd onderzocht. Er worden verschillende typen receptoren voor exciterende aminozuren beschreven, zoals n-methyl-d-aspartaat (NMDA) receptoren, quisqualaat receptoren en kainezuur receptoren. In de nucleus caudatus worden met name quisqualate receptoren gestimuleerd door corticale eindigingen. Het onderzoek naar de functie van dit type receptor wordt bemoeilijkt door het feit dat hiervoor (nog) geen selectieve antagonisten beschikbaar waren. In de in dit hoofdstuk beschreven gedragsstudies werd gebruik gemaakt van de selectieve quisqualaat receptor agonist dl-α-amino-3-hydroxy-5-methyl-isoxazol-4-propion zuur (AMPA). Het effect van deze agonist op het wisselen van patronen werd onderzocht bij katten die op een smalle balk dienden te klimmen, vervolgens over deze balk moesten lopen en tenslotte van de balk op een plank moesten springen. Gedurende de test waren er geen veranderingen in externe prikkels, behalve die de dieren zelf introduceerden als gevolg van hun eigen bewegingen, die het gedrag van de katten konden sturen. AMPA reduceerde de tijd die de dieren nodig hadden om op de balk te klimmen (in dieren die geen abnormale pootbewegingen vertoonden, zie verder); AMPA verhoogde zowel het aantal kopbewegingen als het aantal keren dat het dier startte met lopen. Behalve voornoemde effecten op het wisselen van motorische patronen veroorzaakte AMPA bij een deel van de geteste

dieren afwijkingen in pootbewegingen. De door de agonist veroorzaakte veranderingen in het wisselen konden worden tegengegaan door de breed-spectrum glutamaat receptor antagonist kynurine zuur, maar niet door de selectieve NMDA receptor antagonist d-2-amino-7-phosphonoheptanoaat (AP7). Aan de andere kant, de abnormale pootbewegingen konden worden voorkomen zowel door kynurine zuur als AP7.

In Sectie 3.3 werd een experiment beschreven waarin de interactie tussen dopamine en glutamaat in de nucleus caudatus werd onderzocht. Het effect van apomorfine op de door AMPA geïnduceerde gedragsveranderingen werd geanalyseerd. De door AMPA veroorzaakte veranderingen in het wisselen konden dosis-afhankelijk door apomorfine worden tegengegaan; de AMPA-geïnduceerde abnormale pootbewegingen werden niet beïnvloed door de dopamine agonist. De in Sectie 3.2 en 3.3 beschreven resultaten leiden tot de conclusie dat quisqualaat receptoren in de rostromediale nucleus caudatus betrokken zijn bij het wisselen van motorische patronen terwijl zowel quisqualaat als NMDA receptoren betrokken lijken te zijn bij de door AMPA geïnduceerde pootstoornissen. Het feit dat apomorfine wel in staat bleek de door AMPA geïnduceerde veranderingen in het wisselen van motorische patronen te wijzigen, maar niet de stoornissen in pootbewegingen, onderstreept dat beide fenomenen waarschijnlijk door verschillende uitgangsbanen gemedieerd worden, namelijk het wisselen via de caudato-nigro-colliculaire baan, en de pootstoornissen via de caudato-nigro-thalamo-corticale baan.

Hoofdstuk 4 beschrijft een tweetal studies waarin hersenarealen centraal staan die hetzij direct, hetzij indirect, neuronale informatie ontvangen vanuit de nucleus caudatus, namelijk de substantia nigra pars reticulata en de diepere lagen van de colliculus superior. Caudato-nigrale en nigro-colliculaire vezels bevatten veelal de inhiberende neurotransmitter gamma-aminoboter zuur (GABA). In Sectie 4.1 werd nagegaan of de substantia nigra pars reticulata inderdaad beschouwd kan worden als een uitgangsstation voor de nucleus caudatus en niet, zoals klassiek werd verondersteld, als deel van een (negatief) terugkoppelend systeem naar de oorsprong van de dopaminerge nigro-caudatale vezels, namelijk de dopamine cellen in de substantia nigra pars compacta.

Unilaterale intranigrale toediening van de GABA agonist muscimol induceerde een kontralaterale lichaamshouding, snel kontralateraal cirkelen, en stereotiep likken; unilaterale toediening van de niet-kompetetieve GABA antagonist picrotoxine induceerde een kontralaterale lichaamshouding, traag kontralateraal cirkelen, 'freezing' gedrag, en een onvermogen de achterpoten op te trekken in de 'balk-test'. Het bleek dat haloperidol noch apomorfine, toegediend in de nucleus caudatus, in staat was gedragseffekten te beïnvloeden die veroorzaakt werden door experimenteel-geïnduceerde veranderingen in de GABAerge neurotransmissie van de substantia nigra pars reticulata. Deze waarnemingen onderstrepen de rol van de nigrale pars reticulata als uitgangsstation voor de nucleus caudatus. Het feit dat noch de gedragseffekten na intranigrale (pars reticulata) toediening van de GABA agonist muscimol, noch de gedragsveranderingen geïnduceerd door intranigrale toediening van picrotoxine overeen kwamen met effecten zoals die beschreven zijn na experimentele manipulatie van de nucleus caudatus, geeft aan dat de nigrale pars reticulata neuronale prikkel niet uitsluitend doorgeeft, maar ook transformeert.

De gedragseffekten van intracolliculaire toediening van picrotoxine werden beschreven in Sectie 4.2. Unilaterale toediening van picrotoxine induceerde een kaskade van motorische patronen: beginnend met kleine, kontralaterale oorbewegingen, gevolgd door sequenties van korte kontralaterale kopbewegingen, kontralaterale torsobewegingen en, tenslotte, ook kontralaterale lichaamsbewegingen. Het merendeel van deze bewegingen was dosis-afhankelijk, GABA-specifiek en plaats-specifiek. Bilaterale toediening van picrotoxine veroorzaakte vergelijkbare bewegingen, maar nu gericht naar beneden (ventrocaudaal) of naar beide zijden. De gedragseffekten na experimenteel geïnduceerde veranderingen in de GABAerge activiteit van de diepere lagen van de colliculus superior bleken niet overeen te komen met die effecten die volgden op experimentele manipulatie van de substantia nigra pars reticulata. Hieruit volgt dat ook de diepere lagen van de superior colliculus, als uitgangsstation van de substantia nigra pars reticulata, neuronale prikkels niet alleen doorgeven, maar ook transformeren.

In Hoofdstuk 5 werden een tweetal experimenten beschreven waarin de effecten

werden onderzocht van een progressief dysfunctionerende nucleus caudatus of (diepere lagen van de) colliculus superior op het wisselen van motorische patronen. In Sectie 5.1 werd onderzocht in hoeverre een toename in de mate van stimulatie van dopamine receptoren in de rostromediale nucleus caudatus ook andere delen van de hersenen, buiten de caudatus, kan beïnvloeden. Hiervoor werd het effect bestudeerd van relatief hoge doseringen apomorfine, toegediend in de rostromediale caudatus, op het wisselen van motorische patronen bij het verzamelen van voer pellets in de tredmolen-test. Gezien het feit dat slechts één van de motorische patronen in deze test caudatus-specifiek is (zie Sectie 3.1), kunnen verstoringen binnen de rostromediale nucleus caudatus onderscheiden worden van verstoringen buiten dit gebied. De verzamelde data lieten zien dat apomorfine een successieve afbraak van de patroon sequenties tot gevolg had waarbij niet alleen caudatus-specifieke patronen geremd werden, maar ook niet caudatus-specifieke patronen. Deze 'regressie' in motorisch gedrag werd geïnduceerd via dopamine receptoren gezien het feit dat haloperidol in staat bleek dit apomorfine-effect te blokkeren. Daar apomorfine ook niet caudatus-specifieke patronen kon remmen kon geconcludeerd worden dat ook andere hersenkernen, die (in)direct neuronale prikkels ontvangen van de nucleus caudatus, betrokken zijn bij deze regressie.

De mogelijke betrokkenheid van uitgangsstations van de nucleus caudatus werd verder onderzocht in experimenten beschreven in Sectie 5.2. In deze studie werd nagegaan in hoeverre picrotoxine, toegediend in de diepere lagen van de colliculus superior, in staat was een vergelijkbare regressie in de tredmolen test te veroorzaken. Opnieuw werden getrainde dieren voor en na behandeling, in dit geval intracolliculair toegediend picrotoxine, geobserveerd in de tredmolen test. Picrotoxine bleek dosis-afhankelijk en GABA-specifiek een regressie in motorisch gedrag te kunnen induceren. Deze regressie was vergelijkbaar met de regressie in motorisch gedrag zoals dat gevonden was na intracaudataal apomorfine. Bovengenoemde regressie in motorische gedrag lijkt inherent te zijn aan de hierarchische organisatie van de hersenen.

In Hoofdstuk 6 werden experimenten beschreven waarin werd nagegaan in hoeverre

experimenteel geïnduceerde neuropathologische veranderingen van de (rostromediale) nucleus caudatus leiden tot (1) gedragsveranderingen karakteristiek voor funktie-stoornissen van de rostromediale caudatus, en (2) gedragsveranderingen karakteristiek voor funktiestoornissen van uitgangsstations van de caudatus, zoals de substantia nigra pars reticulata en de diepere lagen van de colliculus superior. Daartoe werd het 'open veld' gedrag geanalyseerd van katten onmiddellijk na unilaterale lokale applicatie van kainezuur, een neuro-exciterende verbinding (Sectie 6.1), en na unilaterale permanente afsluiting van de middelste cerebrale arterie (Sectie 6.2). In Sectie 6.1 wordt beschreven dat intracaudatale toediening van kainezuur een sequentie van gedragingen tot gevolg heeft die karakteristiek zijn voor: (1) een aktivatie van dopamine receptoren in de nucleus caudatus; (2) een aktivatie van GABA receptoren in de substantia nigra pars reticulata; en (3) een remming van GABA activiteit in de diepere lagen van de colliculus superior. Bovendien bleken voornoemde effecten gepaard te gaan met een toename in metabole activiteit op het niveau van deze strukturen zoals gekonstateerd werd met behulp van opname van [^{14}C]-2-D-deoxyglucose.

In Sectie 6.2 werd een geheel andere techniek toegepast teneinde te kunnen nagaan in hoeverre de in Sectie 6.1 waargenomen effecten karakteristiek zijn voor de cerebrale organisatie van de betrokken strukturen, of, alleen eigenschappen van het kainezuur vertegenwoordigen. De gedragseffekten van unilaterale afsluiting van de middelste cerebrale arterie werden bestudeerd tot 21 dagen na afsluiting. De gepresenteerde gegevens laten zien dat deze ingreep ook functionele en metabole veranderingen veroorzaakte in de nucleus caudatus, de substantie nigra pars reticulata en de diepere lagen van de colliculus superior. Bovendien bleken de gedragsveranderingen precies het spiegelbeeld te zijn van de effecten beschreven in Sectie 6.1. Konkluderend kan gesteld worden dat de waargenomen effecten na lokale toediening van kaine zuur of unilaterale afsluiting van de middelste cerebrale arterie inherent zijn aan de hierarchische wijze waarop de onderzochte strukturen geordend zijn in het brein.

Hoofdstuk 7 behandelde een experiment waarin de rol van de rostromediale nucleus caudatus bij het programmeren van spieraktiviteit werd onderzocht. Daartoe werden

katten getraind vanaf een verhoging op een platform te springen. De electromyografische activiteit van een voorpoot extensor spier werd geregistreerd gedurende de landingsfase voor en na intracaudatale toediening van haloperidol. Haloperidol veroorzaakte een reductie in een deel van de EMG activiteit; deze reductie werd gevonden tijdens het landen vanaf verschillende hoogtes. Dit effect was dopaminespecifiek aangezien apomorfine bij 2 van de 3 spronghoogtes in staat bleek deze reductie te blokkeren. Bovendien bleek ook intracolliculair toegediend picrotoxine in staat dit haloperidol effect te kunnen remmen (bij 2 van de 3 spronghoogtes) waaruit blijkt dat de haloperidol-geïnduceerde reductie kennelijk een bepaalde GABAerge activiteit op het niveau van de colliculus vereiste. Gezien het feit dat de haloperidolgeïnduceerde reductie betrekking had op een deel van de spieraktiviteit die 'voorgeprogrammeerd' was, d.w.z. niet gestuurd werd door externe of proprioceptieve prikkels, kan gesuggereerd worden dat de door haloperidol geïnduceerde reductie het gevolg was van een verminderd vermogen eigenmachtig spieraktiviteit te programmeren.

KLINISCHE IMPLICATIES

Op basis van de gegevens beschreven in Sectie 3.1 kan verwacht worden dat patienten die lijden aan een gereduceerde dopaminerge activiteit in de basale ganglia gekenmerkt worden door een verminderd vermogen eigenmachtig wijzigingen aan te brengen in hun bewegingspatronen. Patienten die lijden aan de Ziekte van Parkinson blijken inderdaad gekenmerkt te worden door een selectieve verstoring in hun vermogen te wisselen, 'to shift set', zonder exteroceptieve prikkels, zgn. 'cues', die informatie bevatten ten aanzien van het uit te voeren gedrag. Tegelijkertijd is het wisselen van gedragselementen niet gestoord voor zover exteroceptieve prikkels aanwezig zijn die informatie bevatten ten aanzien van de uit te voeren gedragingen. Dit specifieke deficit is vastgesteld in oog-volgtaken (Crawford, Henderson & Kennard, 1989; White et al., 1988), het wisselen van motorische patronen (Cools et al., 1984b; Rogers & Chan, 1988; Benecke et al., 1987; Flowers, 1976), geheugen taken (Helkala et al., 1988; Brown & Marsden, 1987) en cognitive taken (Brown, 1989; Cools et al.,

1984; Lees & Smith, 1983; Flowers & Robertson, 1985; Taylor, Saint-Cyr and Lang, 1986). De resultaten beschreven in Hoofdstuk 7 geven bovendien aan dat een verminderd vermogen tot het eigenmachtig wisselen van gedragingen tot uiting kan komen in spieraktiviteit *per se*. Een verminderd vermogen tot het eigenmachtig programmeren van gedrag kan in bepaalde gevallen gecompenseerd worden door het programmeren van gedrag met behulp van externe prikkels (zie Sectie 3.1). Het gebruik maken van exteroceptieve en/of proprioceptieve 'cues' bij het sturen van bewegingen zou mogelijk als therapie kunnen worden toegepast bij patienten die lijden aan de Ziekte van Parkinson (zie Stern, Lander & Lees, 1980).

In Sectie 5.1 bleek het 'overstimuleren' van caudatale dopaminerge receptoren een regressie in het gedrag tot gevolg te hebben. Vergelijkbare fenomenen kunnen mogelijk ook optreden bij parkinson patienten die worden behandeld met de dopamine precursor L-dopa. Aanwijzigingen hiervoor volgen uit verschillende klinische studies die hebben aangetoond dat, ondanks een L-dopa therapie, parkinson patienten een verminderde prestatie leveren zowel in cognitieve als in motorische tests waarin eigenmachtig wisselen vereist wordt (zie Bowen et al., 1975; Bowen, 1976; Cools et al., 1984; Flowers & Robertson, 1985). Ondanks de L-dopa therapie functioneert de nucleus caudatus van deze patienten blijkbaar onvoldoende. Bovendien ontwikkelen vele L-dopa-behandelde parkinson patienten het zogenaamde 'On-Off'-fenomeen (Lewitt & Chase, 1983), hetgeen waarschijnlijk niet het gevolg is van de chronische behandeling of de progressie van de ziekte (Lang et al., 1982). Het optreden van 'Off'-fases lijkt samen te vallen met het bereiken van piek plasmaconcentraties van L-Dopa (Fahn, 1974), en sommige patienten geven geen verbetering te zien tijdens een 'Off'-fase door intraveneuse toediening van lisuride of L-Dopa (Hardie et al., 1982). Blijkbaar is het optreden van deze fases geen gevolg van een onvoldoende receptor stimulatie. De gegevens beschreven in Hoofdstuk 5 geven aan dat de 'Off'-fases bij parkinson patienten de consequentie kunnen zijn van een regressie proces als gevolg van een overstimulatie van striatale dopamine receptoren met als resultaat het uitschakelen van de nucleus caudatus en/of uitgangsstations van de nucleus caudatus.

Tenslotte geven de resultaten beschreven in Hoofdstuk 6 aan dat klassieke parkinson symptomen zoals hypokinesie, rigiditeit en tremor mogelijk pas tot uiting komen indien de striatale pathologie geleid heeft tot een verstoord functioneren van striatale uitgangsstations. Deze mogelijkheid geeft aan dat farmaca die het verstoorde functioneren van caudatale uitgangsstations selectief kunnen herstellen mogelijk van groot therapeutisch belang zouden kunnen zijn.

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Rob Jaspers werd geboren 12 november 1955 te Nijmegen. Hij heeft het Jeroen Bosch college te 's Hertogenbosch bezocht waar hij in 1975 het diploma Atheneum B behaalde. Datzelfde jaar begon hij zijn studie biologie aan de Katholieke Universiteit van Nijmegen. In 1979 behaalde hij het kandidaatsexamen biologie. In 1982 legde hij het doctoraalexamen af met als hoofdvakken Psychoneurofarmacologie, Dieroecologie en als bijvak Dierfysiologie. In datzelfde jaar trad hij in dienst van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek te Den Haag (ZWO-BION) en was werkzaam als wetenschappelijk onderzoeker op het Instituut voor Farmacologie, Katholieke Universiteit van Nijmegen. Als lid van de werkgroep Psychoneurofarmacologie, heeft hij onder leiding van Prof. Dr. A.R. Cools onderzoek verricht naar de functie van de nucleus caudatus en van uitgangsstations van de nucleus caudatus in het programmeren van motorisch gedrag van de kat. In 1982 en in 1985 was hij als wetenschappelijk gast-onderzoeker werkzaam onder leiding van Prof. Dr. K.-H. Sontag op het Max Planck Institut für experimentelle Medizin te Göttingen, West Duitsland. In de periode van januari 1986 tot november 1987 was hij in dienst van de Katholieke Universiteit van Nijmegen waar hij, wederom onder leiding van Prof. Dr. A.R. Cools, onderzoek heeft verricht naar de rol van het glutamaterge corticostriatale baansysteem in het motorisch gedrag en naar de effecten van focale cerebrale ischemie op het functioneren van enkele hersenstrukturen. Vanaf 1988 tot december 1989 was hij als wetenschappelijk medewerker verbonden aan het Max Planck Institut für experimentelle Medizin te Göttingen, West Duitsland, waar hij onderzoek heeft verricht naar de effecten van tijdelijke cerebrale ischemie op het programmeren van oriëntatie-gedrag van de rat. Vanaf januari 1990 is hij als wetenschappelijk medewerker verbonden aan het Medisch Biologisch Laboratorium van T.N.O. te Rijswijk. Hij is getrouwd met Hanneke Sanders. Zij hebben twee kinderen, Tessa en Rianne.

Uit gezamelijk onderzoek zijn de volgende publicaties voortgekomen:

Cools, A.R., Jaspers, R., Kolasiewicz, W., Sontag, K.-H. and Wolfarth, S. (1983) Substantia nigra as a station that not only transmits, but also transforms, incoming signals for its behaviour expression: striatal dopamine and GABA-mediated responses of pars reticulata neurons. Behav. Brain Res., 7, 39-49.

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