DEFINING A ROLE FOR THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS IN STRIATAL OUTFLOW

Laura F. Allen

A Thesis Submitted for the Degree of PhD at the University of St. Andrews

1996

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DEFINING A ROLE FOR THE
PEDUNCULOPONTINE TEGMENTAL NUCLEUS
IN STRIATAL OUTFLOW

LAURA F ALLEN

SUBMITTED TO THE UNIVERSITY OF ST ANDREWS FOR THE DEGREE OF PhD
JUNE 1995
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(i) I, Laura Frances Allen, hereby certify that this thesis, which is approximately 58,000 words in length, has been written by me, that it is a record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Signature of candidate: [Signature] Date: 22.6.95

(ii) I was admitted as a research student in October 1992 and as a candidate for the degree of PhD in October 1992; the higher study for which this is a record was carried out in the University of St Andrews between 1992 and 1995.

Signature of candidate: [Signature] Date: 22.6.95

(iii) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of PhD in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

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Date: 22-6-95
Acknowledgements

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A final special thanks must also go to all the rats without whom this work could never have been done.
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<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine.</td>
</tr>
<tr>
<td>ACh</td>
<td>Acetylcholine.</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase.</td>
</tr>
<tr>
<td>ALH</td>
<td>Anterolateral Hypothalamus.</td>
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<tr>
<td>ARAS</td>
<td>Ascending Reticular Activating System.</td>
</tr>
<tr>
<td>CA</td>
<td>Catecholamine.</td>
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<tr>
<td>CG</td>
<td>Central Gray.</td>
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<tr>
<td>ChAT</td>
<td>Choline Acetyltransferase.</td>
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<td>CNF</td>
<td>Cuneiform Nucleus.</td>
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<tr>
<td>CPP</td>
<td>Conditioned Place Preference.</td>
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<td>CP</td>
<td>Caudate Putamen.</td>
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<tr>
<td>DA</td>
<td>Dopamine.</td>
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<tr>
<td>DLCP</td>
<td>Dorsolateral Caudate Putamen.</td>
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<tr>
<td>DLF</td>
<td>Dorsolateral Funiculus.</td>
</tr>
<tr>
<td>DMT</td>
<td>Dorsomedial Nucleus Of The Thalamus.</td>
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<tr>
<td>DpMe</td>
<td>Deep Mesencephalic Nucleus.</td>
</tr>
<tr>
<td>DRN</td>
<td>Dorsal Raphe Nucleus.</td>
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<tr>
<td>EP</td>
<td>Entopeduncular Nucleus.</td>
</tr>
<tr>
<td>GABA</td>
<td>y-aminobutyric Acid.</td>
</tr>
<tr>
<td>GP</td>
<td>Globus Pallidus.</td>
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<tr>
<td>HRP</td>
<td>Horseradish Peroxidase.</td>
</tr>
<tr>
<td>IC</td>
<td>Inferior Colliculus.</td>
</tr>
<tr>
<td>LC</td>
<td>Locus Coeruleus.</td>
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<td>LDTg</td>
<td>Laterodorsal Tegmental Nucleus.</td>
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<td>LH</td>
<td>Lateral Hypothalamus.</td>
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<tr>
<td>LHA</td>
<td>Lateral Hypothalamic Area.</td>
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<td>Abbreviation</td>
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<tr>
<td>LPO</td>
<td>Lateral Preoptic Area.</td>
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<tr>
<td>MEA</td>
<td>Midbrain Extrapyramidal Area.</td>
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<tr>
<td>MLR</td>
<td>Mesencephalic Locomotor Region.</td>
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<tr>
<td>MPT</td>
<td>Medial Prefrontal Cortex.</td>
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<tr>
<td>NA</td>
<td>Noradrenaline.</td>
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<tr>
<td>NAcc</td>
<td>Nucleus Accumbens.</td>
</tr>
<tr>
<td>NBM</td>
<td>Nucleus Basalis Magnocellularis</td>
</tr>
<tr>
<td>NMC</td>
<td>Nucleus Reticularis Magnocellularis.</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide.</td>
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<tr>
<td>NRM</td>
<td>Nucleus Raphe Magnus.</td>
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<tr>
<td>PB</td>
<td>Parabrachial Nucleus.</td>
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<tr>
<td>PD</td>
<td>Parkinson's Disease.</td>
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<tr>
<td>PGO</td>
<td>Pontine Geniculococular.</td>
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<tr>
<td>PHAL</td>
<td><em>Phaseolus Vulgaris</em> Leucoagglutinin</td>
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<td>PPTg</td>
<td>Pedunculopontine Tegmental Nucleus.</td>
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<td>PRF</td>
<td>Pontine Reticular Formation.</td>
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<td>REM</td>
<td>Rapid Eye Movement.</td>
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<tr>
<td>RRF</td>
<td>Retrorubral Field.</td>
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<td>SC</td>
<td>Superior Colliculus.</td>
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<tr>
<td>SI</td>
<td>Substantia Innominata.</td>
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<tr>
<td>SN</td>
<td>Substantia Nigra.</td>
</tr>
<tr>
<td>SNC</td>
<td>Substantia Nigra Pars Compacta.</td>
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<tr>
<td>SNR</td>
<td>Substantia Nigra Zona Reticulata.</td>
</tr>
<tr>
<td>STN</td>
<td>Subthalamic Nucleus.</td>
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<tr>
<td>VLCP</td>
<td>Ventrolateral Caudate Putamen.</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral Tegmental Area.</td>
</tr>
<tr>
<td>VP</td>
<td>Ventral Pallidum.</td>
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<tr>
<td>WGA-HRP</td>
<td>Wheat Germ Agglutinin-HRP.</td>
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Abstract

The pedunculopontine tegmental nucleus (PPTg) lies within the pontomesencephalon and contains cholinergic and non-cholinergic neurones. It has extensive afferent and efferent connections throughout the brain. Early research suggested a role for the PPTg in the mediation of locomotor activity, and it was believed to form the major substrate of the electrophysiologically identified mesencephalic locomotor region (MLR). Studies using selective excitotoxic lesions of the PPTg demonstrated that it has no role in the mediation of spontaneous or nucleus accumbens-induced (NAcc) locomotion. However evidence has suggested that the cuneiform nucleus (CNF) and not the PPTg is the main locus of the MLR. The effects of bilateral ibotenate CNF lesions on spontaneous and amphetamine-induced locomotion stimulated from the NAcc were therefore investigated. CNF lesions had no effect on either type of locomotor activity.

Bilateral ibotenate lesions of the PPTg have been shown to influence the expression of orofacial stereotypies following administration of systemic amphetamine. Oral stereotypies can be elicited reliably by direct stimulation of the ventrolateral caudate-putamen (VLCP). This thesis sought to clarify the role of the PPTg in the mediation of oral stereotypies, by combining bilateral ibotenate lesions of the PPTg with direct microinjection of amphetamine into the VLCP. Lesions of the PPTg caused a shift in the dose response curve to amphetamine resulting in an increase in the incidence and intensity of orofacial stereotypies at lower doses. Thus the PPTg appears to have inhibitory control over the expression of orofacial behaviors.

It is hypothesised that while neither the PPTg nor the CNF have a role in the mediation of locomotor activity per se they may provide an integrative functional role, which influences motor outflow. The role of the CNF in the transmission of nociception and a role for the PPTg in the mediation of striatal outflow is discussed.
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What makes the pedunculopontine tegmental nucleus (PPTg) an interesting structure to study?

In the past fifteen to twenty years enormous advances have been made in histochemical techniques and in the availability of a wide range of neurotoxins with which to make relatively selective lesion studies. The development of choline acetyltransferase (ChAT) immunohistochemistry has allowed the visualisation and identification of cholinergic neurones within the brain. This has allowed researchers to study the connectivity of nuclei containing cholinergic neurones and developments in various lesion techniques have allowed some investigation into the functional role of acetylcholine in the brain. Cholinergic neuronal populations have been localised to two main areas; the basal forebrain and the pontomesencephalon (Ch5 and Ch6 neurones). A role in memory, learning and attentional processes has been hypothesised for basal forebrain cholinergic neurones on the basis of its anatomical connections and from the results of lesion studies (Dunnett et al. 1985; Hepler et al. 1985; Murray and Fibiger 1985). This caused considerable excitement since loss of cholinergic neurones in the basal forebrain has been identified in patients suffering from Alzheimer’s disease, and for a while it was believed that animal models of this disorder could be developed by causing cholinergic depletion in the basal forebrain (Wenk et al. 1987). More recent studies have proved however that the issue is much more complex than it first appeared (Dunnett et al. 1987, 1991; Torres et al. 1994; Björklund and Dunnett 1995; Winkler et al. 1995) and the search for a good animal model of Alzheimer’s disease continues.

The Ch5 cholinergic neurones of the pontomesencephalon are part of the PPTg, which also contains non-cholinergic neurones. The cholinergic PPTg has been implicated as part of the ascending reticular activating system (Morruzzi and Magoun 1949), which mediates general behavioural functions such as attention and arousal, and a role for the PPTg in the control of the sleep/wake cycle has been hypothesised (Sakai 1988; Semba et al. 1990; Woolf 1991). The non-cholinergic PPTg receives substantial innervation from the majority of structures in the basal ganglia (Rye et al. 1987; Steininger et al. 1992). Indeed, the PPTg has reciprocal connections with the basal ganglia (Steiniger et al. 1992; Rye et al. 1987). Cholinergic projections from the
PPTg to the substantia nigra have been shown to influence the release of DA in the striatum (Blaha and Winn 1993) and loss of cholinergic PPTg neurones have been identified in Parkinson's disease and progressive supranuclear palsy, a Parkinsonian-type disorder also characterised by severe cognitive impairments (Halliday et al. 1990, Hirsch et al. 1987; Zweig et al. 1987; Jellinger et al. 1988; Agid et al. 1990). These data indicate a role for cholinergic PPTg neurones in controlling dopamine activity in both normal and pathological conditions. The involvement of non-cholinergic neurones in PD is also highlighted by a study by Mitchell et al. (1989). Overactive pallidal outflow has been identified in monkeys made hemi-parkinsonian by injections of MPTP, and this was shown to chronically inhibit the PPTg (Mitchell et al. 1989).

The non-cholinergic PPTg has amongst other connections, descending output to sites of motor control in the pons and medulla, and some research has hypothesised a role for the PPTg in the mediation of the akinetic disorders seen in PD (Turski et al. 1990). In addition an increased number of PPTg cholinergic neurones has been recorded in the brains of schizophrenic patients (Garcia-Rill et al. 1995; Karson et al. 1991).

The PPTg warrants investigation due to its extensive afferent and efferent connections throughout the brain. It is the only positively identified structure outwith the traditionally defined basal ganglia that provides excitatory input into the basal ganglia, and it is one structure through which the basal ganglia can gain access to sites of motor control in the pons and medulla. The PPTg receives direct and indirect innervation from two main areas within the striatum, the caudate-putamen and nucleus accumbens. Both structures have been rigorously investigated and it has long been accepted that the NAcc mediates reward-related information while the CP appears to be involved in “habit” responding (Taylor and Robbins 1884, 1986; Marsden 1982; Reading and Dunnett 1991; McDonald and White 1993). The PPTg appears to be one site at which motor habits can be integrated with and modified by information concerning the motivational state of the animal. Therefore defining a functional role for the PPTg in the mediation of basal ganglia outflow in general, and striatal outflow in particular could contribute to understanding how information processed by basal ganglia structures is combined and then translated into behaviours appropriate to the environment.
Part I: Anatomical considerations
Chapter 1. A general overview of the basal ganglia with particular reference to the striatum.

1.1 Basal ganglia-thalamocortical loops

The basal ganglia are a group of interconnected subcortical structures which play an integral part in the control of the motor system. The function of the motor system is to "organize and co-ordinate the activities of individual muscles to generate sequences of movement that are integrated into behavioural responses appropriate to the environment" (Mogenson et al. 1980, p75). The component nuclei of the basal ganglia are the striatum, substantia nigra (SN), subthalamic nucleus (STN), and pallidal complex which includes the globus pallidus (GP) and entopeduncular nucleus (EP) in non-primates. Research into the connections of the basal ganglia have suggested the existence of a number of segregated basal ganglia-thalamocortical loops via which information is channelled from parts of the cortex through the basal ganglia and thalamus and back up to the cortex (Alexander et al. 1986). The majority of this work has been done in the monkey, but Groenewegen et al. (1990) has shown that the rat brain is organised in a similar way. To date five parallel basal ganglia-thalamocortical circuits have been identified, all of which centre upon different parts of the frontal lobe. The best documented of these loops is the motor circuit; the others are the oculomotor, lateral orbitofrontal, anterior cingulate and dorsolateral prefrontal circuits. General characteristics of these parallel circuits have been identified. Each circuit has input into discrete parts of the striatum and this anatomical separation continues throughout the loops. However within each loop a certain amount of funnelling goes on. Input into the striatum arises from a number of cortical areas which are functionally related. Some of these inputs are integrated at the level of the striatum and the process of integration continues throughout the loop at the level of the GP and SN and then at the thalamus, until the loop is closed by thalamic projections to the cortex. For example, the motor circuit (Figure 1.1) arises in related areas of the cortex; striatal inputs come from the motor cortex, supplementary motor area, arcuate premotor area and somatosensory cortex. These projections innervate the putamen in a somatotopically organised manner. For example the part of the motor cortex and somatosensory cortex that control "leg" areas both project to the dorsolateral putamen. The putamen then sends projections to the ventrolateral GP
(internal and external segments) and the caudolateral substantia nigra pars reticulata (SNr), with the topography being retained throughout. The internal portion of the GP and the SNr then innervate the oral part of the ventrolateral nucleus of the thalamus (VLo), lateral parts of the magnocellular and parvocellular ventral anterior thalamic nuclei (VAmc and VApC), centromedian nucleus (CM) and the mediodorsal nucleus (MD). The VLo and VAmc connect in turn to the supplementary motor area, the VApC connects with the premotor cortex, and both the VLo and CM project to the motor cortex, thereby closing the loop (Alexander et al. 1986; Alexander and Crutcher 1990; Joel and Weiner 1994). The supplementary motor area innervates the motor cortex, the arcuate premotor area and also directly innervates the spinal cord, while the premotor cortex innervates the motor cortex and the motor cortex has direct outflow to the spinal cord. Thus the motor circuit is well connected to areas which mediate motor activity and evidence suggests it has a role in the organization, initiation, direction and control of movement (Alexander et al. 1986; Alexander and Crutcher 1990).

Less well documented is the anterior cingulate circuit (Figure 1.2). Here the cortical projections arise within the anterior cingulate, prelimbic and orbitofrontal cortex and project to the ventral striatum. The ventral striatum in turn projects to the ventral pallidum (VP) the rostromedial internal section of the GP and rostrodorsal SNr. All these structures innervate the medial part of the MD which then projects back to the anterior cingulate cortex (Alexander et al. 1986; Joel and Weiner 1994). The ventral striatum also receives innervation from limbic areas such as the hippocampus and amygdala and it has been suggested that the cortical areas projecting to the ventral striatum also have limbic functions, though Alexander et al. (1986) have refused to speculate on the behavioural functions this loop could mediate due to the lack of full understanding surrounding the functions of the limbic system.

In a recent review the organization of the basal ganglia-thalamocortical loops has been extended. Unlike the separate parallel systems proposed by Alexander et al. (1986) the authors propose that there is interconnection between the circuits at the level of the cortex (Joel and Weiner 1994). These authors describe an organisation which incorporates "split circuits". In this model there are both closed and open loops. The closed loops are organised in the way described above where thalamocortical projections innervate the part of cortex that sent the corticostriatal
projections thereby closing the loop. In the organization of the open loops the thalamocortical projections innervate a different cortical area than the one from which the corticostriatal projections arose, this allows the thalamocortical projections to project to areas of cortex from which other basal ganglia-thalamocortical circuits arise (Figure 1.3). For example the closed part of the motor circuit arises from the projections of the internal section of the GP, which innervates the VAdc and closes the loop by projecting back to the motor and supplementary motor cortex. The open part of the circuit arises from the projections of the SNr which project to the VAmc and MD which then innervate the associative prefrontal cortex. In a similar way the anterior cingulate circuit (or limbic circuit) has both open and closed loops. The closed loop arises and terminates within the anterior cingulate cortex and is mediated through the VP and MD, while there are two proposed open loops; one which innervates the associative prefrontal cortex via the SNr, MD and VApC and the other which innervates the motor and premotor cortex via the internal part of the GP, and ventral anterior thalamic nucleus (Joel and Weiner 1994). Open loops do not just exist within the motor and anterior cingulate circuits. Joel and Weiner have also outlined their existence in the dorsolateral prefrontal circuit (or associative circuit), and believe that they could exist within other basal ganglia-thalamocortical circuits. A possible functional reason for the existence of open loops is to allow for the intercommunication of the separate circuits, via a series of feedforward and feedback mechanisms. For example this would allow for information about the motivational state of the animal (limbic circuit) to be combined with the selection of appropriate motor programmes (associative circuit) which then allows for the generation of a motor response (motor circuit). Thus intercommunication between the basal ganglia-thalamocortical circuits could be important for the integration of information about the state of the animal and its environment which would then allow for the selection, combination and direction of an appropriate behavioural response.

1.2 The striatum

The striatum is the largest subcortical structure in the brain and forms one component of the basal ganglia. From the description of the basal ganglia-thalamocortical loops it is evident that the striatum receives input from the whole of the cerebral cortex and projects to many subcortical structures, including the GP, SN and thalamus (Graybiel
et al. 1994). The cortical input into the striatum is topographically and somatotopically organised. This in turn determines topographic and somatotopic innervation throughout the rest of the basal ganglia structures (Von Krosigk et al. 1992).

The striatum is said to be an “arousing” structure (Chevalier and Deniau 1990). Both the striatonigral and striatopallidal efferents are believed to contain the inhibitory neurotransmitter \( \gamma \)-aminobutyric acid (GABA). Likewise both the SNr and GP have GABAergic projections to the thalamus, superior colliculus (SC) and mesencephalic tegmentum. When the striatum is at rest, both the GP and SNr are free to exert inhibitory control over these structures. However when the striatum is activated, the GP and SNr are inhibited and their target structures are therefore disinhibited allowing for the generation of movement. While the GP and SNr are inhibited, the thalamocortical and colliculospinal neurones show increased sensitivity to their cerebellar and sensory inputs. Thus Chevalier and Deniau (1990) argue that the role the striatum has in the control of motor output is that of a gating signal, whereby it controls the access of sensory information to motor circuits. It has been suggested that the disinhibition of the basal ganglia itself is not sufficient to generate movement, but that the function of the gating system is to "set a pattern of readiness in premotor networks that will be further activated for the execution of movement" (Chevalier and Deniau 1990).

1.3 Internal architecture of the striatum

The major projecting neurone from the striatum is the medium spiny neurone, which as its name suggests is medium in size with spines covering its dendrites. These neurones account for 96% of the neuronal population in the striatum (Smith and Bolam 1990), and their axons are extensively collateralised within the striatum. The types of neurotransmitter found within the basal ganglia are numerous but their distribution is fairly straightforward. Afferents to the striatum that arise in the cortex are predominantly glutamatergic (an excitatory neurotransmitter) while afferents from the mesencephalon come predominantly from the dopaminergic A8, A9 and A10 neurones. The major efferents of the striatum which project to the SNr and GP are GABAergic (inhibitory). Interneurones within the striatum consist of aspiny neurones that contain either GABA or ChAT, and there is also a population of neurones
containing substance-P which may function as interneurones (Graybiel 1990; Smith and Bolam 1990). Midbrain dopaminergic input into the striatum appears to terminate on the distal dendrites of medium-spiny neurones that also receive innervation from the cortex or hippocampus. This convergence of synaptic contacts onto the medium-spiny neurones that project to the GP and SNr, allow the possibility of dopaminergic modulation of cortical input which then affects outflow to the thalamus and brainstem (Smith and Bolam 1990).

The subdivisions of the striatum are complex. For example the striatum can be compartmentalised on the basis of it’s neurochemistry. These compartments have been termed the striosomes (or patches) and the extrastriosomal matrix. The larger matrix is identified by acetylcholinesterase-rich immunoreactivity, while the striosomes have poor acetylcholinesterase (AChE) content but correspond to patches of opiate-receptor binding sites (Gerfen 1984; Graybiel 1990; Groenewegen et al. 1991). There is differential input into the matrix and striosomes which could translate into functional differences. For example the striosomes along with the ventral striatum appear to derive their innervation from limbic structures and the infragranular areas of the cortex, while the matrix predominantly receives innervation from supragranular cortical areas. Thus both the striosomes and matrix receive cortical input from the same cortical areas but from different cortical layers (Gerfen 1989; Parent 1990). There is also differential dopaminergic (DA) innervation of the striosomes and matrix compartments of the striatum, with the A9 DAergic afferents arising in the substantia nigra pars compacta (SNC) innervating the striosomes and the retrorubral area A8 DAergic and ventral tegmental A10 DAergic neurones innervating the matrix (Groenewegen et al. 1991). The efferents of the two compartments are also distinct, which lends weight to the suggestion that the two compartments are involved in functionally distinct behaviours. While the matrix predominantly innervates the GP and SNr, the striosomal compartments predominantly innervates the SNC. This may affect functional output of the striatum, with the matrix mediating the sensorimotor outflow and the striosomes having some influence on the DAergic input into the striatum. That the striosomes derive their innervation primarily from limbic areas suggests one way in which the limbic system may modulate dopaminergic input into the striatum, via the SNC (Gerfen 1984; Graybiel 1990; Groenewegen et al. 1991).
There is a further level of complication with regards to the organization of the matrix. Most of the projections to the GP and SNr appear to arise within the matrix. All these projections contain GABA, but if the distribution of neuropeptides within these projection neurones is also taken into consideration, it appears that there are distinct neuronal projections to the internal and external segments of the GP. For example the internal GP (or rodent EP) is rich in substance P and dynorphin while the external GP (or rodent GP) is rich in enkephalin and neotensin (Penny et al. 1986; Gerfen et al. 1990; Graybiel 1990, Parent 1990). This division may help to explain some of the motor anomalies found in disorders in which the basal ganglia are involved. There is growing evidence that there is a modular organization within the matrix, which would account for the patchy distribution of the cortical input into the matrix and for the apparent clustering of output cells which project to distinct areas (Graybiel 1990; Malach and Graybiel 1986; Gimenez-Amaya and Graybiel 1990; Jimenez-Castellanos and Graybiel 1989; Penny et al. 1986). What functional use could the compartmentalisation of the striatum (termed matrisomes by Graybiel et al. 1991) have? Ann Graybiel argues that "by means of these compartmental divisions, and the restricted cross-compartment connections present, the basal ganglia can selectively bring together - or keep separate - their different inputs, and reorganise activity patterns before distributing the information to the rest of the brain.” (Graybiel 1990, p252).

1.4 The role of dopamine in the modulation of striatal outflow

As described above there is differential neuropeptide expression in striatonigral and striatopallidal neurones. Both these output pathways contain GABA, but the striatopallidal neurones which project to the GP contain enkephalin and neurotensin, while the striatonigral neurones which project to the SNr and EP contain substance P and dynorphin. Indirect evidence has also indicated that dopamine receptors D1 and D2 are also differentially expressed with D1 receptors found predominantly on striatonigral neurones while D2 receptors are located on striatopallidal neurones (Gerfen et al. 1990). Dopamine has differential effects on the regulation of these neuropeptides, as shown by rats bearing 6-OHDA lesions of the striatum. Dopamine depletion appears to cause an increase in the expression in enkephalin and D2 receptors on the striatopallidal projection neurones, while concurrently decreasing the
number of substance P and D1 receptors found on the striatonigral neurones (Gerfen et al. 1990). D1 and D2 receptors appear to have differential effects on SNr neurones whereby excitation of the striatonigral pathway results in the inhibition of the SNr which in turn allows the disinhibition of the thalamus, SC and pedunculopontine tegmental nucleus (PPTg), allowing motor behaviours to occur. However excitation of the striatopallidal neurones increases the activity of the SNr and therefore further inhibits the thalamus, SC and PPTg. This occurs due to disinhibition of the STN. The striatopallidal neurones inhibit the GP, this then disinhibits the STN which has excitatory input into the SNr, which in turn increases the inhibitory output of the SNr (Gerfen et al. 1990) (Figure 1.4).

The differential expression of the D1 and D2 receptors has been questioned recently as more has been discovered about dopamine receptors. Five dopamine receptors have now been identified (D1-D5) these are classified into two groups by virtue of their affinity to the D1 and D2 receptors (Surmeier et al. 1993). Studies by Surmeier et al. (1993) suggest that there is extensive co-localisation of the D1 and D2 family of receptors on striatal GABAergic neurones. This co-localisation could be in the region of 60-80%. However the co-localisation of these receptors on the medium spiny neurones has been found on the somatodendritic membrane but has not been investigated in the terminal regions of the neurones. Thus it is possible that the dopaminergic receptor segregation suggested by Gerfen et al. (1990) which results in differential SNr activation by the striatonigral and striatopallidal neurones could still be upheld. Some experimental results investigating voltage-dependent Ca$^{2+}$ currents supports the explanation of differential dopamine effects on the striatopallidal and striatonigral neurones, where loss of dopamine decreases expression of substance P in striatonigral neurones and increases enkephalin levels in striatopallidal neurones (Berretta et al. 1992).

1.5 Connections of the ventral and dorsal striatum

From the distribution of cortical input, the striatum can be subdivided into the dorsal striatum, which is approximately synonymous with the caudate-putamen (CP) and the ventral striatum which includes the nucleus accumbens (NAcc), the ventromedial CP and the olfactory tubercle. The dorsal and ventral striatum receive innervation from different areas in the brain and are functionally distinct.
1.5.1 *Afferent and efferent projections of NAcc*

The NAcc is a distinctive striatal subregion that is located in the rostroventral part of the striatum where it surrounds the anterior horn of the lateral ventricle, extending dorsally into the septum. It is generally accepted that the NAcc serves as a functional interface between the limbic system and the basal ganglia, helping turn motivational goal-directed information into an appropriate activational response. The NAcc receives cortical innervation from areas reputed to have limbic functions, including the anterior cingulate, prelimbic and orbitofrontal cortex in the primate (Alexander et al. 1986; Joel and Weiner 1994) and medial prefrontal, insular and entorhinal cortex in the rat (Deniau et al. 1994). The limbic system consists of several nuclei including the amygdala, hippocampus, anterior thalamic nuclei, parts of the hypothalamus, limbic cortex and the fibre bundles that interconnect these regions; the medial forebrain bundle and fornix. The limbic system is associated with behaviours involving "drive" and "emotion" such as hunting for food, escape from predators, copulation, and thermoregulation. Structures of the limbic system (primarily the hippocampus and amygdala) project either directly to the ventral striatum or indirectly via the ventral tegmental area (VTA). The VTA also innervates the NAcc by means of DA-containing A10 (mesolimbic) neurones. Kelley et al. (1982) studied the amygdalostrital projection in the rat, and found that it had an extensive distribution throughout the striatum, with dense labelling occurring in the caudal striatum, and the ventromedial part of the striatum rostral to the anterior commissure (which includes the NAcc). The innervation is much more sparse in the dorsolateral part of the rostral striatum, the area which receives its primary input from the sensorimotor cortex. In addition to the amygdalostrital projection, the antero-ventromedial striatum is also extensively innervated by the VTA, prefrontal cortex and hippocampal formation via the fornix which suggests that this area is predominantly concerned with limbic structures. The early subdivision of the NAcc as the area responsible for the mediation of limbic information, and the CP as the area responsible for the mediation of motor information has been questioned by a number of anatomical studies which have identified much more widespread limbic connections throughout the striatum. For example the widespread amygdalostrital connections identified by Kelley et al. (1982) and the distribution of VTA afferents which extend beyond the boundaries of
the NAcc into the entire ventromedial portion of the rostral striatum and also into the caudal striatum (Beckstead et al. 1979).

The efferent outflow of the NAcc is predominantly inhibitory utilising the neurotransmitter GABA (Mogenson et al. 1980). The NAcc innervates the ventral GP and also sends fibres through the substantia innominata (SI), and lateral preoptic area (LPO) to innervate the SN and retrorubral field (RRF) (Von Krosigk et al. 1992). The SI, VP and nucleus basalis of Meynert are components of the ventral pallidal region (VPr) and Mogenson et al. (1983), have demonstrated that the NAcc also innervates these areas. Furthermore autoradiographic evidence suggests that these projections are topographically organised, medial parts of the NAcc projecting to medial parts of the ventral GP, lateral NAcc to lateral parts of the ventral GP. This topography is maintained in the dorsoventral gradient. The NAcc also innervates the lateral nucleus of the amygdala and lateral habenular nucleus (Nauta et al. 1978), however this innervation is very sparse in comparison to the nigral and pallidal efferent connections. Within the NAcc there are inputs from limbic structures that terminate on nigrostriatal neurones, which innervate the medial SNc. This could provide one route whereby the information coming from the limbic system could exert its influence upon dopaminergic input into the dorsal sensorimotor part of the striatum, thus allowing for some integration between motivational and motor information (Groenewegen et al. 1991; Smith and Bolam 1990).

More recent work by Zahm and Heimer suggests that the NAcc can be subdivided into shell and core areas on the basis of acetylcholinesterase and substance P immunohistochemistry (Zahm and Heimer 1993). The core and shell of the NAcc have distinct afferent and efferent connections (Table 1.1). While the core has connections that closely parallel those of the CP, the shell, in addition to reciprocal connections with basal ganglia structures also communicates with limbic system structures. There is a further subdivision within the NAcc that cannot be divided into core and shell on the basis of immunohistochemistry. This anterior part of the NAcc has been labelled the "rostral pole" by Zahm and Heimer (1993) who have conducted tracing studies within this area of the NAcc. They have concluded on the basis of connectivity that the medial rostral pole is closely aligned to the shell whereas the lateral rostral pole is aligned with the core.
Afferents to the NAcc can be localised to core and shell areas. For example projections from subcortical structures such as the thalamic paraventricular nucleus, CM and MD and hippocampus are all directed to the core of the NAcc, which also receives input from the GP, VP, STN and A8, A9 and A10 DA-containing neurones. Afferents to the shell also include the DA-containing neurones and VP but widespread innervation also arises from the LPO, amygdala, ventral subiculum, brainstem reticular formation and CG (Groenewegen et al. 1991; Zahm and Brog 1992).

The efferent projections of the NAcc that take account of the core and shell division have also been extensively studied. NAcc efferents have been investigated using electrophysiological and anatomical tracing techniques (Deniau et al. 1994; Zahm and Heimer 1993). The whole of the NAcc has been shown to innervate the mesencephalic DA neurones, with the core predominantly innervating the A9 DA-containing neurones and the shell innervating the A8 and A10 DA-containing neurones (Groenewegen et al. 1991; Zahm and Brog 1992). The whole of the NAcc also innervates the midbrain extrapyramidal area (non-cholinergic PPTg) in the brain stem though the shell provides the most substantial innervation (Zahm and Brog 1992). The shell of the NAcc substantially innervates the ventromedial VP, the LPO, the rostrocaudal extent of the lateral hypothalamus (LH) and an area termed the extended amygdala, which includes the medial amygdala and sublenticular SI. The shell also substantially innervates mesencephalic structures including the retrorubral nucleus (RR), the central gray (CG) and lateral mesencephalic tegmentum. Sparser efferents to the SNc and EP have been identified. The core of the NAcc has substantial innervation of the medial LH, the EP, the dorsal part of the SNc and dorsolateral VP. There are some sparse afferents to the GP, lateral VTA, SNr, medial VP and STN (Zahm and Heimer 1993; Zahm and Brog 1992). Other studies have identified a more substantial innervation of the dorsomedial SNr from the core of the NAcc. This part of the SNr then innervates the MD and forms part of the limbic circuit (Deniau et al. 1994). Although it has been suggested that the NAcc-SNr projection is one way in which the limbic input into the ventral striatum can gain access to the basal ganglia motor systems, Deniau et al. (1994) have shown that the part of the SNr that is innervated by the NAcc, itself innervates the MD which is connected with the prefrontal cortex (Groenewegen et al. 1990). Other efferent
targets of the NAcc namely the VP and GP also innervate the MD (Groenewegen et al. 1990). In the rat the prelimbic part of the prefrontal cortex is known to have direct projections to the spinal cord where it influences autonomic functions (Hurley-Gius and Neafsey 1986) suggesting that the NAcc could have some influence over the autonomic system. However the robust connections of the core to the EP and dorsolateral VP allow the NAcc to gain access to the motor circuit of the basal ganglia (Zahm and Brog 1992).

1.5.2 Afferent and efferent connections of the CP

The CP is a larger, more heterogeneous structure than the NAcc and receives innervation from the entire neocortex, including the association and visual cortex, and thalamic nuclei (Alexander et al. 1986; Joel and Weiner 1994; Graybiel et al. 1994). In the primate the putamen forms part of the motor circuit described above and receives innervation from the motor cortex, premotor cortex and supplementary motor area (Alexander et al. 1986). Groenewegen et al. (1990) have identified similar parallel organization within the rat. Different areas within the CP appear to have different afferent innervation. For example the dorsolateral CP receives innervation almost exclusively from sensorimotor cortex, while the middle ventrolateral CP is innervated by the perirhinal cortex and amygdala (Kelley et al. 1982). Sensorimotor innervation of the dorsolateral CP extends across the entire rostrocaudal axis and is somatotopically organised. For example in primates, the dorsolateral part of the putamen appears to be activated by movement of the lower parts of the body, while the neurones of the ventrolateral part of the putamen respond to orofacial movements. Neurones located somewhere between these areas respond to upper body movements (Alexander et al. 1986). The CP also receives a major DAergic innervation from the SNc (A9 DAergic neurones). This projection is of great importance, since it is loss of nigrostriatal DA neurones and subsequent depletion of striatal DA levels that is the primary pathological feature of Parkinson's disease.

The GP and SNr are the two major targets of CP outflow. In the primate, the putamen innervates the ventrolateral part of the internal GP (EP in rats) and caudolateral SNr and both these sites then innervate thalamic nuclei, in particular the VLo, the VAmc and VApC, CM and MD. These thalamic nuclei then project to cortical motor areas (Alexander et al. 1986; Alexander and Crutcher 1990; Joel and
Weiner 1994). However there are two separate output streams from the CP that appear to modulate activity of the motor circuit. These are the direct and indirect striatonigral and striatopallidal pathways identified on the basis of their neurochemistry (Penny et al. 1986; Gerfen et al. 1990). The direct pathway contains substance P and projects to the SNr and internal GP, while the indirect pathway contains enkephalin and projects to the external GP.

The matrix of the striatum projects to the SNr and GP and there is evidence that there is a modular organization within the matrix (matrisomes) where the projection neurones appear to be clustered according to their efferent target areas (Graybiel et al. 1990; Malach and Graybiel 1986). However evidence from a retrograde tracing study where tracer was injected in either the internal or external GP has suggested a different level of organization within the matrisomes of the primate putamen (Flaherty and Graybiel 1993, 1994). In an extensive tracing study, Flaherty and Graybiel (1994) showed that matrisomes distributed throughout the primate putamen received innervation from single sites of the motor and somatosensory cortex that were related to specific body parts, and that the inputs from the motor cortex and somatosensory cortex were overlapping. They reported in a previous study that each matrisome contained neurones which projected to the internal and external GP, however there was no neuronal collateralisation, meaning single neurones within a matrisome projected only to the internal or external GP (Flaherty and Graybiel 1993). From their subsequent tracing study they discovered that the matrisomes which received the diverging cortical input from discrete sites within the motor and somatosensory cortex then re-converged onto single sites within the GP (Flaherty and Graybiel 1994). Flaherty and Graybiel (1993, 1994) have suggested that the purpose of the matrisomal divisions which receive diverging cortical input that is then re-converged at the level of the GP, may be to co-ordinate the flow of information through the direct and indirect output pathways of the striatum. Since it has been suggested that the striatonigral and striatopallidal pathways have disinhibitory and inhibitory effects respectively, co-ordination of these two pathways could be vital for the control of normal movement and associative motor learning (Gerfen et al. 1990; Flaherty and Graybiel 1993, 1994).

Mogenson et al. (1983) demonstrated a projection from the CP to dorsal parts of the GP and to the SN but not the subpallidal region in the rat. As with the NAcc,
the CP-GP connection showed a topographical relationship, this time in the rostrocaudal axis. Von Krosigk et al. (1992) have looked more closely at the efferent projections of a subregion of the CP, the ventrolateral caudate putamen (VLCP). They injected an anterograde tracer into the VLCP and observed profuse labelling within the caudolateral GP and dorsolateral SNr. Furthermore using electron microscopy and postembedding immunohistochemistry, they demonstrated that the VLCP projection converges onto neurones in the SNr (nigroreticular neurones) which also receive innervation from the GP and that both these projections utilise the inhibitory neurotransmitter GABA. The SNr has projections to medullary and pontomedullary regions which suggests a possible route for the mediation of motor behaviours (in this case orofacial movements) which do not involve the basal ganglia-thalamocortical loops. The striatum has also been shown to innervate nigrothalamic neurones and a converging striatal and GP innervation has been demonstrated on nigrocollicular neurones (Smith and Bolam 1991).

1.6 Functional role of the basal ganglia: a brief summary
The basal ganglia are no longer considered to have purely motor functions but are also believed to have a role in the mediation of limbic, and cognitive functions (Graybiel et al. 1994). It appears that the function of the basal ganglia are not to generate the physical mechanisms for movement per se, but to determine and direct the combination of movements to be made by selecting the appropriate neurones to be activated. This is achieved through neurochemical diversity, compartmentalisation and gating signals (Goldman-Rakic and Selemon 1990). However the striatum also has descending projections directed towards the brain stem which may provide another pathway through which the basal ganglia could mediate motor behaviour. The role this descending projection has in the mediation of motor behaviours is still poorly understood. Understanding it's role is important since it will provide more information on how the brain controls the generation of movement and it may help some resolve basal ganglia disorders.
Table 1.1. Some of the Afferent and Efferent Projections of the NAcc Core and Shell

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<th>Efferents from the NAcc</th>
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<td>Core</td>
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Figure 1.1. Simplified version of the motor circuit as described by Alexander et al. (1986). The arrow leading from the SNr and GPi to the thalamic nuclei, represents innervation to all the thalamic nuclei shown. Where one arrow represents input from two structures this does not indicate collaterals but is used for the sake of simplicity to represent two separate inputs. For a fuller description of the connectivity of the motor circuit refer to the text.
Figure 1.2. Simplified version of the anterior cingulate circuit as described by Alexander et al. (1986). For fuller description of the connectivity of the anterior cingulate circuit refer to the text.
Figure 1.3. Simplified version of the open and closed basal ganglia-thalamocortical loops as described by Joel and Weiner (1994). This diagram shows the connectivity of the motor circuit and anterior cingulate circuit. The heavy lines represent the closed loops of the motor and anterior cingulate circuits. The single arrows (thin lines) represent one of the two open loops of the anterior cingulate circuit, which innervates the associative prefrontal cortex. The double arrows show the open loop of the anterior cingulate circuit which innervates the motor cortex and the triple arrows show the open loop of the motor circuit which innervates the associative prefrontal cortex. This shows how cross-communication between the basal ganglia thalamocortical loops could occur (Joel and Weiner 1994). For a fuller description refer to the text.
Figure 1.4. This diagram has been copied from Gerfen et al. (1990), from *Science* Volume 250, page 1431.
Fig. 4. Diagram of the connections of striatal output neurons. The degree of shading of the cells denotes relative peptide mRNA levels, and the thickness of the lines indicates relative activity measured in studies of 2-deoxyglucose measurements (24). (A) Control: The cortex and thalamus provide an excitatory input to the striatum. Striatal neurons that contain enkephalin (ENK) and the D₂ dopamine receptor provide an inhibitory input to the globus pallidus (GP). Pallidal neurons provide an inhibitory input to the subthalamic nucleus (STN), which provides an excitatory input to the substantia nigra pars reticulata (SNr). Striatal neurons that express the D₁ dopamine receptor, dynorphin (DYN) and substance P (SP) provide an inhibitory input to the substantia nigra/entopeduncular nucleus (SNr/EP). SNr/EP GABAergic neurons inhibit neurons in the thalamus, superior colliculus, and pedunculopontine nucleus (PPN). Normal behavioral activity (arrows at the bottom of the diagram) is dependent on coordinated striatonigral and striopallidal outputs that regulate SN-output.
Chapter 2. Anatomical connections of the pedunculopontine tegmental nucleus.

2.1. Definition of the boundaries of the PPTg

The pedunculopontine tegmental nucleus (PPTg) was originally identified in the human brain by Olszewski and Baxter in 1954 where it was said to consist of a group of medium to large dark stained neurones situated close to the superior cerebellar peduncle in the pontomesencephalic tegmentum. Since then areas homologous to the human PPTg have been identified in the primate, cat and rat. The rat PPTg extends caudodorsally from the caudal substantia nigra to the parabrachial nucleus (Figure 2.1). It is bordered dorsally by the cuneiform nucleus, ventrally by the oral pontine reticular nucleus, laterally by the nuclei of the lateral lemniscus and medially by the superior cerebellar peduncle (Figure 2.2). Although small, the PPTg has been receiving increasing attention over the last few years. It encompasses an area in the mesopontine tegmentum that contains the Ch5 acetylcholine (ACh) neurones and non-cholinergic neurones. There has been controversy surrounding the anatomical specificity of the PPTg. Some authors (for example Steininger et al. 1992; Rye et al. 1987, 1988) define the PPTg as being synonymous with the cholinergic neurones in the mesopontine tegmentum, using the term "midbrain extrapyramidal area" (MEA) to describe the other neurones lying alongside those of the "cholinergic PPTg". However this implies two entirely separate structures and is misleading since the cholinergic and non-cholinergic neurones of the mesopontine tegmentum are intermingled (Spann and Grofova 1992; Lavoie and Parent 1994a). Furthermore, some authors (Rye et al. 1988) use a definition of the PPTg which also includes the cholinergic neurones that lie ventral to the superior cerebellar peduncle and form the subpeduncular tegmental nucleus (Paxinos and Watson 1986). Other authors (Garcia-Rill 1991, Jackson and Crossman 1983, Sugimoto and Hattori 1984) reject such a constricted definition of the nucleus and claim that the PPTg encompasses both cholinergic and non-cholinergic neurones. In this thesis the broader definition of the PPTg is used although it is recognised that the PPTg can be separated into two component parts: cholinergic neurones which are part of the ascending reticular activating system (ARAS) and non-cholinergic neurones which receive output from the basal ganglia and associated limbic structures.
2.2. Morphology and neurochemistry

The PPTg has a diverse morphology and neurochemistry and a topographical organization which is similar in all commonly studied species including the rat (Rye et al. 1987; Spann and Grofova 1992), cat (Vincent and Reiner 1987), primate (Lavoie and Parent 1994a) and human (Mesulam et al. 1989). The cholinergic neurones of the PPTg, form a continuous column of cells (Woolf 1991). According to Rye et al. (1987) the PPTg contains around 1600-1800 cholinergic neurones. The ChAT positive cells in the PPTg are slightly larger than those in the basal forebrain, but apart from size and spacing all cholinergic cells are morphologically similar (Woolf 1991).

The PPTg cholinergic neurones have somata that are either angular or pyramidal in shape. They have 3-5 primary dendrites which radiate in several directions, each giving rise to 2 or 3 secondary dendrites (Woolf 1991; Lavoie and Parent 1994a). Dendritic overlap allows neighbouring ChAT positive cells to communicate and also provides a basis for different cholinergic subsystems to interconnect. Within the PPTg the cholinergic cells are variable in size. In the dorsocaudal part of the PPTg the cholinergic cells are densely packed and some authors refer to this as the PPTg pars compacta, naming the more diffusely grouped neurones the pars dissipatus (Rye et al. 1987, 1988; Sugimoto and Hattori 1984; Swanson et al. 1984). The cholinergic neurones within the PPTg pars dissipatus are smaller and more elongated than those in the pars compacta (Spann and Grofova 1992). Three different sizes of cholinergic neurone were noted by Sugimoto and Hattori (1984) on the basis of cell volume; large neurones measured 2000-6000 \( \mu \text{m}^3 \), medium neurones 500-2000 \( \mu \text{m}^3 \) and small neurones less than 500 \( \mu \text{m}^3 \). The small and medium sized neurones provided 85% of the total neuronal number though the large neurones accounted for up to 90% of the total volume of the PPTg. Approximately 20% of the cholinergic neurones in the PPTg are also stained positively for substance P (Vincent et al. 1983; Rye et al. 1987).

The non-cholinergic neurones are more variable in size. Some reports identify the non-cholinergic neurones of the PPTg as small to medium sized and either round or fusiform in shape (Kang and Kitai 1990; Rye et al. 1988). However in an extensive light and electron microscopic study, in addition to small and medium sized neurones, Spann and Grofova (1992) also found large non-cholinergic neurones in rats, as have Lavoie and Parent (1994a) in primates. These large non-cholinergic neurones have
some similarities to the cholinergic neurones in terms of their shape and internal structure, but are different with respect to their synaptic boutons (Spann and Grofova 1992). PPTg neurones have also been classified into three different types on the basis of their membrane characteristics, although identifying the neurochemical content of these three types of neurone has proved controversial (Kang and Kitai 1990; Leonard and Linas 1990; Leubke et al. 1992). Kang and Kitai (1990) argued that in the rat Type I and III neurones had burst firing patterns and were non-cholinergic, whereas type II neurones were morphologically different from the type I and III neurones, had tonic firing patterns and were identified as cholinergic. However using a variety of neuronal tracers in the guinea pig, Leonard and Linas (1990) identified both type II and III neurones as cholinergic, while Leubke et al. (1992) argued that they had identified a proportion of all three types of neurones as cholinergic, using a combination of biocytin tracing and NADPH-diaphorase histochemistry in the neonatal rat. These discrepancies may be due to the different types of animal used in each experiment, however this is an area that warrants further clarification. The different sized non-cholinergic neurones identified by Spann and Grofova (1992) and the different firing patterns of the type I and type III neurones identified by Kang and Kitai (1990), suggest that the non-cholinergic neurones may be dissociable into two heterogenous groups, while the cholinergic, type II neurones form a homogeneous group. Although the neurochemistry of the non-cholinergic neurones remains to be verified, glutamate-immunoreactive neurones which are intermingled with cholinergic PPTg neurones have been reported in the primate, while GABA-immunoreactive neurones have not (Lavoie and Parent 1994a). These glutamate-immunoreactive neurones suggest that neurones using glutamate as a neurotransmitter may constitute one of the heterogenous non-cholinergic cell populations of the PPTg.

2.3. Afferent and efferent connections of the PPTg

The PPTg has extensive afferent and efferent projections (Tables 2.1 and 2.2). Afferents to the PPTg have been identified from the telencephalon, mesencephalon, pons and medulla and the efferent projections of the PPTg are no less extensive. They spread widely and provide innervation of limbic structures, the basal ganglia, all the thalamic nuclei, sites within the pontomedullary reticular formation, and even the spinal cord. Thus the PPTg has a large number of connections which allow it to have
an impact on very different types of behavioural control. The PPTg can be divided into the cholinergic PPTg and non-cholinergic PPTg on the basis of their neurochemistry. Although both the non-cholinergic and cholinergic PPTg have some afferent and efferent projections in common, they can also be separated on the basis of their differing afferent and efferent connections. These differential connections result in different functional consequences; with the cholinergic PPTg considered part of the ascending reticular activating system involved in behaviours associated with activation and arousal, and the non-cholinergic PPTg which receives substantial innervation from basal ganglia structures reputed to mediate descending motor outflow.

2.3.1. Afferents and efferents of the cholinergic PPTg

Since afferent innervation of the cholinergic PPTg arises from several widespread areas, input into the PPTg can be described by grouping the structures by their location in the brain. A sparse projection from the medial prefrontal cortex has been identified (Semba and Fibiger 1992) but the main afferent innervation arises from subcortical structures. For example, the cholinergic PPTg receives afferent innervation from sites within the telencephalon, including moderate projections from the SI, amygdala and bed nucleus of the stria terminalis (Swanson et al. 1984; Semba and Fibiger 1992; Steininger et al. 1992). Within the diencephalon, the PPTg receives extensive afferent projections from the hypothalamus, in particular the LHA and in contrast to the high level of efferent innervation from the PPTg to the thalamic nuclei, afferents to the PPTg arose only in the caudal part of the thalamus (Swanson et al. 1984; Semba and Fibiger 1992; Steininger et al. 1992). The dorsal raphe nucleus (DRN), central tegmental field (CTF), deep mesencephalic nucleus (DpMe) and central gray (CG) are the structures within the mesencephalon that gave rise to the most dense projection to the PPTg. Moderate innervation from the SC, RRF, and SNc has also been reported (Semba and Fibiger 1992; Steininger et al. 1992). From the pons afferents from the pontine reticular formation (PRF) were identified including a projection from the oral pontine reticular nucleus and the caudal pontine reticular nucleus (also called the gigantocellular tegmental field) and a number of retrogradely neurones were also seen bilaterally in the laterodorsal tegmental nucleus, though the ipsilateral projection was heaviest (Semba and Fibiger 1992; Steininger et
al. 1992). It has also been shown that the dendrites of PPTg cholinergic neurones extend into local fibre systems such as the superior cerebellar peduncle, and the medial and lateral lemniscus (Rye et al 1987). Sensory and motor information from these fibres probably activate PPTg cholinergic neurones. Thus the cholinergic PPTg receives extensive afferent innervation including synaptic input from "reticular formation" nuclei such as the gigantocellular reticular nucleus of the medulla and the caudal pontine reticular nucleus (Jones and Yang 1985; Semba and Fibiger 1992; Steininger et al. 1992). The reticular formation is a large "net-like" structure which consists of many nuclei located in the core of the medulla, pons and midbrain and which project to the cerebral cortex, thalamus and spinal cord. All these nuclei interconnect resulting in a complex, diffuse network of communication. The reticular formation nuclei receive sensory information and through extensive output pathways co-ordinate the activity of the reticular formation to play a role in sleep, arousal, attention, movement and muscle tone. Furthermore the LHA and CG are believed to have roles in the regulation of autonomic functions and pain control respectively (Saper et al. 1976; Behbehani and Fields 1979). Thus the afferent innervation to the cholinergic PPTg could combine such information with that of the input from the ascending reticular activating system, which in turn would determine the sequence of outflow from the PPTg which would serve to activate other brain areas such as the thalamus, and result in the generation of appropriate behaviours.

The efferent connections of the PPTg are no less widespread. Afferents to the medial prefrontal cortex have been identified from the PPTg though the major cholinergic innervation of this site was localised to the LDTg and subsequent studies failed to demonstrate a cortical projection from the PPTg at all (Vincent et al. 1983a; Satoh and Fibiger 1986; Woolf et al. 1990; Wolff 1991). Ascending PPTg neurones innervate the SI in the telencephalon, and diencephalic structures such as the LH and thalamus (Hallanger et al. 1987; Lee et al. 1988). Cholinergic PPTg neurones project most heavily to the thalamus, innervating the majority of thalamic nuclei in the rat (Sugimoto and Hattori 1984; Hallanger et al. 1987; Semba et al. 1990; Newman and Ginsberg 1994), primate (Lavoie and Parent 1994b) and human (Mesulam et al. 1989). Projections have also been identified from the cholinergic PPTg to the basal
forebrain\(^1\) (Semba et al. 1988; Jones and Cuello 1989). Both the basal forebrain and the thalamus are believed to have a major, complementary role in cortical activation and in the regulation of behavioural state. Both these structures receive heavy innervation from cholinergic neurones within the PPTg and a number of collaterals from the PPTg to neurones within the thalamus and basal forebrain have been identified (Losier and Semba 1993). Such collateralization would allow for the concurrent stimulation of both the thalamus and basal forebrain by the cholinergic neurones of the PPTg. This could contribute to the co-ordination of cortical activation and regulation of the behavioural state of the animal. There is also evidence identifying cholinergic projections to the pontine reticular formation (PRF), an area that is involved in several aspects of sleep, including the initiation of REM sleep, PGO spikes and EEG desynchronization (Rye et al. 1988; Semba et al. 1990). These efferents come from both the PPTg and LDTg, though the PPTg projection is of greater density; approx 13-31\% of cholinergic neurones (Semba et al. 1990). The vast majority of the cholinergic PPTg neurones which project to the PRF collateralize to innervate the thalamus (Semba et al. 1990). Cholinergic innervation of the thalamus is known to enhance cortical activation by blocking the rhythmic oscillatory thalamocortical neurones (Semba et al. 1990; Woolf 1991). Thus collateralized cholinergic PPTg neurones may have yet another role in behaviour state control, this time in the mediation of REM sleep while simultaneously blocking the rhythmic oscillatory activity of the thalamocortical neurones which are associated with slow wave sleep (Semba et al. 1990). The cholinergic PPTg has also been shown to innervate several areas within the PRF and medulla, including the oral pontine and caudal pontine reticular nuclei, medullary reticulospinal regions and gigantocellular field of the medulla (Rye et al. 1988; Spann and Grofova 1991). The projection to the medullary gigantocellular field has been shown to mediate state-dependent respiratory depression during REM sleep (Lydic and Baghdoyan 1993). Descending cholinergic efferents have also been reported to innervate the anterior ventrolateral medulla, an

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\(^1\) The anatomy of the basal forebrain is extremely complex. The basal forebrain comprises the cholinergic nucleus basalis magnocellularis, the medial septum, the ventral pallidum, substantia innominata and vertical and horizontal limbs of the diagonal band (Jones and Cuello 1989). The horizontal limb of the diagonal band is also known as the magnocellular preoptic nucleus, while the substantia innominata has also been described as a widespread area that includes the nucleus basalis magnocellularis, ventral pallidum and sublenticular extended amygdala (Alheid and Heimer 1988). Therefore as with the nomenclature of the PPTg, care should be taken when interpreting references to structures within the basal forebrain.
area that is involved in the regulation of blood pressure (Yasui et al 1990). Blood pressure can be increased following injection of cholinergic agonists into the ventrolateral medulla, while previous treatment with cholinergic antagonists can block the increased blood pressure seen when injecting peripheral anticholinesterases (Yasui et al 1990).

Within the mesencephalon cholinergic PPTg neurones innervate the RRF and SN (Lee et al. 1988; Lavoie and Parent 1994b, 1994c). A major cholinergic innervation to the SN has been localised to the PPTg, though it is controversial whether such a connection exists. Cholinergic neurones arising predominantly from the ipsilateral PPTg pars dissipatus which target the SN have been identified by Beninato and Spencer (1987). Tracing studies using retrograde tracers such as WGA-HRP (Gerfen et al. 1982; Jackson and Crossman 1983; Moon Edley and Graybiel 1983), fluorescent tracers like true blue and flurogold (Woolf and Butcher 1986; Gould et al. 1989; Lavoie and Parent 1994c) and anterograde tracers such as PHA-L and leucine (Lavoie and Parent 1994b) have demonstrated the existence of such a projection. In their tracing study Gould et al. (1989) identified cholinergic projections to both SNc and SNr, though the innervation of the SNc was twice as dense as that to the SNr. Bolam et al. (1991) reported that cholinergic neurones formed synaptic contact with the dendrites and soma of dopaminergic neurones in the ferret SN. However others have argued that no such projection exists (Rye et al. 1987; Lee et al. 1988). A functional study using in vivo microdialysis and electrochemistry has also demonstrated the existence of a cholinergic projection from the PPTg to the SN that affects the activity of nigral DA-containing neurones (Blaha and Winn 1993). Injections of neostigmine were made into the SN and DA efflux in the striatum was measured. Neostigmine is an acetylcholinesterase inhibitor, which prevents the breakdown of acetylcholine (ACh) and thus increases the level of endogenous ACh in the SN. This treatment increased the level of DA release in the striatum, as did microinjections of nicotine, a direct ACh receptor agonist. However lesions of the PPTg which removed the cholinergic innervation of the SN, resulted in a marked decrease in the release of striatal DA following injections of neostigmine (Blaha and Winn 1993). Conversely microinjections of nicotine into PPTg-lesioned rats greatly potentiated the efflux of DA in the striatum, suggesting that the lesion had produced postsynaptic receptor supersensitivity. These data indicate that not only does a
cholinergic projection exist from the PPTg to the SN, but that it also influences the activity of DA-containing neurones within the SNC. There is still little direct evidence that the PPTg cholinergic efferents actually terminate in the SN, except for the data of Bolam et al. (1991) and a double labelling study where retrograde tracers fluorogold or rhodamine labelled microspheres were injected into the SN and retrograde tracing identified in ChAT-immunostained PPTg neurones (Oakman et al. 1995). However the evidence described above argues strongly in favour for the existence of a cholinergic projection. Cholinergic innervation of the SN is important because it provides an extra-striatal excitatory input into the SN which affects the activity of the DA-containing neurones which themselves have a role in the modulation of striatal activity. Neurones within the PPTg have also been observed to project directly to the striatum in primates (Nakano et al. 1990; Lavoie and Parent 1994b). Although these neurones were not formally identified as cholinergic in either case, Nakano et al. (1990) reported that only large neurones within the PPTg were stained following injections of the retrograde WGA-HRP tracer into the putamen and head of the caudate nucleus. A similar direct projection from the PPTg to the striatum has also been identified in the rat (Saper and Lowey 1982). These striatal and nigral projections, like those to the thalamic nuclei and basal forebrain could be interpreted as forming part of the ascending reticular activating system, whereby neurones within the pons and brainstem have excitatory influences over forebrain structures and the basal ganglia which modulate the sleep/wake cycle. Thus the cholinergic PPTg has widespread afferent and efferent connections which allow it to influence many different behaviours.

2.3.2. Afferents and efferents of the non-cholinergic PPTg

The afferent projections to the non-cholinergic PPTg can also be segregated in a similar way to the afferents to the cholinergic PPTg, although the afferent innervation from basal ganglia structures will be described separately. From the telencephalon a moderate projection to the non-cholinergic PPTg was reported from the SI while the most dense projection from the diencephalon arose in the lateral habenular nucleus, and slightly less dense labelling was seen in the LHA (Steininger et al. 1992). The non-cholinergic PPTg received a large number of projections from sites within the mesencephalon. These included a dense innervation from the CG and DRN which was
of approximately the same density as the afferents to the cholinergic PPTg. Following injections of the tracer wheat germ agglutinin horse radish peroxidase (WGA-HRP) into the non-cholinergic PPTg dense retrograde tracing was also seen in the contralateral SC, while moderate labelling was found in the CTF and VTA (Steininger et al. 1992). Afferents from the pons and medulla were less extensive in the non-cholinergic PPTg than those described for the cholinergic PPTg. The highest density of afferent innervation arising in the pons came from the parabrachial nuclei. Within the PRF a moderate projection was seen in the oral pontine nucleus, and a moderate projection also arose in the LDTg. Only a small number of neurones in the gigantocellular field of the medulla were labelled by tracers injected into the non-cholinergic PPTg (MEA) (Steininger et al. 1992).

The major output pathways of the striatum are the SN and GP. The non-cholinergic PPTg receives innervation from both of these basal ganglia structures and also from other nuclei within the basal ganglia. The caudal part of the GP innervates the PPTg, and this projection has been shown to utilise the inhibitory transmitter GABA (Rye et al. 1987; Moriizumi and Hattori 1992). However there are discrepancies in the amount of labelling reported by different tracing studies. For example, Rye et al. (1987) reported fairly dense labelling of the non-cholinergic PPTg from GP and sparse labelling from the EP in rats. Sparse labelling from the EP was also reported in cats (Moon-Edley and Graybiel 1983). These findings are in contradiction to the large primate innervation of the PPTg from the internal segment of the GP which is homologous to the EP in rat and cat (Charara and Parent 1994), while Steininger et al. (1992) reported sparse retrograde labelling to the GP and moderate labelling in the EP following retrograde tracers injected into the PPTg. Projections to the PPTg from the SNr have also been reported (Beckstead et al. 1979; Gerfen et al. 1982; Moon Edley and Graybiel 1983; Spann and Grofova 1991; Deniau and Chevalier 1992; Semba and Fibiger 1992) and though it is unclear which population of PPTg neurones this projection appeared to target, it is likely that the non-cholinergic neurones were in receipt of this innervation since more recent studies which make a distinction between the non-cholinergic and cholinergic PPTg have identified dense innervation of the non-cholinergic PPTg by the SNr (Rye et al. 1987; Steininger et al. 1992). Also the study conducted by Spann and Grofova (1991), demonstrated that the projection from the SNr terminated predominantly in the PPTg.
pars dissipatus which they define as containing both cholinergic and non-cholinergic neurones. Furthermore the part of the PPTg that received the most dense input from the SNr was the area that sends afferents to the basal ganglia structures (Spann and Grofova 1991). The projection from the SNr is also an inhibitory, GABAergic one. Although Steininger et al. (1992) did not report any innervation from the STN other authors have done so (Nauta and Cole 1978; Jackson and Crossman 1981, 1983; Saper and Lowey 1982; Moon Edley and Graybiel 1983). Innervation of the PPTg by basal ganglia structures suggests a possible descending subcortical route whereby motor information could gain access to the brainstem and spinal cord.

Reciprocal non-cholinergic innervation of all the basal ganglia structures by the non-cholinergic PPTg has also been reported. Non-cholinergic projections to the SN have been identified (Jackson and Crossman 1983; Sugimoto and Hattori 1984; Beninato and Spencer 1987; Rye et al. 1987; Steininger et al. 1992; Lavoie and Parent 1994c) while efferents to the STN, GP and EP and striatum (including the NAcc and CP) have also been reported (Saper and Lowey 1982; Jackson and Crossman 1983; Moon Edley and Graybiel 1983; Sugimoto and Hattori 1984; Rye et al. 1987; Lee et al. 1988). The PPTg also projects to the SC and ventral thalamic nuclei (Saper and Lowey 1982). Although many of these studies didn't identify the neurochemical content of the PPTg neurones, from the evidence provided by other studies (Steininger et al. 1992; Rye et al. 1987; Lavoie and Parent 1994c) it is highly likely that the projections they identified were non-cholinergic.

Descending projections innervate the medulla, caudal pons (part of the PRF) and spinal cord (Jackson and Crossman 1983; Jones and Yang 1985; Rye et al. 1988; Garcia-Rill 1991; Woolf 1991). Injections of retrograde tracer into the medullary reticular formation labelled the non-cholinergic neurones of the PPTg, with injections into the magnocellular field yielding the greatest density of labelling (Rye et al. 1988). The innervation of the spinal cord by the PPTg is controversial, with some research arguing that these projections to the spinal cord arise from structures adjacent to the PPTg such as the Köllicker-Fuse nucleus and parabrachial nuclei (Spann and Grofova 1991). However other research has identified a PPTg-spinal cord projection (Skinner et al. 1990a). For example Rye et al. (1988) argue that the spinal cord derives its innervation almost exclusively from the mesopontine tegmentum including the non-cholinergic PPTg, subceruleal region and the Köllicker-Fuse parabrachial subnucleus.
Other authors have suggested that the PPTg projection to the spinal cord is cholinergic (Swanson et al. 1984). However an extensive double labelling study by Rye et al. (1988), demonstrated that a projection arises from the MEA (synonymous with the non-cholinergic PPTg). A large number of neurones arising in the mesopontine tegmentum project their axons to the pons and medulla through Probst's tract (Rye et al. 1988). Some of the axons from the PPTg, both cholinergic and non-cholinergic, project through the ventromedial part of Probst's tract to innervate the medullary gigantocellular field, where they largely overlap. The medullary reticulospinal region is innervated most heavily by the PPTg, both cholinergic and non-cholinergic neurones, and subceruleal region (Rye et al. 1988). The nuclei of the medullary gigantocellular field project to motor areas in the medulla and the cranial and autonomic nerves of the spinal cord which suggests another, less controversial route through which the PPTg could affect sites of motor control (Spann and Grofova 1991).

The non-cholinergic PPTg receives extensive afferent innervation from sites within the basal ganglia that are involved in the transmission of information regarding motor control. The efferent innervation of the non-cholinergic PPTg predominantly targets sites within the pons, medulla and spinal cord which themselves have descending connections to areas that are involved in motor outflow (Jones and Yang 1985). Thus the PPTg is a good candidate for the first site of a relay station outwith the basal ganglia, which forms part of a subcortical motor pathway that receives basal ganglia motor information which can then be translated into appropriately directed motor behaviours. This would allow for the existence of two motor pathways, first the basal ganglia thalamocortical loop which forms the motor circuit and a descending subcortical pathway that innervates sites within the medulla and spinal cord.
Table 2.1. Summary of the afferent and efferent connections of the cholinergic PPTg

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<tr>
<th>Cholinergic PPTg</th>
<th>Afferents</th>
<th>Efferents</th>
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<td><strong>Telencephalon</strong></td>
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<td>Telencephalon</td>
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<td>Basal Forebrain nuclei</td>
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<td>Caudal thalamus</td>
<td>LH</td>
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<td>Gigantocellular reticular nucleus of the medulla</td>
<td>Gigantocellular field of the medulla</td>
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<td>Ventrolateral medulla</td>
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Table 2.2. Summary of the afferent and efferent connections of the non-cholinergic PPTg

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<th>Efferents</th>
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<td>Striatum</td>
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<td>Diencephalon</td>
<td>Lateral habenular nucleus</td>
<td>Ventral thalamic nuclei</td>
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<td>CTF</td>
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<td>VTA</td>
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<tr>
<td>Pons and Medulla</td>
<td>Parabrachial nuclei</td>
<td>Magnocellular field of the medulla</td>
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<tr>
<td></td>
<td>LDTg</td>
<td>Spinal cord</td>
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<tr>
<td></td>
<td>Gigantocellular field of the medulla</td>
<td>Gigantocellular field of the medulla</td>
</tr>
<tr>
<td></td>
<td>Oral pontine reticular nucleus</td>
<td>Medullary reticulospinal region</td>
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Figure 2.1. Sagittal section showing the position of the PPTg in the rostrocaudal plane in the rat brain. The PPTg extends caudodorsally from the rostral SNr to the parabrachial nuclei. ac = anterior commissure, CNF = cuneiform nucleus, CP = caudate putamen, NAcc = nucleus accumbens, PB = parabrachial nucleus, scp = superior cerebellar peduncle, SNr = substantia nigra pars reticulata. Simplified diagram redrawn from the atlas of Paxinos and Watson (1986).
Sagittal Plane

Lateral. 1.90 mm

CP
ac
NAcc
CNF
PB
SNr
PPTg
scp
Figure 2.2. Coronal section showing the position of the caudal PPTg in the mediolateral and dorsoventral planes in the rat brain. The PPTg is bordered dorsally by the CNF, ventrally by the oral pontine reticular nucleus (PnO), laterally by the lemniscal fibres and medially by the superior cerebellar peduncle (scp). CG = central gray, IC = inferior colliculus, DLL, ILL, VLL = lemniscal nuclei, ll = lateral lemniscal fibres, SPTg = subpeduncular tegmental nucleus. Simplified diagram redrawn from the atlas of Paxinos and Watson (1986).
Chapter 3. Anatomical connections of the cuneiform nucleus.

3.1. Definition of the boundaries of the cuneiform nucleus

The cuneiform nucleus (CNF) was first identified in the guinea pig by Castaldi in 1926 and since then has been shown to exist in the cat (Taber 1961; Berman 1968) and rat (Beitz 1982a). Lately increasing attention has been directed at the CNF. Although it has been considered a part of the pain control system for many years, other evidence has indicated that it may also constitute part of the mesencephalic locomotor region (MLR) (see Chapter 7).

There exists some controversy regarding the delineation of the boundaries of the CNF. Taber (1961) defined a widely dispersed CNF extending rostrally as far as the SN, lying dorsolateral to the red nucleus and RRF, and caudally beyond the decussation of the superior cerebellar peduncle, lying ventral to the inferior colliculus (IC) and dorsal to the PPTg. In this thesis the definition used is the one depicted in the rat stereotaxic atlas of Paxinos and Watson (1986), which gives a relatively constrained definition of the CNF (Figures 3.1 and 3.2). This corresponds to Taber's caudal CNF, whereas Taber's (1961) rostral CNF is homologous with the deep mesencephalic nucleus of Paxinos and Watson (1986) or the mesencephalic reticular nucleus of Swanson (1992). The Paxinos and Watson (1986) CNF extends rostrally from the lateral parabrachial nucleus to the caudal border of the SC and is bordered medially by the CG, laterally by the dorsal nucleus of the lateral lemniscus, dorsally by the caudal part of the IC and ventrally by the PPTg.

3.2. Morphology and neurochemistry

There is relatively little information which describes the cytoarchitectural and cytochemical structure of the CNF. Neurones within the CNF are small to medium in size and either oval, triangular or pear-shaped (Castiglioni et al. 1978; Rye et al. 1987; Gioia and Bianchi 1987a, 1987b). In cats the neurones can be classified into two types on the basis of their dendritic arborization, with fusiform neurones giving rise to 1-2, and multipolar neurones giving rise to 3-7 spiny, primary dendrites (Gioia and Bianchi 1987a, 1987b). This is similar to the data described for humans (Gioia and Bianchi 1987b). The primary dendrites of the more numerous multipolar neurones also give rise to widespread secondary dendrites and the axons and
dendrites of these neurones project outwards the CNF, while the dendrites of the fusiform neurones remain within the boundaries of the CNF (Gioia and Bianchi 1987a). Thus the multipolar neurones of the CNF have been hypothesised to be the main output neurones of the CNF while the fusiform neurones appear to have an interneuronal function (Gioia and Bianchi 1987a). Neurones of the CNF are densely packed within the nucleus (Rye et al. 1987, Berman 1968; Taber 1961) and contain a number of neurochemicals including serotonin, neurotensin, substance P and enkephalin, which behaves pharmacologically like opiates (Simantov et al. 1977; Beitz 1982b; Moss and Basbaum 1983; Moss et al. 1983), though this may be an overestimation since some of these studies use Taber's (1961) definition of the CNF boundaries, which includes the DpMe of Paxinos and Watson (1986).

3.3. Afferent connections of the CNF

The major connections of the CNF have been identified by a number of tracing studies (Table 3.1). Bernard et al. (1989) used the tracer WGA-HRP to identify both the afferent input to and efferent outflow from the CNF. They found that the major afferent projections to the CNF arose from the forebrain and from the rostral midbrain. Forebrain afferents include projections from the nucleus centralis of the amygdala, ZI, ventromedial hypothalamus (VMH) and periventricular gray matter (Garcia-Rill et al. 1981; Bernard et al. 1989; Zemlan and Behbehani 1984). A sparse projection from the subpallidal region has also been recorded (Garcia-Rill et al. 1981; Swanson et al. 1984). The subpallidal region consists of parts of the SI, the LPO and LHA, and this region receives a dense projection from the NAcc (Jones and Mogenson 1980). From the midbrain a high density of substantia nigra pars lateralis afferents have been identified as has an afferent input from the lateral and dorsal central gray (CG), and afferents of lesser density have been observed from the SNc and peripeduncular area (Garcia-Rill et al. 1981; Bernard et al. 1989; Zemlan and Behbehani 1984; Redgrave et al. 1988). A small direct innervation to the medial CNF has also been identified from the cat EP using electrophysiological recording and confirmed with fluorescent retrograde tracing (Garcia-Rill et al. 1981). Other retrogradely labelled sites were the STN and precruciate cortex while a weak projection to the contralateral CNF was also identified. The CNF also receives major innervation from the deep and intermediate grey layers of the SC, which is involved in
attention to sudden moving stimuli and the mediation of the rapid fight or flight response (Redgrave et al. 1987; Dean et al. 1988; Redgrave et al. 1988; Zemlan and Behbehani 1984). Tracing studies revealed that the CNF receives only a slight innervation from sites within the pons and medulla, including reciprocal innervation from the NRM and caudal pontine reticular nucleus, but no innervation from the spinal cord (Bernard et al. 1989). However a projection from the spinal cord to the CNF has been identified electrophysiologically in rats (McMahon and Wall 1985). In this study the authors reported that lamina I of the spinal cord gives rise to ascending collateralized axons which course through the dorsolateral funiculus of the spinal cord (DLF) to a number of nuclei in the midbrain, including the CNF and CG (McMahon and Wall 1985).

3.4. Efferents connections of the CNF

Efferents from the CNF were observed to terminate almost exclusively in the pons and medulla. A moderate density of labelled fibres have been observed in the gigantocellular and magnocellular reticular formation, the caudal pontine reticular nucleus and the pontomedullary locomotor strip (PLS) (Steeves and Jordan 1984; Zemlan and Behbehani 1984; Bernard et al. 1989). Further efferents were identified in the dorsolateral CG and the peripeduncular area (Redgrave et al. 1988; Steeves and Jordan 1984; Zemlan and Behbehani 1988). A retrograde tracing study in the primate using HRP in the spinal cord has also identified a substantial connection with the CNF (Castiglioni et al. 1978) while projections from the CNF to the spinal cord have also been reported in rats (Carlton et al. 1983). However Bernard et al. (1989) failed to identify any direct spinal projections following their tracing study using WGA-HRP and the existence of a direct CNF-spinal cord pathway remains controversial. Innervations of the contralateral CNF, the SC, SN and the parafascicular nucleus have been observed in a number of anatomical studies (Redgrave et al. 1988; Zemlan and Behbehani 1988) as has labelling within the LC, hypothalamus and pallidum (Garcia-Rill et al. 1981).

However the majority of efferent fibres target the nucleus raphe magnus (NRM) and nucleus reticularis magnocellularis (NMC), two structures which mediate the transmission of nociceptive information. This suggests that the CNF is involved in the endogenous pain control system (Takagi 1980; Beitz 1982b; Carlton et al. 1983;
Zemlan and Behbehani 1984, 1988; Bernard et al. 1989). Both the NRM and the NMC (also termed the nucleus paragigantocellularis) lie in the ventromedial medulla and Carlton et al. (1983) have reported that a similar number of efferents project to both these sites. The CNF is considered to have a role in the transmission of nociceptive messages (Behbehani and Fields 1979; Dostrovsky et al. 1982; McMahon and Wall 1985; Zemlan and Behbehani 1988). The two major targets of the efferent projections of the CNF, the NRM and NMC, provide a major innervation of the spinal cord via the dorsolateral funiculus (DLF) (Basbaum et al. 1978; Zemlan et al. 1984). The NMC targets laminae IV-VII and lamina X of the spinal cord (Zemlan et al. 1984) while the NRM makes contact with almost all levels of the spinal cord, with particularly dense projections to lamina I and lamina V, which are the sites which are most responsive to noxious stimulation (Basbaum et al. 1976). The NRM utilises the inhibitory neurotransmitter serotonin and is the major source of the serotonergic projection to the spinal cord (Oliveras et al. 1977; Basbaum and Fields 1978). A study by Carlton et al. (1983) revealed that of the structures that provided afferent innervation of the NRM, only the CG projected to the DLF. They interpreted this as being an indication that the NRM is the final common site for the integration of neurones concerned with nociception, before this information is sent to the spinal cord, although they did not rule out the existence of other possible parallel pathways. In addition to the role in nociception hypothesised for the CNF on the basis of it’s projections to the NRM, NMC and CG, other efferent connections suggest that there is a further possibility that the CNF could effect sites of motor control within the pons and medulla. For example the CNF innervates the nucleus reticularis gigantocellularis, which projects via the ventrolateral funiculus to cranial motor nuclei VI, VII, and XII, which influence motor outflow (Basbaum et al. 1976, 1978).

3.5. Summary
The CNF nucleus is connected to a number of structures in the brain, with the majority of afferent projections to it arising in the midbrain and forebrain, while the major efferent projections of the CNF descend to contact a number of sites within the pons and medulla. On the basis of these anatomical connections a possible role for the CNF in the transmission of nociceptive messages and the mediation of analgesia has been suggested (Behbehani and Fields 1979; Dostrovsky et al. 1982; McMahon and
Wall 1985; Zemlan and Behbehani 1988). Other anatomical and behavioural evidence has indicated that in addition to nociception the CNF may also exert influence over motor behaviours and some research has indicated that the CNF may well constitute the major part of the mesencephalic locomotor region (MLR) (Steeves and Jordan 1984; Mori et al. 1977).
Table 3.1. Summary of some of the afferent and efferent connections of the CNF

<table>
<thead>
<tr>
<th>CNF</th>
<th>Afferents</th>
<th>Efferents</th>
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<tr>
<td>Telencephalon</td>
<td>SI</td>
<td>Hypothalamus</td>
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<td>Amygdala</td>
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<td>LPO</td>
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<td>Diencephalon</td>
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<td></td>
<td>VMH</td>
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<td>Mesencephalon</td>
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<td></td>
<td>CG</td>
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<tr>
<td></td>
<td>SN pars lateralis</td>
<td>SN</td>
</tr>
<tr>
<td></td>
<td>CNF (contralateral)</td>
<td>CNF (contralateral)</td>
</tr>
<tr>
<td></td>
<td>SNc</td>
<td>Pallidum</td>
</tr>
<tr>
<td>Pons and Medulla</td>
<td>NRM</td>
<td>NRM</td>
</tr>
<tr>
<td></td>
<td>Caudal pontine reticular nucleus</td>
<td>Caudal pontine reticular nucleus</td>
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<tr>
<td></td>
<td>Spinal cord</td>
<td>Spinal cord</td>
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<tr>
<td></td>
<td></td>
<td>NMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gigantocellular reticular nucleus</td>
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</tbody>
</table>
Figure 3.1. Saggital section showing the position of the CNF in the rostrocaudal plane in the rat brain. The CNF is bordered caudally by the parabrachial nucleus and extends rostrally to the caudal border of the superior colliculus. ac = anterior commissure, CP = caudate putamen, IC = inferior colliculus, NAcc = nucleus accumbens, PB = parabrachial nucleus, scp = superior cerebellar peduncle, SC = superior colliculus. Simplified diagram redrawn from the atlas of Paxinos and Watson (1986).
Figure 3.2. Coronal section showing the position of the CNF in the dorsoventral and mediolateral planes in the rat brain. The CNF is bordered dorsally by the inferior colliculus, ventrally by the PPTg, medially by the central gray and laterally by the lateral lemniscal fibres. CG = central gray, ll = lateral lemniscal fibres. Simplified diagram redrawn from the atlas of Paxinos and Watson (1986).
Part II. General Functional Considerations
Chapter 4. Behavioural functions of the dorsal and ventral striatum with particular reference to the role of dopamine.

4.1. General functional differences between the NAcc and CP

From the anatomical review in chapter 1, it is clear that the dorsal and ventral striatum can be characterised by their different afferent and efferent connections. However, not only do the different parts of the striatum have different connections, there is also a large amount of evidence that the dorsal and ventral striatum also have different functional roles. Since the majority of research has concentrated on either the NAcc (the major component of the ventral striatum) or the CP (which is the primary component of the dorsal striatum), the rest of this chapter will be confined primarily to discussing the functional role of these two structures.

Most of the early research into the functional role of the striatum concentrated on the actions of dopamine (DA) since it was well established that both the NAcc and CP receive substantial DAergic innervation from the mesencephalic A10 or A8 and A9 DAergic neurones respectively and disturbance of striatal DA is a major pathological feature of many basal ganglia disorders such as Parkinson’s disease and schizophrenia. Studies were initially conducted using electrolytic lesions but these destroyed both intrinsic neurones and damaged fibres of passage. Therefore when 6-OHDA was discovered, a neurotoxin which selectively destroyed cell bodies while leaving axon fibres travelling through the site of injection relatively intact, this became the lesion method of choice. 6-OHDA works at DA and noradrenaline reuptake sites, where it is taken up by the neurone and degraded into DA and hydrogen peroxide, the hydrogen peroxide being toxic to the neurone. These lesion studies were commonly combined with either central or peripheral administration of amphetamine, a catecholaminergic stimulant. Amphetamine is known to stimulate the release and block the reuptake of both DA and noradrenaline (NA).

In a series of experiments done from 1973-1975 Susan Iversen and her colleagues drew attention to a possible differential role for the NAcc and CP. They showed that the behavioural response of rats to different doses of systemic amphetamine could be blocked by selective lesions in the CP or NAcc using 6-OHDA (Creese and Iversen 1973, 1975). Creese and Iversen (1975) showed that depletion of nigrostriatal DA levels following administration of 6-OHDA into the SN in adult rats
abolished the stereotypies shown in response to relatively high doses of amphetamine, though the locomotor response was not blocked even when DA levels in the CP were depleted by 90%. When given apomorphine (a direct DA receptor agonist) these rats showed an increased locomotor response and intense stereotyped behaviours. Furthermore rats with lesions of the dorsal and ventral NA pathway showed no change in their response to amphetamine. Creese and Iversen (1975) drew several conclusions from these data. First, that both the stereotypy and locomotor responses produced by amphetamine are reliant only on the DA system, a finding that was confirmed by Kelly and Iversen (1976). Second that postsynaptic DA receptors in the striatum are still intact and the response to apomorphine indicates that they have become supersensitive; and finally that amphetamine is an indirect agent that acts presynaptically to release DA or block its reuptake.

Kelly et al. (1975) took this a step further when they investigated whether the different behaviours stimulated by different doses of amphetamine were mediated from the same or different neural substrates. Creese and Iversen (1975) had concluded that the actions of amphetamine were dependent on the functional integrity of the nigrostriatal pathway, but they were not entirely correct. It is well known that relatively low doses of amphetamine enhance locomotor activity while higher doses produce stereotyped behaviours such as sniffing, rearing, licking and gnawing. Kelly et al. (1975) made 6-OHDA lesions of either the rat NAcc or CP and then investigated the effect of different doses of systemic amphetamine. NAcc lesions abolished the enhanced locomotor activity at a low dose (1.5 mg/kg) but did not abolish stereotyped behaviours at a high dose (5.0 mg/kg). DA-depleting lesions of the CP had the opposite effect, abolishing stereotypies at the high doses but not the locomotion elicited from low doses. Indeed at the highest dose of amphetamine (5.0 mg/kg) the CP lesioned rats maintained high levels of locomotor activity throughout the test session. Thus amphetamine has differential effects on behaviours mediated from the dorsal and ventral striatum. This finding was reinforced by Joyce and Iversen (1984) who made lesions within the CP and studied their effect on locomotion, stereotypy and anorexia. They also reported that rats with lesions of the CP demonstrated an attenuated response to amphetamine-induced stereotypies, and exhibited anorexia, but locomotion at low doses was unaffected, and increased in line with controls.
Since these early experiments which have shown that the CP and NAcc have different functional roles, research has concentrated on discovering the roles that the NAcc and CP play in the generation of different behaviours.

4.2. Functions of NAcc
A role for the NAcc has been implicated in a large number of behaviours. This overview concentrates on the part it plays in the mediation of locomotion and motivated behaviour, which includes both appetitive and aversive behavioural tasks.

4.2.1. NAcc-stimulated locomotor activity
It is now widely believed that the NAcc is associated with the mediation of locomotor activity stimulated by mesolimbic DA neurones. There is a large body of evidence to support this. Pijnenburg and Van Rossum (1976) conducted a study of locomotion stimulated from the NAcc following injection of several different chemicals including DA, DA metabolites such as homovanillic acid and amphetamine. They found that amphetamine was the most effective for producing a locomotor response, DA was less effective and DA metabolites had no effect at all. They concluded that the DAergic stimulation of the NAcc can result in locomotor activation. Injections of DA into the NAcc in rats produces a robust increase in locomotor activity in the open field (Mogenson et al. 1980). This locomotor response can also be produced by DAergic stimulants such as apomorphine and amphetamine and is blocked by systemic or central injections of neuroleptics such as haloperidol (Pijnenburg et al. 1975), or the catecholaminergic neurotoxin 6-OHDA injected directly into the NAcc (Joyce et al. 1983). NAcc-stimulated locomotion can also be attenuated by injections of GABAergic agonists into the VP (Mogenson et al. 1980). Swerdlow and Koob (1984) hypothesized that a GABAergic NAcc-VP connection could be the primary output for the expression of mesolimbic DA-stimulated locomotion. They blocked the supersensitive locomotor response to apomorphine, shown by rats with 6-OHDA NAcc lesions, by injecting muscimol, a GABA agonist, into the VP region. Furthermore, injections of picrotoxin, a GABA antagonist, into the VP region have been reported to initiate locomotor activation (Swanson et al. 1984).

Although mesolimbic DAergic stimulation results in locomotion, this does not tell us much about the role of mesolimbic DA in normal behaviour. Fink and Smith
(1979, 1980) studied catecholamine (CA) depletion and its effect on exploratory locomotion. They injected 6-OHDA into the anterolateral hypothalamus (ALH) and studied the subsequent effect of CAergic depletion on exploratory behaviour using various different tasks, for example placing the rat in a novel open field, or placing novel objects in the open field or home cage. They reported deficits in investigatory behaviour, but no effect on global motor behaviour (Fink and Smith 1979). Their results however showed extensive CAergic depletion throughout the brain and did not determine the relative importance of specific CAergic terminal fields. Fink and Smith did speculate that the forebrain CAergic terminal fields were probably the most important for the mediation of exploratory behaviour, since the mesolimbic and mesocortical areas receive dense CAergic innervation. They investigated this hypothesis by studying exploratory behaviour following DAergic depletion of mesolimbic and mesocortical neurones using 6-OHDA in the ALH (Fink and Smith 1980). In this study the NAergic neurones were protected from the toxic effects of 6-OHDA by pretreating the rats with desmethylimipramine (a NA reuptake blocker), allowing for the study of DAergic depletion alone. Again they reported a decrease in exploratory behaviour and extended this evidence by demonstrating that systemic doses of apomorphine restored levels of exploratory behaviour to novel stimuli. It would thus appear that the mesolimbic and mesocortical DA neurones are critical for the mediation of exploratory behaviour. However there are problems with this work, the major criticism being that 6-OHDA injected into the ALH depletes levels of DA in a large number of structures, including cortical areas, limbic areas, the NAcc, the CP, and elements of the thalamus and hypothalamus. Winn and Robbins (1985) drew attention to this problem in a study which attempted to clarify this issue. They also investigated the effects of 6-OHDA lesions to either the ALH or NAcc on locomotion, exploratory behaviour and body weight. Winn and Robbins (1985) found that rats with lesions of the ALH were hypoactive and showed blocked locomotor and stereotypy responses to amphetamine. The rats also displayed profound loss of body weight. Loss of body weight and the abolished stereotyped response to amphetamine are indicative of damage to the CP, which was borne out by the histological analysis. By contrast the rats with NAcc lesions displayed no weight loss, hypokinesia, or exploratory deficits, and the stereotypies in response to amphetamine remained unaltered. They did however exhibit a reduced locomotor response to
amphetamine. This study shows that mesolimbic dopamine depletion does affect locomotor activity produced by psychostimulants, but does not affect exploratory behaviour or body weight. It also contradicts the findings of Fink and Smith (1980) demonstrating that 6-OHDA lesions of the ALH produce a widespread non-specific DA depletion, from which it is impossible to conclude that specific DA target structures are responsible for the mediation of exploratory behaviour. From the evidence cited above it appears that the NAcc has no part to play in exploration. However the experiments conducted by Winn and Robbins (1985) are not above criticism, the main one being that the Carlson box is not the best behavioural method for investigating exploratory behaviour. Thus the exact role of the NAcc in exploration of novel environments remains uncertain.

4.2.2. NAcc and motivated behaviours

In addition to locomotor activity, the NAcc plays an important part in the acquisition of reinforcing behaviours such as intracranial self-stimulation, conditioned place preference and responding for conditioned reinforcement (Cador et al. 1991, Carr and White 1983, Taylor and Robbins 1984, 1986).

Intra-accumbens amphetamine will potentiate responding for conditioned reward which has become motivationally significant through prior association with a primary reinforcer such as food or water. That the NAcc is a critical substrate for the enhanced response to reward-related stimuli following stimulation by amphetamine, was shown by Taylor and Robbins (1984). Three groups of water deprived rats were trained on a conditioned reinforcement paradigm to associate a light with the delivery of water. In the test phase pressing one of two levers produced the light but no water (the conditioned reinforcer). During the test phase the rats were microinjected with different doses of amphetamine into either the NAcc, CP or medio-dorsal nucleus of the thalamus. Only the rats who received microinjections of amphetamine into the NAcc showed a dose-dependent increase in responding for the conditioned reinforcer. Taylor and Robbins (1986) confirmed this finding by making 6-OHDA lesions in either the NAcc or CP. Using a conditioned reinforcement paradigm, they investigated the effects of intra-accumbens amphetamine on the response rate for the conditioned reinforcer. They found that lesions in the NAcc attenuated the potentiated response normally seen following intra-accumbens amphetamine, while
lesions of the CP did not. Thus enhancement of responding for the conditioned reinforcer appears to be dependent on the DAergic activation of the NAcc and not CP. Kelley and Delfs (1991) also demonstrated that the NAcc was the most effective site for the enhancement of conditioned responding following injections of intra-accumbens amphetamine. They conducted a more extensive study, microinjecting amphetamine into seven different striatal subregions and investigating their effect in a conditioned reinforcement paradigm. They also found the NAcc to be the most effective site for the enhancement of response, though those with injections in the anterior dorsal striatum showed some enhancement to the highest dose of amphetamine. The other sites failed to elicit any enhancement of response. Kelley and Delfs (1991) concluded that sites within the striatum that are progressively further from the NAcc are ineffective in potentiating the response to a conditioned reinforcer, while those sites that are progressively nearer the NAcc support such enhancement.

The acquisition of behavioural responses to reward-related stimuli has been related to the mesolimbic dopaminergic system. DA (but not NA) receptors in the NAcc are believed to be one of the substrates for the rewarding properties of psychostimulants such as amphetamine and cocaine. This has been demonstrated by Cador et al. (1991) who conducted a series of experiments using the conditioned reinforcement paradigm to investigate whether NA had any role to play on the acquisition of responding. In the first experiment they compared similar doses of DA or NA injected into the NAcc, and found that while DA potentiated the rate of response NA had no effect. In a follow-up experiment they investigated whether NAergic innervation of the ventral striatum could be implicated in the increased response to intra-accumbens amphetamine. They did this since the 6-OHDA lesions characteristically made in the NAcc to demonstrate attenuated responding to conditioned reinforcement are not specific to DA neurones but also destroy NA and because amphetamine also affects NAergic transmission. Lesions were made in the dorsal noradrenergic bundle (DNAB) with 6-OHDA. In this area the 6-OHDA spares DA neurones but produces almost complete depletion of NA in the NAcc and frontal cortex. They found that these lesions did not affect the potentiated response to the conditioned reinforcer. The conclusion drawn from this work was that the potentiated response to conditioned reinforcers following amphetamine injected into the NAcc depends upon a dopaminergic substrate within the NAcc. Investigation into the role
of specific DAergic receptors on responding for conditioned reinforcement has also been investigated (Chu and Kelley 1992). Selective D1 and D2 DA receptor agonists were injected into the NAcc and were found to have no effect on the response for conditioned reinforcement. However if these agonists were administered together the rats showed a marked enhancement in their response. This study provides evidence that D1 and D2 DA receptors must be activated simultaneously, if they are to effect changes in responding for conditioned reinforcement (Chu and Kelley 1992).

There are also electrophysiological experiments looking at patterns of neural activity to reward-related responding. Electrophysiological recording has identified neurones within the ventral and dorsal striatum which respond to primary reward (Schultz et al. 1993). Single cell recording in macaque monkeys have shown that neurones in both the dorsal and ventral striatum will respond unconditionally to food or liquid reward which are delivered either during a trained behavioural task such as a delayed go-no-go task or independent of a behavioural task (Schultz et al. 1993). Neurones within the dorsal and ventral striatum will also respond to the expectation of reward during the delayed go-no-go task (see section 4.3.2. for a fuller description) and in anticipation to the initiation of responding for cocaine delivery (Carelli et al. 1993, Carelli and Deadwyler 1994; Chang et al. 1994). Conversely, mesencephalic A8, A9 and A10 DA neurones do not respond unconditionally to primary reward. Although these neurones will initially increase firing to primary reward, during the learning of a behavioural task the neural response is transferred from the primary reward to the conditioned stimulus (Schultz et al. 1993; Mirenowicz and Schultz 1994). It is hypothesised that these DAergic neurones do not respond to primary reward itself but to the salient properties of the stimulus which is coupled with the primary reward during task learning (Schultz et al. 1993; Mirenowicz and Schultz 1994).

Recent evidence suggests that the NAcc is not only involved in the mediation of reward-related behaviours, but is also the critical substrate for the mediation of conditioned avoidance (Rebec et al. 1992, McCulloch et al. 1993). Rebec et al. (1992) made ibotenic lesions in the ventral, medial and dorsolateral striatum of a group of rats and studied their effect on a signalled avoidance task where the rats had been trained to press a lever on hearing a signal to avoid receiving a foot-shock. They found that lesions of the ventral striatum only, affected the rats' response, by
decreasing the number of successful avoidances. This could not be explained by a locomotor deficit since the latency to respond on the lever following the signal was not affected. McCulloch et al. (1993) made 6-OHDA lesions of the NAcc in a group of rats that had been trained on a lever press avoidance task. In this task a 5s electric shock was presented every 30s which the rats could avoid by pressing a lever. They reported that following DAergic depletion in the NAcc, there was a significant decrease in the frequency with which the rats pressed the lever to avoid the shock. In another experiment, McCulloch et al. (1993) used in vivo dialysis to measure the amount of extracellular DA in the NAcc of a group of rats trained in the lever avoidance paradigm. They were compared with two groups of controls, one of which received periodic shock without previous training and the other group who received no shock. They found that the amount of extracellular DA and its metabolites increased linearly with successful performance on the lever avoidance task. Thus it appears that the NAcc is crucial in the mediation of motivated behaviour which can be driven either by positive or negative stimuli.

Rats will self-administer amphetamine and dopamine into the NAcc (Hoebel et al. 1983; Guerin et al. 1984), and can acquire conditioned place preference (CPP). Carr and White (1983) gave rats intra-accumbens injections of amphetamine prior to placing them in one of two chambers. The following day the rats received saline or no treatment and were placed into the other chamber. This procedure was continued 6 times, after which the rats were allowed free access to either chamber. They showed a significant preference for the chamber that had been paired with the amphetamine. This indicates that the effect of the amphetamine itself was rewarding since there was no other motivational factor such as food or water involved. The same procedure was then carried out with injections in the CP but the rats showed no preference for the chamber paired with the amphetamine. This shows again that the NAcc is the critical substrate for mediating the rewarding properties of amphetamine and that amphetamine not only potentiates the response to conditioned reinforcers but is also inherently rewarding in itself. Spyraki et al. (1982) conducted an interesting experiment using CPP. Food-deprived rats were divided into two groups, one received injections of saline just before training the other group was given injections of haloperidol, a DA antagonist. The rats were then placed in one of two chambers one with food in it or one without. This was repeated 8 times and on the test session
the rats were allowed to choose between the chambers. The rats who were trained under saline showed a CPP for the chamber where they had received food. The rats treated with haloperidol did not demonstrate any preference for the chamber where the food had been. Thus haloperidol blocked CPP, but it did not block motor activity since the rats treated with haloperidol, were able to eat the food in the chamber during the training sessions. This is interesting for it suggests that NAcc DA is involved in the mediation of reward but it may not be implicated in locomotion per se. The locomotor response shown to NAcc amphetamine may be the result of general activation, and the animal exhibits locomotor activity because there is nothing in the cage to which it may direct it's attention.

4.3. Functions of the CP
From the work of Gerfen (1990), described in chapter 1, it is clear that DA within the CP has differential modulatory effects upon activity within the striatonigral and striatopallidal pathways. Activation of the striatonigral pathway inhibits the SNr which results in disinhibition of its target nuclei, and thus outflow from sites in the thalamus, superior colliculus and pons are facilitated. The striatopallidal pathway has the opposite effect on the SNr leading to increased inhibition of its target nuclei (Gerfen 1990). It has been hypothesised that concurrent activation of the striatonigral pathway and inhibition of the striatopallidal pathway by DAergic stimulants increases the impact of cortically stimulated activity in the striatum which in turn facilitates transmission from thalamic nuclei to the frontal cortex and from the pontomedullary nuclei which then effect behavioural outflow (Robbins and Everitt 1992). From this functional organization questions arise as to which behaviours the CP could influence.

4.3.1. The CP and sensorimotor behaviours
As described above, the research of Kelly et al. (1975) demonstrate that one of the behaviours influenced by the CP is the mediation of stereotypies produced by stimulation of amphetamine. However, the research conducted by Kelly et al. (1975) constitutes only one example of the behavioural differences between the NAcc and CP. There are numerous other behaviours mediated by the CP. A number of studies have investigated the behavioural impairments resultant upon lesioning the nigrostriatal DA pathway with 6-OHDA. In contrast to the locomotion and
reinforcement attributed to the NAcc, DA in the CP is believed to have a role in sensorimotor processes. The deficits observed following unilateral lesion of the nigrostriatal DAergic pathway cannot be categorised as simply motor or sensory in character but have been interpreted as a failure to co-ordinate information about sensation with an appropriate motor response (Turner 1972; Blackburn et al. 1992; Dunnett and Robbins 1992). That DA depletion does not interfere with motor responding per se is evidenced by the fact that animals can still exhibit escape behaviours and perform forced swim tasks following striatal DAergic depletion (Arnt 1982; Beninger et al. 1989; Keefe et al. 1989). Turner (1972) and Marshall and Teitbaum (1974) provided evidence for the sensorimotor nature of these deficits by showing that rats with electrolytic lesions could still perceive sensory stimuli on the side contralateral to the lesion, for example they could vocalise, close their eye in response to tactile stimulation of the contralateral side of the head, and the pupil of the contralateral eye reacted following presentation of a bright light. However the rats failed to orient towards any contralateral stimuli though they were not simply motorically unable to do so since they could still use the limbs contralateral to their lesion during activities such as locomotion and grooming.

This type of sensorimotor impairment was also described following lesions in the medioventral striatum (Dunnett and Iversen 1982a). Unilateral lesions were made in 5 different striatal areas using 6-OHDA or kainic acid and their effect studied on a battery of sensorimotor tests developed by Marshall and Teitbaum (1974) which investigate sensorimotor orientation and co-ordinated limb use on each side of the body. Rats with lesions of the medioventral striatum showed a long-lasting deficit in sensorimotor orientation which was characterised by neglect of stimuli presented to the side contralateral to the lesion. All other lesions produced a transient deficit which disappeared within a month of surgery. No impairments were reported on the co-ordinated limb use test in any rat. Thus it appears that lesions of the medioventral striatum can produce sensorimotor impairments, while other parts of the striatum remain unaffected.

Many authors feel that the term 'sensorimotor' which is readily used to describe the behavioural functions of the CP is too indiscriminate a label, since many aspects of behaviour can be encompassed under this umbrella term (Carli et al. 1989; Dunnett and Robbins 1992). For example the failure to orient towards a unilateral
stimulus could be due to unilateral neglect of the side contralateral to the lesion, a failure to co-ordinate this information with an appropriate response, or a failure in the initiation of the response (Dunnett and Robbins 1992; Blackburn et al. 1992). Thus many recent experiments have concentrated on refining the definition of the effects produced by both stimulation and lesion studies which investigate the role of the CP in different experimental paradigms.

4.3.2. The CP and cognitive processes

In addition to the sensorimotor functions attributed to the CP, a role in cognitive functioning has also been hypothesised. There is a large body of evidence showing that lesions confined to specific parts of the CP that receive afferent innervation from specific parts of association cortex cause the same disruption on cognitive tasks as do lesions in these cortical areas (Dunnett and Robbins 1992). Loss of DA has been shown to result in deficits in establishing and switching attention, in the acquisition and retention of visual discrimination tasks and in some forms of memory (Robbins and Everitt 1987; Robbins et al. 1990; McDonald and White 1993).

Neurones in the putamen and caudate nucleus of primates have been shown to fire while performing on a go/no go response paradigm (Apicella et al. 1992). In this procedure, monkeys were trained to hold one hand still on a touch-sensitive lever. Presentation of a light stimulus cued the animal to respond or withhold a response following presentation of the trigger-stimulus, which was presented after a random delay. If the cued stimulus was green, the monkey was required to make a response, if red the monkey was to withhold the response. On presentation of the trigger-stimulus, a yellow light, the monkey had either to respond appropriately on the test lever or keep his hand on the touch-sensitive lever. If the response was correct the monkey received a juice reward. The cued stimulus, therefore served two functions. First it provided the monkey with prior knowledge as to which response was required for the reward. Second, it led to the expectation that the trigger-stimulus would appear, while the trigger-stimulus in turn led to the expectation that a reward would occur. Thus it could be argued that training on this task led to learning of a predictive sequence of events, on which the animal could base it's expectation of a subsequent event occurring. Presentation of one stimulus, would lead to the activation of a memory store which would predict the occurrence of the next event. In addition to
those neurones which fired prior to the initiation of movement (which shall be discussed in section 4.3.3.) a number of neurones were found to fire in relation to the expectation of an event happening. The authors argued that these could be distinguished from those neurones which fired prior to making a movement response. This neural activity was reported as being unrelated to muscle activity, but possibly connected to memory for the task conditions which were shaped by prior experience (Apicella et al. 1992).

There is further evidence that the CP could have a role in simple stimulus-response pairings. Rats with electrolytic or neurotoxic lesions of the CP have been shown to be impaired on a win-stay memory paradigm (McDonald and White 1993). Food-deprived rats were placed in an 8 arm radial maze, where 4 arms were baited and cued by light illumination. The rat was required to collect these food pellets and the same arms of the maze were then rebaited. Once the rat had retrieved the second food pellet, the light in the arm was switched off, and the experiment terminated when all the baited arms had been visited a second time and the food pellets retrieved. That the deficit was not one of motor or motivational impairment was shown by the fact that the lesioned rats performed normally on a win-shift task which also used the 8 arm radial maze. In this experiment however, the rat was required to learn that it would receive a food reward on visiting each arm of the maze only once. Visually salient extra-maze cues were placed on the walls of the testing room, which the rat could use to help discriminate which arms it had already visited. The win-shift paradigm investigates spatial memory, since the rat is required to learn the relationship between several visual cues and each maze arm. The authors argue that this type of task is not a simple stimulus-response task, since adjacent arms of the maze have some of the visual cues in common, and therefore for successful performance of the task several visual cues are required to be associated with any particular arm. In comparison, successful performance on the win-stay task requires “habit” memory, since the rat is required to learn the association between the appearance of the light and making the required behavioural response which is then reinforced by food reward. This is a stimulus-response task, which is independent of the spatial location of the maze arms, in fact for each trial different arms of the maze were used. What this experiment failed to distinguish between is whether the deficit on the win-stay task is due to a failure to acquire the stimulus-response association,
or whether the rats have acquired the task but are unable to express the appropriate behavioural response (McDonald and White 1993).

The CP may also have some role in the facilitation of memory for learned events (Carr and White 1984). Water deprived rats were given access to a water tube in a test chamber and during the time spent in the chamber, a tone was presented 5 times. The rats were placed 24h later into a different chamber where the tone was paired with an electric shock delivered through the grid floor. Two groups of rats received 2 tone-shock pairings, and immediately following the task they received injection of amphetamine or saline directly into the dorsolateral CP. Another group of rats received 12 tone-shock pairings but did not receive any treatment. After another 24 h the rats were exposed again to the test chamber, and the effect of the tone on the suppression of drinking investigated. Carr and White (1984) discovered that the rats who had received 12 tone-shock pairings suppressed their drinking when the tone was presented, while the rats which received 2 tone-shock pairings and the saline injection did not. However the rats which received amphetamine directly following the 2 tone-shock pairings, suppressed their drinking in the same way as the untreated group. This result was interpreted as indicative of amphetamine having an enhancing effect leading to the improved consolidation of memory for learned events (Carr and White 1984). Administration of direct DA agonists had a similar effect (Packard and White 1990).

Dunnett and Iversen (1982b) investigated the effects of 6-OHDA lesions of the lateral striatum on acquisition and performance on both continuous reinforcement and differential reinforcement of low rates (DRL) of responding paradigms. In the continuous reinforcement task, food deprived rats were trained to press a lever to obtain a food reward, while in the DRL task, the rats were required to allow for a delay of at least 20 sec before pressing the lever to obtain food. The lesioned rats were not impaired on the acquisition or the performance of the continuous reinforcement task. However they did show impairments in acquisition and performance on the DRL task. Control rats successfully inhibited their response on the lever for the required delay, with the probability of their responding on the lever low when the inter-response time was low. As the 20 sec inter-response time elapsed the probability of their responding on the lever increased. Although the lesioned rats could withhold their responding, they were less successful at doing so and showed an
increased probability of responding on the lever when the inter-response time was low, though their performance was no different to controls as the inter-response time increased. Dunnett and Iversen (1982b) argued that the lesioned rats did not have a motivational deficit since they were not impaired on the acquisition or performance of the continuous reinforcement schedule. They interpreted the lesioned rats failure to withhold their response as a cognitive deficit in either the temporal ordering of their reactions or as a failure to switch their strategy to one that was appropriate to the task requirements.

Rats with 6-OHDA lesions which reduced DA in the CP were also shown to have deficits in both the acquisition of and performance on a conditioned discrimination task (Robbins and Everitt 1987). In this experimental paradigm, the rats were trained to respond for a reward by pressing one of two levers. Their response on the right or left lever was determined by the presentation of either a fast or slow light (or tone) stimulus, which they were required to discriminate in order to respond on the correct lever. Following lesions of the CP, rats were impaired on the acquisition of the discrimination task, and also on maintaining the discrimination performance if previously trained on the task to set criterion levels (Robbins and Everitt 1987). In a similar experiment lesions of the CP, or receptor blockade with DAergic antagonists, were also found to impair acquisition and performance on a visual discrimination task, with rats also showing slowed responses in both pressing on the levers and in collecting food reward (Robbins et al. 1990). Interpretation of these data are difficult but could be explained by a combination of impairments in sensorimotor, motivational or attentional integration (Robbins and Everitt 1987; Robbins et al. 1990).

Two similar experiments by Carli et al. (1984, 1989) also looked at the effects of CP lesions on performance on a previously learnt discriminative paradigm but came to different conclusions to the experiment described above. Carli and her colleagues trained rats to hold their heads in a central position on either side of which were two holes in the apparatus. On the presentation of a brief and unpredictable visual stimulus, the rats had to either respond by poking their nose into the hole on the side the stimulus was presented (same discrimination) or poke their nose into the hole contralateral to the stimulus presentation (opposite discrimination). Rats then received unilateral 6-OHDA CP lesions. Following the lesions the rats showed a
pronounced bias of responding towards the side ipsilateral to the lesion, regardless of the prior training received or the side of the presentation of the stimulus. The rats could make head movements contralateral to the lesion but were slower to initiate these, though once initiated there was no difference in the time taken to execute the movement. Thus rather than a deficit in making the discrimination, the rats showed a deficit in the initiation but not the execution of movement in response to goal-directed stimuli (Carli et al. 1984, 1989).

4.3.3. The CP and the initiation of movement

The CP is part of the basal ganglia thalamocortical motor circuit which receives cortical input from motor, premotor and sensory cortex, and projects via the pallidum and thalamus to the supplementary motor area (for references see chapter 1). This circuit has a role in the preparation and execution of motoric behaviours, which leads to a readiness to respond or 'response set' (Robbins and Everitt 1992).

Support for an initiation impairment like that described by Carli et al. (1984, 1989) comes from a number of studies. Dunnett and Bjorklund (1983) investigated the effects of unilateral 6-OHDA lesions of ascending forebrain DA and NA neurones on conditioned rotation. They trained water deprived rats to rotate either clockwise or anti-clockwise to obtain a reward of sugar-water. Lesions were then made either contralateral or ipsilateral to the side the rats was trained to rotate around. They found that rats who had lesions ipsilateral to the side of rotation, still performed at pre-operative levels for the sugar-water, whereas rats who received lesions contralateral to the side of rotation were impaired. If the task was reversed, requiring the rats with the contralateral lesions to turn ipsilateral to the lesion, and vice versa, they found that the impaired rats rapidly learnt and were able to turn ipsilateral to the lesions to obtain sugar-water, while the previously unimpaired rats failed to learn to turn contralaterally to the lesion to obtain the reward. However, in both groups, once the rat had initiated turning to the side contralateral to the lesion, the speed and execution of the movement was normal. Dunnett and Bjorklund (1983) interpreted these data, as indicative of the rats suffering from an initiation impairment. They argued that the deficit was not the result of a primary motor impairment since once initiated the rats could move normally toward the side contralateral to the lesion. Nor was the deficit one of motivation since the rats who were trained to respond
ipsilateral to the lesion showed no impairments in turning for reward, while those rats that were initially trained to turn contralateral to the lesion, rapidly learnt the reversal condition. This initiation impairment was attributed to loss of striatal DA since rats with various levels of damage to NAergic neurones, showed similar impairments on the task, while those rats with less than 85% striatal DA loss were not impaired.

There are several, more recent studies that have also indicated a problem of initiating movement following DA depletion. For example Amalric and Koob (1987) investigated reaction time performance following either DAergic depletion or administration of a dopamine antagonist in the CP or NAcc. The rats were required to press a lever until a light appeared when they had to release the lever immediately to gain a food reward. Rats were trained to criterion on this task prior to surgery or administration of the antagonist. Following either experimental manipulation to the CP but not NAcc, the rats showed a decreased number of correct responses and an increased latency to release the lever. This increase in reaction time is also suggestive of an initiation deficit (Amalric and Koob 1987).

That rats with unilateral striatal DAergic depletion show deficits in the initiation of movement in the absence of deficits in the perception of cues or execution of movement was elegantly demonstrated by Brown and Robbins (1991). They trained rats to perform on simple and choice reaction times to investigate whether loss of striatal DA resulted in differential differences between the two types of reaction-time task. Rats were trained to place their noses into a hole which initiated the presentation of a bilateral light stimulus. The rats were trained to respond by poking their noses into one of two adjacent holes, with the brightness of the stimuli dictating which hole they were to respond to. Rats were trained to make the response immediately on the presentation of an auditory tone, which was presented at randomly varied delays. In the simple reaction time, the lights were presented prior to the tone, allowing the rat prior warning as to which direction to respond. In the choice reaction, both the light and tone stimuli were presented simultaneously. A number of measures were recorded including the number of correct trials, the time taken to respond to the tone and the effect of the different delays in presenting the stimuli. Preoperatively, rats showed a longer reaction time to the choice reaction task than to the simple reaction task, and achieved more correct responses following the simple reaction task. In addition the speed of the reaction time increased as a function
of increasing the delay for the presentation of the stimuli. This is attributed to the increasing 'readiness to respond' as the probability for the occurrence of the stimuli appearing increases as time elapses. Following unilateral DA depletion which predominantly involved loss of DA in the CP, rats showed an interesting pattern of performance. Postoperatively, the rats still showed increased reaction times to the choice compared with the simple reaction task, and increasing the length of the stimulus delay increased the speed of the reaction time. However when looking at the side of response some differences appear. When rats were required to respond ipsilateral to the lesion regardless of task, they still showed the increased speed of reaction time as the stimulus delay increased, however this did not occur when the rats were required to make a response contralateral to the side of their lesion. As the stimulus delay increased the reaction time to make a contralateral response was much slower than for the ipsilateral response. Response accuracy was not affected by the lesion, and rats remained more accurate at responding to the simple than the choice reaction task, indicating that the lesions did not affect the ability to perceive and use the prior information provided in the simple reaction task. The time taken to make a movement, either ipsilaterally or contralaterally was also unaffected by the lesion, indicating that a purely motor deficit is unable to explain the slowness in reaction time towards the contralateral side despite the increased delay in stimulus presentation. Brown and Robbins (1991) explained this failure to show an increase in the speed in reaction following increasing stimulus delay as a deficit in internally generated motor readiness, in other words a deficit in the increased readiness to generate a motor response. Primates with MPTP lesions also show deficits in reaction times for the initiation of movement linked to environmental stimuli and in the initiation of spontaneous movement (Schultz et al. 1989). This agrees with the Parkinsonian literature (Robbins and Brown 1990) and the above experiments, which describe deficits in the initiation of voluntary movement.

Electrophysiological studies lend credence to a role for the CP in the initiation of movement. Excitation of neurones in discrete areas of the primate putamen result from movement of separate parts of the body (Alexander & DeLong 1985) while individual body parts are represented in the dorsolateral CP of rats, though a number of neurones in this area respond to whole body movement (Carelli and West 1991). Single unit recording of neurones in the primate putamen have been shown to respond
prior to the execution of movement (Schultz et al. 1989; Apicella et al. 1992; Schultz and Romo 1992). In an electrophysiological study, the activity of neurones within the primate caudate nucleus were recorded (Rolls et al. 1983). It was found that few neurones in this area could be classified as sensory or motor, though neurones which responded to movement were found in the putamen. However a large number of neurones did respond to environmental cues which the monkeys were able to use to prepare a response in a given task. Schultz and Romo (1992) recorded neurones within both the primate caudate nucleus and putamen in both an environmentally cued task, the delayed go/no go task described above, and following self-initiated behaviours which were not linked to external stimuli. They found that 33% of the neurones recorded in both areas fired in response to the initiation of movement in either condition. However the other 66% recorded fired only to self-initiated movements. Thus it would appear that neurones within the CP respond in relation to environmental cues which are used in preparation of the initiation of movement, though they could also be involved in directing attention or in memory functions (Rolls et al. 1983; Shultz et al. 1989; Schultz and Romo 1992).

The hypothesis that the CP is involved in the initiation of movement and the generation of learned response set is supported by the several electrophysiological experiments which show that neurones within the primate CP respond preparatory to the initiation of movement, and also by the CP's place within the basal ganglia thalamocortical motor circuit which has a role in the planning, initiation and execution of movement. From the experimental evidence described above it appears that the role of DA in the CP is a facilitatory one which may function to increase motor readiness for the preparation of movement, rather than affect motor performance per se.

4.3.4. The CP and oral motor behaviours

Feeding can be stimulated by injections of low doses of amphetamine either systemically or directly into the CP (Winn et al. 1982; Kelley et al. 1989a). However, ventral and lateral parts of the CP have also been shown to have a more specific role in oral motor behaviours which is consistent with the work of Alexander and DeLong (1985) who showed that the primate putamen is topographically organised with neurones in the dorsolateral part responsive to movements of the leg, ventral parts
responding to orofacial movements, and neurones located in-between responsive to arm movements. Electrophysiological recording in the rat CP has also demonstrated a dorsoventral topography, with neurones in the dorsolateral striatum responsive to limb movement, while neurones lying ventral and lateral to these neurones respond to movement of the head and face (Carelli and West 1991). Furthermore single unit recordings in awake freely moving rats demonstrated that neurones in the ventrolateral part of the striatum fired in response to orofacial behaviours (Mittler et al. 1994). The majority of neurones recorded responded only in relation to licking movements while others responded both to licking and to at least one other orofacial movement such as sniffing or mouth movements (Mittler et al. 1994).

These data correspond well to a lesion study of the lateral striatum conducted by Pisa (1988). He reported that ibotenate lesions of the lateral striatum resulted in deficits in tongue use and forelimb reaching in rats. Pisa (1988) presented food-deprived rats a spatula covered with mash food which they were allowed to lick for one minute. Rats with ventral lesions of the lateral striatum showed deficits in tongue reaching, specifically with regard to tongue protrusion and the relation of tongue protrusion to head positioning. Reaching and grasping of food pellets was also investigated and rats with dorsal lesions of the lateral CP had impairments in the coordination of reaching and grasping for the food. Pisa (1988) drew attention to the similarities of these sensorimotor impairments to the deficits observed following damage to the sensorimotor cortex.

Injections of amphetamine directly into the ventrolateral caudate putamen (VLCP) produces long-lasting, intense oral stereotypies, in particular, licking, biting and self-gnawing (Kelley et al. 1988; Dickson et al. 1994). A subsequent study investigated the effects of amphetamine injected directly into either the dorsolateral caudate putamen (DLCP) or VLCP on locomotion and feeding behaviour (Kelley et al. 1989a). Spontaneous locomotion did not occur following injection into the DLCP or VLCP. A slight non-significant increase in feeding bouts was observed following injection into the VLCP but not the DLCP. The most interesting result was the increase in stereotyped oral behaviour elicited from the VLCP. At the highest dose of amphetamine intense self biting of the forepaws was observed, as was an increase in the incidence of gnawing and licking. No such stereotyped behaviours were observed from the DLCP. The lack of behavioural responses elicited from the DLCP was most
probably due to the fact that the behaviours studied were not appropriate to demonstrate the functions of the DLCP (Kelley et al. 1989a). In a further study to clarify the underlying neural substrate of the pronounced oral stereotypies elicited from the VLCP, Delfs and Kelley (1990) conducted an experiment which investigated whether the activation of a particular DA receptor subtype (D1 or D2) was responsible for the elicitation of these oral stereotypies since it is hypothesised that D1 and D2 DA receptors may have different effects on the control of striatally mediated behaviours (Gerfen et al. 1990). Delfs and Kelley (1990) used specific receptor agonists and antagonists to study this question and concluded that simultaneous activation of both D1 and D2 receptors was required to elicit intense oral stereotypies within the VLCP. Furthermore this DA-mediated stereotypy itself appears to be affected by glutamatergic projections to the VLCP which could arises from cortical, thalamic or limbic areas (Kelley and Delfs 1994). The influence of glutamatergic innervation on DA-mediated stereotypy was shown by pre-treatment of the VLCP by injection of excitatory amino acid antagonists, which work at NMDA, AMPA, kainate or quisqualate receptor subtypes, prior to infusion of amphetamine. This reduced or blocked amphetamine-induced stereotypy (Kelley and Delfs 1994). These data also fit with anatomical studies which have identified converging synaptic innervation from mesencephalic DA-containing and cortical glutamate-containing neurones onto the dendritic spines of striatal output neurones, and suggests that the modulatory effect of DA works to potentiate glutamatergic excitation of the same striatal neurones. Injection of cholinergic agonists into the VLCP also induces chewing-like mouth movements, but unlike those stimulated by amphetamine, these are not directed towards any particular target, but are vacuous in nature (Kelley et al. 1989b). This experiment is of interest since chronic administration of neuroleptics can cause abnormal oral motor side effects in rats which can be prevented by administration of anticholinergic drugs (Rupniak et al. 1983).

The above experiments suggest that in the rodent the lateral striatum influences oral motor behaviours with the VLCP subregion playing a primary role, and that the rodent VLCP could form a homologue of the primate ventral putamen.
4.4. Combined functional role for NAcc and CP

The anatomical distinctions that characterise the afferent and efferent connections of the NAcc and CP are paralleled by functional differences. However the notion of the NAcc and CP as being entirely distinct from one another is perhaps too extreme. Some behaviours seem to depend upon recruitment of both parts of the striatum. In his overview of the functions of striatal DA Salamone (1992) argued that "it should also be recognized that there is considerable overlap between motivational and motor processes...Broekkamp et al. (1977) stated that it was arbitrary to draw an absolute dichotomy between brain mechanisms involved in motor control and motivation." (Salamone 1992, p165).

One of the behaviours which is dependent on simultaneous recruitment of both the CP and NAcc is rotation (Dunnett and Robbins 1992). Rats bearing unilateral 6-OHDA lesions will show postural asymmetry towards the lesioned side. When stimulated with systemic amphetamine the rat will show rotational behaviour that is ipsilateral to the side of the lesion. This is explained by the fact that amphetamine operates at presynaptic DA terminals, which are absent on the lesioned side. Therefore the amphetamine stimulates the unlesioned striatum, and ipsilateral rotation is the result. Following doses of apomorphine rotation contralateral to the side of the lesion is stimulated due to postsynaptic DAergic receptor supersensitivity on the lesioned side (Dunnett and Robbins 1992). It has been hypothesised that both the CP and NAcc are required to produce these rotational effects, with the CP contributing the postural asymmetry which results in rotation, while stimulation of the NAcc is required for the activation of locomotor activity. Support for this hypothesis comes from a study where systemic amphetamine, but not apomorphine will produce rotation in rats with unilateral CP injections. However if this lesion is combined with bilateral lesions of the NAcc rotation will be produced following administration of apomorphine but not amphetamine (Dunnett and Robbins 1992).

However the interaction of NAcc and CP in behaviour is often even more complex. The CP appears to be involved in preparation for action and the generation of learned sequences of movement (response set), while the NAcc appears to be involved in the mediation of reward-related information. Another hypothesised function of the CP is that of the mediation of habitual responding, when a learned response becomes so well trained that it is automatically generated (Marsden 1982;
Reading and Dunnett 1991; Apicella et al. 1992; McDonald and White 1993). Training in a conditioned behavioural task incorporates both motivational features as well as the learning of a sequence of motor responses (Salamone 1992).

An experiment looking at the effects of NAcc lesions on a delayed matching to position paradigm (DMTP) provides evidence for this kind of interaction between the NAcc and CP (Reading and Dunnett 1991). Food-deprived rats were trained to press on a lever when it was inserted into an operant chamber. They were rewarded by the delivery of food which they could then collect by pushing back the panel covering the food chamber. Once they had achieved a good level of performance the task was modified. The rat was required to press on the lever when it entered the chamber, a nose poke at the food panel, instead of delivering food, resulted in two levers being inserted into the cage. The rat then had to press the lever which had previously appeared in the cage to obtain reward (matching to position). Once they reached a criterion of $>90\%$ correct responding, a random delay between the disappearance of the first lever and introduction of the two levers was instigated (DMTP). Rats with bilateral ibotenate lesions of NAcc were much slower than controls to extinguish their responding on DMTP tasks, when reward for correct trials was no longer available. Thus the lesioned rats showed deficits in altering their behavioural response when the task was no longer rewarding (Reading and Dunnett 1991). This could be explained by the fact that the motivational part of the task, mediated by the NAcc had become uncoupled from the habitual response mediated by the CP and therefore the animal continued to respond even in the absence of reward. This provides evidence for the requirement of striatal interaction in performance on a conditioned response experiment. The NAcc is primarily important for learning about the incentive value of conditioned reward, but then as the response becomes well trained the CP which is involved in turning learned responding into habitual responding becomes involved in generating the behavioural response set for the task. Thus the habitual response necessary for the task becomes uncoupled from reward relevant information which is why responding can still occur in the absence of reward.

"After relatively stereotyped tasks are sufficiently well trained, the information concerning external signals, required behavioural reactions and occurrence of reward is thought to be centrally represented in a noncognitive, procedural form"
closely linked to the way it is used for task performance" (Apicella et al. 1992, p958).

4.5. Summary

The striatum has a complex role in the mediation of many different behaviours including locomotor activity, responding for conditioned reinforcement, conditioned avoidance, response switching, feeding, sexual behaviour, associative memory, initiation of movement and oral motor behaviours. An exhaustive review of all these behaviours, and indeed the many others that the striatum appears to have a role in, is outwith the scope of this chapter, as is a review of the third major ascending DAergic pathway which arises from the A10 DA neurones and innervates the frontal cortex. This mesocorticolimbic system is hypothesised to have a role in cognitive processes. For more extensive descriptions of this system and the other behavioural roles of the striatum, the reader is directed to reviews by Robbins and Brown (1990), Blackburn et al. (1992), Dunnett and Robbins (1992), Robbins and Everitt (1992), and Amalric and Koob (1993). However a word should be said about the role of DA in many behaviours. This chapter describes some of the diverse behaviours that the striatum helps mediate. It’s role is complex: for example DA seems to be preferentially involved in responding for secondary reinforcers (conditioned reinforcement) rather than in primary reinforcers (Blackburn et al. 1992). Responding for secondary reinforcers in appetitive experimental designs, is more sensitive to DAergic facilitation and disruption than responding to primary rewards (Robbins et al. 1989; Salamone 1992). There is also evidence that DAergic antagonists disrupt conditioned avoidance but have little effect on escape behaviours (Arnt 1982; Beninger et al. 1989). Furthermore DA appears to have a more fundamental role in controlling initiation and preparatory behaviours rather than in consummatory behaviours or the execution of motor behaviours (Blackburn et al. 1992; Dunnett and Robbins 1992). For example the initiation of feeding but not the latency to feed is disrupted by DAergic antagonists (Wise and Colle 1984; Hoffman and Beninger 1986), while the initiation of movement but not the ability to execute the movement once initiated is impaired following DAergic lesions (Dunnett and Bjorklund 1983; Amalric and Koob 1987; Brown and Robbins 1991).
It is difficult, if not unrealistic, to attribute many different behaviours to the activity of one neurotransmitter, when a number of other transmitters not only input to the striatum, but also exist within the striatal interneurones, and there is differential distribution of DAergic receptors on the efferent striatal projections (Brown and Robbins 1990; Blackburn et al. 1992). However the role of DA in the striatum could be generalised as being one of facilitation, which enhances behavioural activation through its connections with numerous other structures within the brain, and which at a behavioural level increases an animal’s behavioural response to both internal and externally guided stimuli (Blackburn et al. 1992, Dunnett and Robbins 1992; Salamone 1992). Furthermore, for many tasks, especially those that require a complex response, the production of co-ordinated, appropriately generated behaviours relies upon the recruitment and interaction of a number of striatal areas, which make various contributions to the establishment and generation of appropriate behavioural sequences.
Chapter 5. General behavioural functions of the pedunculopontine tegmental nucleus (PPTg).

Cholinergic neurones form a diffuse neuronal network in the central nervous system. Their influence is extensive in both the somatic and autonomic nervous systems and they contribute to a great many functions such as memory and the sleep-wake cycle (Woolf 1991). PPTg cholinergic neurones are part of this ascending reticular activating system (ARAS), which is defined by its electrophysiological properties and has general excitatory functions (Morruzzi and Magoun 1949). The ARAS is thought to mediate complex behaviours such as attention, arousal and readiness to respond (Morruzzi and Magoun 1949). The actual role of the PPTg in the ARAS has not yet been clearly defined, though it is hypothesized that it may influence complex behavioural states by affecting thalamic nuclei which process sensory information. The PPTg has been ascribed several different functional roles.

5.1. PPTg and the sleep/wake cycle

The role of PPTg cholinergic neurones in the control of the sleep/wake cycle has received considerable attention in recent years. The sleep/wake cycle can be divided into three basic components; waking, slow wave sleep and rapid eye-movement sleep (REM). Slow wave sleep is characterised by rhythmic thalamocortical oscillatory activity while waking and REM sleep are characterised by thalamic single-spike firing (Woolf 1991). REM sleep, also called paradoxical sleep, is further characterised by EEG desynchronisation, muscle atonia, rapid eye-movements and ponto-geniculo-occipital spikes (PGO spikes) (Rye et al. 1988; Sakai 1988; Semba et al. 1990).

Descending PPTg cholinergic projections to the pontine reticular formation (PRF) are associated with the control of REM sleep, including muscle atonia and state-dependent respiratory depression present during REM (Rye et al. 1988; Sakai 1988; Lai and Siegel 1990; Semba et al. 1990; Lydic and Baghdoyan 1993). Approximately 13-31% PPTg cholinergic neurones project to the PRF and the majority of these neurones collateralise to innervate the thalamus (Semba et al. 1990).

Many studies support a cholinergic mediation of REM sleep arising from the pontomesencephalon. Kainic acid lesions within the pontomesencephalon, which destroyed the cholinergic neurones of both PPTg and LDTg, have been reported to
disrupt REM sleep states in cats (Webster and Jones 1988). Following the lesion the
time the cats spent in REM sleep was reduced as were the number of PGO spikes.
Muscle atonia was absent and fewer eye movements were made during REM sleep.
However interpretation of these data as being specifically linked to a lack of
acetylcholine is difficult due to the enormous size of the lesion that also led to damage
in several other structures including the parabrachial nuclei, locus coeruleus, central
tegmental field and central gray which contain different neurochemical populations.
However other evidence is available. For example injection of the cholinergic agonist
carbachol, into the pontine gigantocellular field induces a REM sleep-like state in
awake freely moving cats (Quattrochi et al. 1989). Retrograde labelling traced the
source of cholinergic input into this site to the PPTg and LDTg (Quattrochi et al.
1989). Injections of carbachol into the region of the PPTg also induced dose
dependent REM sleep and could be blocked by simultaneous administration of
atropine, a muscarinic antagonist (Gnadt and Pegram 1986; Sakai 1988; Vanni-
Mercier et al 1989).

In addition to the cholinergic control of REM sleep which can be elicited from
the PRF, cholinergic involvement of state-dependent respiratory depression which
also accompanies REM has also been demonstrated (Lydic et al. 1991; Lydic and
Baghdoyan 1993). The area involved in state-dependent respiratory depression has
been localised to neurones of the gigantocellular tegmental field which receive
cholinergic input from the PPTg (Quattrochi et al. 1989). Direct electrical stimulation
of the PPTg has been shown to elicit the release of acetylcholine in the gigantocellular
reticular field while simultaneously promoting state-dependent respiratory depression
(Lydic and Baghdoyan 1993). The decrease in muscle atonia reported following
lesions of the pontomesencephalon (Webster and Jones 1988) may also be mediated
via the cholinergic PPTg. Injection of acetylcholine or carbachol into the caudal
medulla has been shown to promote muscle atonia in cats (Lai and Siegel 1990).

*Ascending* cholinergic projections from the PPTg modulate oscillatory activity
in the thalamus associated with slow wave sleep (Woolf 1991). Oscillatory activity
generated during slow wave sleep is stimulated from the reticular thalamic nucleus
and the waves recorded there are termed spindle waves (Steriade et al. 1987). During
waking and REM sleep these spindle waves are replaced by single spike firing.
Stimulation of the peribrachial region, which includes both PPTg and LDTg neurones,
results in inhibition of the spindle waves in the reticular thalamic nucleus. This can be blocked by peripheral administration of scopolamine, a cholinergic antagonist which acts at muscarinic receptors (Hu et al. 1989). Furthermore in vivo microdialysis in the thalamus recorded high levels of acetylcholine during waking and REM sleep, but not during slow-wave sleep, and this acetylcholine release was traced to the cholinergic innervation from the PPTg (Williams et al. 1994). Cholinergic neurones also mediate ponto-geniculo-occipital (PGO) spikes; fast neural potentials which occur during REM sleep. The dorsolateral geniculate nucleus which lies adjacent to the reticular thalamic nucleus receives a direct projection from the PPTg cholinergic neurones, and this pathway is believed to transmit the PGO spikes (Nelson et al. 1983; Jones 1991; Hobson et al. 1993). Since PPTg cholinergic neurones collateralise to innervate both the PRF and thalamus, it has been suggested that these cholinergic collaterals may function to mediate REM sleep in the PRF while simultaneously blocking slow wave sleep by suppressing thalamocortical oscillation (Steriade et al. 1990; Hobson et al. 1993).

While pontine cholinergic mechanisms are implicated in the REM sleep, basal forebrain-cortical cholinergic projections have been associated with waking and arousal. ‘Arousal’ is used here to mean cortical EEG desynchronisation (Robbins and Everitt 1982; Semba 1991). Stimulation of basal forebrain neurones leads to the release of acetylcholine in the cortex which enhances both sensory processing and EEG output (Semba 1991). The PPTg and sites within the basal forebrain have reciprocal connections, and approximately 8% of cholinergic PPTg neurones are collateralized projections to the thalamus and basal forebrain (Losier and Semba 1993). Both the basal forebrain and thalamus are believed to have a fundamental role in cortical activation and arousal. An interaction between arousal and REM sleep influenced by cholinergic mechanisms was identified by Baghdoyan et al. (1993). They reported that carbachol injected into the basal forebrain of awake freely moving cats increased wakefulness, while carbachol injected into the pontine gigantocellular field induced REM sleep. However simultaneous injection of carbachol into both the basal forebrain and pontine gigantocellular field, reduced the amount of REM sleep compared to a single injection into the pons alone. The exact mechanisms through which stimulation of the basal forebrain can partially inhibit REM sleep are unknown, but it is possible that the stimulation within the basal forebrain creates a state of
arousal which inhibits cholinergic output from the PPTg to the pontine gigantocellular field, thereby suppressing REM sleep. Conversely stimulation of the PPTg may function to inhibit thalamocortical oscillation and basal forebrain activation via its ascending collaterals, while simultaneously enhancing REM-sleep (Steriade et al. 1990; Hobson et al. 1993). Reciprocal inhibitory effects of basal forebrain neurones and PPTg neurones are supported by studies which show that acetylcholine inhibits cholinergic PPTg neurones (Leonard and Llinas 1994), and it also inhibits basal forebrain neurones in an *in vitro* preparation (Khateb et al. 1991). The inhibitory actions of PPTg ACh may also be mediated via an indirect action on GABAergic receptors in the basal forebrain which in turn inhibit cholinergic basal forebrain neurones (Bertorelli et al. 1991).

However, acetylcholine is not the only neurotransmitter that has influence over the sleep/wake cycle. A noradrenergic (NAergic) role in the mediation of wakefulness is also well documented (Robbins 1984; Hobson et al. 1993). NAergic neurones of the locus coeruleus (LC) and serotonergic neurones (5-HT) within the dorsal raphe nucleus (DRN) are active during waking, decrease their rates of firing during slow wave sleep and are completely inhibited during REM sleep (Hobson et al. 1975; McGinty and Harper 1976; Aston-Jones and Bloom 1981; Lydic et al. 1987). The 5-HT neurones have an inhibitory effect on the cholinergic neurones of the PPTg/LDTg in the guinea pig (Leonard and Llinas 1994). A number of experimental studies supports this, for example if the 5-HT neurones of the DRN are pharmacologically inhibited or cooled, it results in the production of PGO waves and/or REM sleep (Hobson et al. 1993). While a reciprocal inhibition of cholinergic PPTg/LDTg neurones with DRN and LC neurones has been hypothesised (Sakai 1988; Jones 1991), there is as yet no evidence for cholinergic inhibition of the DRN while cholinergic innervation of the LC is excitatory (Egan and North 1986). However the implication suggested by the above evidence that a necessary requirement for the initiation of REM sleep is the silencing of LC neurones and the disinhibition of cholinergic PPTg/LDTg neurones by the DRN can not be ruled out (McCarley and Hobson 1975; Aston-Jones and Bloom 1981).
5.2. PPTg and Cognitive Processing

In addition to a role for the PPTg in the sleep/wake cycle the ascending cholinergic efferents to the basal ganglia, and basal forebrain and descending efferents to the PRF suggest that the PPTg is involved in the co-ordination of a wide range of behavioural functions (Woolf and Butcher 1986). Investigation into the PPTg's role in cognitive processing is a relatively new field of research. This has proved a difficult area to interpret due to a large number of complicating factors. For example it is very difficult to dissociate purely cognitive processes from arousal, motivational and motor functions in which the PPTg is also implicated.

5.2.1. PPTg and attention

Cholinergic mechanisms are not the only neural processes involved in cognitive functioning: many neurotransmitters interact to co-ordinate these (Decker and McGaugh 1991) and the PPTg could have an indirect role in attentional processing via connections with the basal forebrain (Dunnett et al. 1991), the basal ganglia, (Foote and Morrison 1987) and the LC (Decker and McGaugh 1991).

The PPTg may have an indirect effect on attentional processes mediated from the nucleus basalis magnocellularis (NBM) via its cholinergic innervation of the NBM. While ibotenate lesions of the NBM or systemic injections of cholinergic antagonists have been shown to impair performance on a wide range of memory and learning tasks (Dunnett et al. 1985; Hepler et al. 1985; Murray and Fibiger 1985; Spencer et al. 1988) more recent evidence has questioned whether such widespread mnemonic functions can be attributed to the basal forebrain (Dunnett et al. 1987; Dunnett et al. 1991). More specific cholinergic lesions using quisqualic acid or AMPA have demonstrated that the basal forebrain cholinergic neurones are not implicated in such a wide range of memory disorders as previously believed although interpretation is still difficult since these toxins are not entirely specific to cholinergic neurones but also disrupt other neurochemical populations (Dunnett et al. 1987; Robbins et al. 1989; Dunnett et al. 1991). However a recent study using a more selective cholinergic toxin has confirmed the results found using AMPA and quisqualic acid (Torres et al. 1994). The toxin saporin conjugated to the monoclonal antibody 192-IgG, attaches preferentially to nerve growth factor receptors which, in the basal forebrain, are exclusively located on cholinergic neurones. In an extensive
study Torres et al. (1994) ascertained that selective lesions of cholinergic neurones could be made in different parts of the basal forebrain, which caused substantial hippocampal and cortical ChAT depletion, but which did not cause any additional neuronal loss. Initial behavioural studies indicated that 92-IgG-saporin lesions of the NBM failed to disrupt performance on a variety of memory tests, including the Morris water maze and delayed non-matching to position task (Torres et al. 1994).

However there is some evidence that links the NBM to processes of attention (Dunnett et al. 1985). Dunnett et al. (1985) investigated the effects of unilateral ibotenate lesions of the NBM, and cholinergic-rich ventral forebrain tissue grafts or non-cholinergic-rich hippocampal tissue grafts placed in the frontal and parietal cortex of NBM-lesioned rats on a modified battery of sensorimotor tests developed by Marshall and Teitelbaum (1974). They found that the rats with lesions of the NBM only, and the rats that had also received the non-cholinergic hippocampal grafts showed significant impairments contralateral to the lesion on all the tests. In contrast the NBM-lesioned group that received cholinergic-rich ventral forebrain grafts, showed significant improvement on three of the tests. These rats showed a reduced contralateral (to the lesion) turning deficit, when placed on a table top. When placed head-down on a grid angled at 45° these rats also showed a reduction in the contralateral deficit shown by the other groups when turning to face head-up the grid, and they reacted to stimuli applied to various ipsilateral and contralateral points of their body, unlike the contralateral sensorimotor neglect characterised by the other two groups (Dunnett et al. 1985). These results could be interpreted as indicative of an attentional deficit following neural loss of the NBM. Despite the lack of specificity of the ibotenate acid, the results of improved performance on the sensorimotor tests shown by the rats who had received the cholinergic-rich grafts suggests that this attentional function is mediated at least in part by cholinergic NBM neurones. The PPTg’s influence over the cholinergic neurones of the basal forebrain already discussed in section 5.1. suggests that the PPTg could exert some control over attentional processing via this connection.

The mesencephalic DA neurones of the SN/VTA project to the cortex, primarily the prefrontal cortex (Foote and Morrison 1987) which is believed to have a role in higher-order processing, such as planning, mnemonic functions and perseverative processes (Fuster 1989). The role of DA in the prefrontal cortex is not
yet fully understood, but it may have a modulatory function (Foote and Morrison 1987). Evidence of a role for DA in the mediation of ‘frontal lobe’ attentional processes comes from a patient study comparing the performance of patients with different types of dementia (Sahgal et al. 1992). Senile dementia of the Alzheimer type (SDAT) and Lewy body type (SDLT) have been found to have different underlying neuropathological features. SDLT is characterized by loss of DA-containing neurones in the SN, and also by the presence of senile plaque formation, whereas SDAT involves the presence of neurofibrillary tangles, but no nigral DAergic loss. Using a visual search matching to sample task which required the utilisation of attentional mechanisms, Sahgal et al. (1992) demonstrated that patients suffering from SDLT showed significant impairments on this task, while patients with SDAT performed on a level with age-matched controls. In addition patients with SDLT showed impairments on a complex set-shifting attentional paradigm, that were similar to those shown by PD patients (Owen et al. 1992). PPTg cell loss and neurofibrillary tangles have been found in the brains of patients suffering from PD, SDLT and other neurodegenerative diseases such as supranuclear palsy (Tomlinson et al. 1984; Hirsch et al. 1987; Zweig et al. 1987; Mufson et al. 1988; Decker and McGaugh 1991). This suggests that altered cholinergic input into the SN from the PPTg may play some role in the attentional deficits shown by the PD and SDLT patients.

PPTg could also have a role in attentional processes via connectivity with the LC which innervates the NBM and several cortical areas (Foote and Morrison 1987; Decker and McGaugh 1991; Semba 1991). ACh excites LC neurones which are believed to have a role in wakefulness and general arousal (Aston-Jones and Bloom 1981; Robbins 1984; Foote and Morrison 1987). The term ‘arousal’ is problematic in that it is poorly defined and often used as a general catch-all phrase. With regard to LC function, a definition of arousal as a “non-specific tonic state of neural activity which modulates not only the sleep/waking cycle, but also the efficiency of performance in the waking state” was given by Robbins (1984, p14). Evidence suggest that NA facilitates neural responses to sensory information (Aston-Jones and Bloom 1981; Robbins 1984; Foote and Morrison 1987). NAergic neurones of the dorsal noradrenergic bundle (DNAB) arising in the LC are also believed to have a modulatory role. They compensate for the distracting influences of over- or under-arousal by directing attention towards a task (Robbins 1984). As with PPTg lesions,
NAergic depletion results in impairments in acquisition of behavioural tasks, though post-operative performance is not affected if the animal has learnt the task prior to surgery (Robbins 1984). This disruption may be explained by the possibility that acquisition of a task requires higher levels of arousal than the execution of a previously learned task, and depletion of NA reduces the ability to maintain selective attention (Robbins 1984). Although NA depletion does not affect performance on pre-learned cognitive tasks, it does interact with basal forebrain cholinergic systems to facilitate the disruptive effect of cholinergic antagonists on working memory tasks such as the radial arm maze (Decker and Gallagher 1987).

Investigations of a more direct role for the PPTg in attention have been conducted. For example a study by Steckler (1993) investigated the effect of ibotenate or quinolinate lesions of the PPTg on sustained attention. Rats were trained on an operant task which required them to respond on one of two levers when a dim light stimulus appeared above the target lever. Presentation of the stimulus was brief and unpredictable and this paradigm is believed to provide a measure of sustained attention. Training occurred prior to the rats receiving bilateral lesions of the PPTg. Following surgery both lesion groups showed initial impairments on the task although they had recovered their performance levels in line with the sham-lesioned group by the 5th day of post-operative testing (Steckler 1993). Interpretation of the deficit as being one of sustained attention is controversial, since a deficit in arousal could not be completely ruled out.

An experiment by Dellu et al. (1991) emphasises the importance of using a number of behavioural paradigms to investigate complex cognitive processes. They attempted to dissociate the differences between attention, learning and memory in their experiment where the effects of quisqualic lesions of the PPTg were assessed on 3 types of memory task. Two of these were designed to investigate spatial memory, and the third assessed working memory. They found that lesioned rats performed on par with controls on a cross maze task, where one of four arms was baited, while the other three arms were used as starting points for entry to the maze. Fixed spatial cues on the walls were provided which the rat was required to use as a point of reference in order to access the baited arm successfully. However the lesioned rats were badly impaired on the other spatial memory task. In this a platform was submerged in a swimming pool of opaque water. The position of the platform could be learnt by
referring to fixed spatial cues in the room. The PPTg-lesioned rats also showed severe impairments on the 8-arm radial maze, a test designed to assess working memory. Each of the 8 arms of the maze were baited, the most efficient strategy for collecting the pellets is to visit each arm only once. This task requires the animal to remember which arms of the maze he has already visited. Since the rats were impaired on only one of the two spatial memory tasks, Dellu et al. (1991) argued that the deficits could not be attributed to memory interference but were linked to task difficulty. Consequently they ascribed the failure of the lesioned rats to acquire the swimming pool and radial maze tasks as a deficit in sustained attention. They argued that sustained attention was required to complete both these complex tasks, while it was unnecessary for successful performance on the simpler cross maze task (Dellu et al. 1991). However another possible explanation for these deficits is that of a learning impairment. This is supported by the fact that no pre-operative training was given and the lesioned rats completely failed to acquire the swimming pool task. Their performance on the 8 arm radial maze also failed to improve over several trials and they tended to make more errors compared with their controls on the simpler cross maze task. Other experiments discussed below show that rats with PPTg lesions show impairments on the acquisition of tasks if they have no pre-operative experience (a deficit in learning), while they show no impairments on performance if they are trained to criterion prior to surgery, which demonstrates that memory is unaffected by the lesion.

5.2.2. PPTg and learning

The data of Dellu et al. (1991) described above suggests that the PPTg may also have a role in learning. In addition to the experiment by Dellu et al. (1991), there are numerous experimental studies which support this view.

Bilateral ibotenate lesions of the PPTg have been shown to disrupt performance on both active and passive avoidance tests (Fujimoto et al. 1990, 1992). The rats were trained following surgery. In the passive avoidance step-through task, rats were placed in a light chamber and given free access to both it and a dark chamber. On entry to the dark chamber the rats received a mild footshock, after which they remained in the chamber for 15s before being returned to their home cage. This was repeated until the rats completely avoided going into the dark chamber throughout a
test session lasting more than 300s. The influence of motor impairments on this and the active avoidance task could be ruled out since the rats showed the same latency to enter the dark chamber as the control rats on the initial trial. The active avoidance task took place in a two-way shuttle box, the rat could avoid the footshock by running to the opposite compartment. Rats with PPTg lesions failed to acquire these avoidance strategies. This evidence fits with a previous study examining the effects of bilateral kainate PPTg lesions on the same active avoidance test (Fujimoto et al. 1989). In this study the rats also failed to acquire the task successfully, despite motor impairments being ruled out. These results could be explained as a deficit in learning in that the rats were impaired in forming the association between the dark compartment and footshock in the passive avoidance task, and in learning to make the association between moving into the opposite compartment and avoiding the footshock in the active avoidance task (Fujimoto et al. 1990, 1992).

Habituation of the acoustic startle response is a robust behaviour, which has been shown to be mediated via two distinct neural pathways (Groves et al. 1974; Jordan and Leaton 1983). While short-term habituation results in a simple stimulus-response reaction, which is not retained on the cessation of the session, longer-term exposure to a predictable stimulus results in long term habituation that can last for days (Groves et al. 1974; Jordan and Leaton 1983). This long-term habituation could be defined as a kind of learning, in that the animal learns to ignore the stimulus since it has not been associated with any event of particular relevance to it. Lesions of the mesencephalic reticular formation, which encompassed the PPTg, had no effect on short-term habituation to the acoustic startle response, but did disrupt the acquisition of long-term habituation (Jordan and Leaton 1983). The authors speculated that long-term habituation arising from the mesencephalic reticular formation may be produced by inhibition of the caudal reticular pontine nucleus, a site which receives afferent innervation from the PPTg (Rye et al. 1988; Spann and Grofova 1991) and which mediates the acoustic startle response (Jordan and Leaton 1983; Ebert and Ostwald 1991). Lesions of the PPTg may disrupt the formation of association between the stimulus and the acoustic startle response, and thus be interpreted as a learning deficit.

Learning deficits have also been documented in patient studies. Patients with SDAT and SDLT showed deficits in a conditioned learning of paired associates task,
with patients who suffered from SDLT quantifiably worse than SDAT patients (Galloway et al. 1992). An earlier study also showed that patients with SDAT or PD were impaired on a conditioned visuospatial associative learning task (Sahakian et al. 1988). Similar deficits have been found in patients and monkeys with frontal lobe damage and it is hypothesised that these deficits are linked to frontal lobe degeneration (Petrides 1982, 1985). Since conditioned learning deficits have been identified in non-demented PD patients (Gotham et al. 1988) and are also demonstrated most strongly by SDLT patients (Galloway et al. 1992) it is possible that such tasks are mediated through the mesocortical connections with the prefrontal cortex which the PPTg could have an indirect modulatory role in via its cholinergic innervation of the SNc.

5.2.3. PPTg and memory
Rats with PPTg lesions fail to learn behavioural tasks if they are trained following surgery. However one way to test the PPTg's role in memory, is to train the rat prior to surgery and then on recovery test for retention of information. In a second part to the experiment by Fujimoto et al. (1992) the effects of bilateral ibotenate lesions to the PPTg given after training the rats to criterion on both the passive and active avoidance tasks were investigated. On testing the lesioned rats following surgery, Fujimoto et al (1992) found that the rats still maintained their preoperative performance levels on both tasks as did the sham-lesioned controls. They concluded therefore that while PPTg lesions disrupt the acquisition of novel information they have no effect on the retention or retrieval of information already stored in memory.

Steckler (1993) investigated long-term spatial memory in a water maze task. Rats were trained to locate a platform in the water maze before receiving either ibotenate or quinolinate lesions of the PPTg. Following surgery neither lesion group showed any deficits in performance on the spatial task reaching the platform in a time which corresponded with their pre-operative performance times. Short-term spatial memory was also unaffected by PPTg lesions, rats were pre-trained on a delayed non-matching to position task before receiving bilateral radio frequency lesions and following surgery there were no differences between the two groups in performance accuracy (Steckler et al. 1994).
It appears therefore that while an intact PPTg is necessary for the acquisition of information, it is not necessary for the retention of information that was learnt previous to the lesion.

5.3. PPTg, motivation and reward
5.3.1. Interactions of acetylcholine and dopamine in the substantia nigra

A role for the cholinergic PPTg in mediating the motivational components of basic behavioural functions such as eating, drinking and sexual behaviour has been suggested (Steckler et al. 1994). Although bilateral excitotoxic lesions have failed to produce gross motivational deficits in spillage, food and water intake (Dunbar et al. 1992, Inglis et al. 1994a; Allen and Winn 1995) an indirect role for the PPTg in behaviours for which there is a pre-existing tendency is suggested by the PPTg's cholinergic innervation of the SNc.

A cholinergic/DAergic interaction at the level of the SNc has been suggested by demonstrating that cholinergic agonists or acetylcholinesterase blockers injected into the SN increased eating in sated rats (Winn and Redgrave 1979, 1981; Winn et al. 1983; Winn 1990). Winn (1990) showed that intranigral carbachol increased the incidence of eating and drinking palatable foodstuffs (dry spaghetti and saccharin water) in sated rats and also affected sexual performance. Winn (1990) concluded that cholinergic stimulation of SN does not have specific effects but has a more generalised function in modulating the expression of behaviours for which there is already a pre-existing tendency to respond and for which the baseline response rate is low (Winn et al. 1983; Winn 1990). That this effect relies on a pre-existing tendency was demonstrated by the fact that only sated rats who had been exposed to spaghetti prior to injection of carbachol increased their consumption, while rats who had no prior experience of this palatable foodstuff did not.

Further evidence for a cholinergic/DAergic interaction comes from studies which show that haloperidol abolishes the action of carbachol in SN (Taha and Redgrave 1980) as does intranigral atropine (Winn et al. 1983). Direct evidence for carbachol's effects being centred upon the nigrostriatal DA neurones was demonstrated by Parker et al. (1991). They showed that 6-OHDA lesions of the nigrostriatal DA-containing neurones abolished the eating response to intranigral carbachol. Cholinergic/DAergic interactions in the SN have long been suspected due
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experiments by Bechara and Van der Kooy (1989, 1992a, 1992b) suggests that
deficits seen in CPP following PPTg lesions are not caused by impairments in learning
or memory but that they reflect disruption of the neural circuits underlying the
rewarding effects of the conditioned reinforcer.

Bilateral ibotenate PPTg lesions disrupted CPP to morphine and amphetamine
in rats trained following the lesion but did not affect the retention of CPP in rats
trained prior to the lesion, implying that PPTg lesions do not affect memory processes
(Bechara and Van der Kooy 1989). Furthermore if the rats with training prior to the
lesion had their CPP extinguished and were then re-trained, they failed to re-acquire
CPP to morphine. Bechara and Van der Kooy (1989) did not attribute this deficit to
an impairment in learning but to a disruption in perception of the rewarding properties
of the morphine. Support for this interpretation comes from another experiment
(Bechara and Van der Kooy 1992a).

In this complex study Bechara and Van der Kooy (1992a) investigated the
effects of bilateral ibotenate PPTg lesions on the formation of CPP to morphine or
food, in drug-naive or drug-dependent rats (ie. rats that had been chronically
pretreated with morphine), and food-deprived or sated rats (food condition). In all
cases the lesions were made prior to any experimental testing and following surgery
rats were divided into 4 groups, one group received repeated morphine injections,
another group was food deprived while the final two received no special treatment.
They were then run through a classic CPP paradigm; the morphine-dependent and
morphine naive rats were given 4 pairings of drug prior to being placed in one side of
the CPP box, and on alternate days 4 pairings of saline prior to being placed in the
other compartment. The same procedure was conducted with the food-deprived and
sated rats but instead of receiving morphine or saline injections food was present in
one compartment and not the other.

Bechara and Van der Kooy (1992a) found that while sham-lesioned morphine-
aive and sated rats formed CPP, PPTg lesions blocked the formation of CPP in both
these groups. In contrast the lesions did not affect CPP in morphine-dependent or
food-deprived rats who showed no difference in performance to their sham-lesioned
controls. Evidence from this and other experiments have led to the hypothesis that
separate neural substrates mediate the rewarding effects of conditioned reinforcers in
naive/sated or dependent/deprived rats and that the PPTg forms a critical part of the
neural circuit which mediates the rewarding effects in non-dependent conditions (Bechara and Van der Kooy 1992a; Bechara et al. 1992). However recent evidence has shown that PPTg-lesioned rats did not show CPP to saccharin solution, regardless of whether they were water-deprived or not (Stefurak and Van der Kooy 1994). This questions the hypothesis of separate neural circuits mediating state-dependent rewarding effects. That the PPTg is a critical substrate for the rewarding effects of the conditioned reinforcer is further shown by an experiment where PPTg lesions failed to block the acquisition of conditioned taste aversion to amphetamine and morphine (Bechara and Van der Kooy 1986).

However more recent data suggest that rats with lesions of the PPTg can perceive stimuli as rewarding and that their problem is one of a failure to integrate information related to the rewarding/motivational effects of a stimuli with a motor response appropriate to achieving the reward. This was shown in an experiment which investigated the effects of bilateral ibotenate lesions on responding for conditioned reinforcement (Inglis et al. 1994b). The rats received training to criterion on a conditioned reinforcement paradigm both prior to and following surgery. Food-deprived rats were trained to associate the appearance of a light in the food hopper, the click of the food hopper and the disappearance of the cage light with the delivery of food. In the test condition pressing on one of two levers resulted in the presentation of these stimuli though food was not delivered (conditioned response), while pressing on the other lever had no effect (unconditioned response). Following intra-accumbens d-amphetamine injections rats with lesions of the PPTg responded equally well as controls on the conditioned lever, indicating that they had no gross motor deficits and also that they were motivated to work for presentation of the stimulus. The lesioned rats also showed no differences in their response on the food hopper panel. However unlike their sham-lesioned controls the lesioned rats also showed increased responding on the unconditioned lever. That this response could be interpreted as due to the non-specific activational influence of the amphetamine was ruled out by the authors since the rats did not respond indiscriminately on the food hopper panel. Instead Inglis at al. (1994b) interpreted this pattern of responding as a specific failure to dissociate between the two levers. Thus the rats were motivated to work for the presentation of the conditioned stimulus but failed to differentiate between the two levers. This deficit was ascribed to a failure to integrate properly the
reward-related information with the appropriate motor sequence required to generate a successfully directed response (Inglis et al. 1994b)

5.4. PPTg and sensorimotor gating

The PPTg has been hypothesised to play a part in an indirect component of the primary acoustic startle pathway. Pre-pulse inhibition (PPI) is shown in a number of species including humans (Davis 1984). PPI is an inhibitory response whereby a low, non-startling stimulus presented milliseconds prior to a startling stimulus reduces the amplitude of the acoustic startle response (Hoffman and Searle 1968; Graham 1975). This cannot be interpreted as a form of conditioned learning since PPI is seen on the first trial and the interval between the pre-pulse and startling pulse is too brief to allow conscious inhibition of the acoustic startle response (Russo et al. 1975; Hoffman and Ison 1980). PPI is believed to occur through a form of sensorimotor 'gating' whereby the preceding signal sets off a sequence of neural events that function to inhibit the acoustic startle response (Koch et al. 1993). Overactivity of mesolimbic DA disrupts PPI and a proposed neural pathway through which the gating effects of PPI is mediated incorporates the NAcc-VP region-PPTg pathway (Swerdlow et al. 1990a, 1990b, 1992; Swerdlow and Geyer 1993). The primary acoustic startle response circuit has been located to the PRF and the caudal pontine reticular nucleus is a fundamental component of this pathway (Jordan and Leaton 1983). Previous studies (Jordan and Leaton 1983; Ebert and Ostwald 1991) suggested a role for the PPTg in the mediation of this response and anatomical evidence identified a projection from the PPTg to the pontine reticular formation (Grofova and Keane 1991). In a double labelling tracing study Koch et al. (1993) identified the cholinergic PPTg and LDTg as a source of innervation to the pontine reticular nucleus. They also conducted electrophysiological tests which showed that application of cholinergic agonists to pontine reticular neurones inhibited the acoustic startle response of the these neurones (Koch et al. 1993). Bilateral quinolinate lesions of the PPTg adversely affected the PPI response although in contrast to the study by Jordan and Leaton (1983), the lesions failed to impair long-term habituation, or diminish the acoustic startle response in the absence of a pre-pulse stimulus. This may be due to the fact that the lesions in Jordon and Leaton's study were electrolytic and could have caused more extensive damage to the area, whereas the quinolinic lesions
in Koch et al's. (1993) experiment were more circumscribed. Swerdlow and Geyer (1993) also reported that lesions of the PPTg disrupted PPI without affecting habituation. These data suggest that the PPTg has an inhibitory role over the acoustic neurones in the pontine reticular formation and therefore serves to gate the acoustic startle pathway without playing a fundamental part in the primary acoustic startle response circuit.

5.5. PPTg and nociception

A number of sites within the pontomesencephalon have been implicated in the transmission of nociceptive information, including the CNF and PAG (Behbehani and Zemlan 1986; Zemlan and Behbehani 1988). The PPTg has also been implicated in the transmission of nociceptive messages. While bilateral NMDA PPTg lesions had no effect on the pain response to the formalin test or on the analgesic effects of morphine (Olmstead and Franklin 1993), a cholinergic role for the PPTg in transmission of nociceptive messages has been suggested (Iwamoto 1989, 1991). The PPTg projects to the areas in the medulla which have been associated with the transmission of nociception such as the nucleus raphe magnus (NRM) (Rye et al. 1988; Spann and Grofova 1991). Nicotine injected into the PPTg dose-dependently increased the latency to respond to painful stimuli in both the tail flick and hot-plate tests (Iwamoto 1989, 1991). This decreased sensitivity to the painful stimuli was reversed by prior administration of a drug that interfered with the release and storage of acetylcholine in brain tissue (Iwamoto 1989). Atropine and scopolamine which act at muscarinic receptors also antagonised the nicotine-induced antinociception implicating a role for both nicotinic and muscarinic receptors in nociceptive transmission (Iwamoto 1989). However naloxone, an opiate receptor blocker, failed to have any effect on the analgesia produced by nicotine in the PPTg, supporting Olmstead and Franklin's (1993) data that there is no interaction between the cholinergic and opioid mechanisms at this level (Iwamoto 1991). However ibotenate lesions of the PPTg abolished the analgesic influence of nicotine injected into this region in both the tail flick and hot-plate tests (Iwamoto 1991). Iwamoto (1989, 1991) concluded that stimulation of nicotinic receptors in the PPTg releases acetylcholine which acts upon muscarinic receptors located on NRM neurones to induce an antinociceptive response.
5.6. Summary

The PPTg’s extensive anatomical connections and no less numerous functional roles make it clear that it occupies a central position within the pontomesencephalon. Not only does it contain a cholinergic population of neurones which form part of the ARAS, the PPTg also receives innervation from a majority of basal ganglia structures. In this chapter the PPTg has been shown to have widespread influence over behaviours implicated in functions as diverse as REM sleep, attention, learning, nociception and in responding for conditioned reinforcement. The question remains as to how the PPTg’s diverse functions can be translated into a coherent functional hypothesis.

A role for the CNF has been hypothesised in the transmission of nociceptive information and a number of studies have also suggested that the CNF constitutes the major substrate for the mesencephalic locomotor region (MLR), a functionally defined structure located in the pontomesencephalon and thought to control locomotor outflow. The evidence which supports the CNF as the locus of the MLR will be reviewed in chapter 7. In this chapter the CNF’s part in the transmission of nociceptive information will be discussed.

6.1. General overview of descending pathways involved in nociception

A number of brain areas have long been regarded to have some part to play in the control of pain transmission, and clarification of the mechanisms underlying endogenously generated analgesia are important since such knowledge may contribute to understanding the processes involved in chronic pain. The mechanisms which control endogenous nociception are organised in three interconnected levels of the brain (Basbaum and Fields 1978; Fields and Basbaum 1978). These brain levels include sites within the pontomesencephalon, the medulla and spinal cord.

There have been several reports of the analgesic effects of electrical stimulation of some mesencephalic areas in rats, primates and humans (Reynolds 1969; Mayer et al. 1971; Mayer and Liebeskind 1974; Pert and Yaksh 1974; Hosobuchi et al. 1977). Electrical stimulation of the dorsolateral central gray (CG) has been used in place of pharmacologically-induced anaesthesia in abdominal surgery in rats (Reynolds 1969). That the electrical stimulation produced analgesia and not paralysis was demonstrated by showing the rats could still emit startle responses to sudden auditory stimuli. The rats also responded to aversive stimuli approximately 5 minutes after the cessation of the electrical stimulation. Such analgesic effects from electrical stimulation of the CG are supported by numerous experimental studies. For example electrical stimulation was effective in blocking responses to noxious thermal stimulation and electrical shocks delivered to the tail, and to pinching the limbs and tail with a sharp pair of forceps (Mayer et al. 1971; Mayer and Liebeskind 1974). Both studies also reported analgesia in the absence of any other sensory or motor impairment, and also showed that there were receptive fields to the stimulus-
produced analgesia with the analgesia only effective in certain parts of the body, which was determined by the part of the CG stimulated, while noxious stimuli applied outside this receptive field produced normal aversive reactions. Results similar to those described above were also found when stimulating in the CG and dorsal raphe nuclei (DRN) of cats, though the stimulus-produced analgesia was only effective as long as stimulation was maintained and ceased almost immediately following stimulation (Oliveras et al. 1974).

At the level of the medulla, Proudfit and Anderson (1975) have demonstrated that stimulus-produced analgesia can also be elicited by electrical stimulation of the nucleus raphe magnus (NRM). This nucleus that lies in the ventral medulla and receives afferent innervation from both the CG and CNF (Behbehani and Fields 1979; Zemlan and Behbehani 1984, 1988; Bernard et al. 1989). The third brain level involved in the transmission of nociception is the dorsal horn of the spinal cord, in particular laminae I and V (Fields and Basbaum 1978) (Figure 6.1.). That midbrain areas can exert inhibitory control over the spinal cord is demonstrated in a study by Oliveras et al. (1974). They identified neurones in lamina V of the dorsal horn of the spinal cord which were responsive to noxious stimuli, such as pinching the skin with forceps, thermal stimulation or electric shock. Electrical stimulation in the midbrain, in the area of the CG selectively blocked the response of the dorsal horn lamina V neurones to noxious stimuli, while being ineffective in blocking their response to more innocuous stimuli, such as gentle tactile manipulation.

The results from stimulation studies have also been confirmed by studies investigating the effects of chemical manipulation using opiate drugs such as morphine. Receptors which bind endogenous neurochemicals such as enkephalin, which behaves pharmacologically like opiates, and opiate receptors have a distribution throughout the mesencephalon and pons which corresponds well to the areas that elicit stimulus-produced analgesia (Mayer and Liebeskind 1974; Pert and Yaksh 1974, Simantov et al. 1977). Opiate agonists such as morphine work by stimulating the production of serotonin (5-HT) and other neurotransmitters such as the catecholamines, though the catecholaminergic role in nociception is controversial and outwith the scope of this chapter. It is hypothesised that the 5-HT then inhibits transmission of nociceptive messages through a pain pathway. That this pain pathway involves the three different levels of the brain mentioned above is supported by
numerous studies. Opiate antagonists can block stimulation of analgesia from sites within the midbrain, pons and medulla (Pert and Yaksh 1974; Akil 1976; Hosobuchi et al. 1976). Naloxone has been shown to partially block analgesia stimulated from the CG in rats (Akil 1976), primates (Pert and Yaksh 1974) and completely block CG-stimulated analgesia in humans (Hosobuchi et al. 1976). Naloxone also completely blocks analgesia stimulated from the NRM (Oliveras et al. 1977b). These studies also suggest that the sites from which stimulus-produced analgesia can be elicited and the sites of the action of opiate drugs are not only similar but also recruit the same pain pathway. 5-HT antagonists injected systemically block the analgesic effects of morphine injected directly into the CG, but do not effect morphine analgesia when they are injected directly into the same part CG (Yaksh et al. 1976). It is known that the NRM sends a major serotonergic, inhibitory projection via the dorsolateral funiculus (DLF) to the dorsal horn of the spinal cord, in particular to laminae I and V, and this pathway is believed to be one of the pathways involved in inhibiting the transmission of nociceptive messages (Fields et al. 1977). Evidence from a number of studies gives credence to this hypothesis. For example lesions of the DLF block analgesia stimulated by peripheral administration of morphine (Basbaum et al. 1976; Hayes et al. 1978). More specifically, lesions of the DLF also block stimulus-produced analgesia stimulated from the CG and NRM (Basbaum et al. 1976; Fields et al. 1977). Decreasing the amount of serotonin (5-HT) in the spinal cord by injection of 5,6-dihydroxytryptamine, a drug which destroys 5-HT neurones, reduces morphine analgesia (Vogt 1974) while lesions of the NRM block the analgesic effects of morphine and also result in the lesioned rat becoming more responsive to the perception of painful stimuli as shown by the reduced latency to show a tail flick response to a noxious stimulus (Proudfit and Anderson 1975).

Thus behavioural evidence indicates that stimulus-produced analgesia can be elicited from the midbrain, pons and medulla which can be blocked by opiate and serotonergic antagonists. Electrophysiological studies have demonstrated inhibition of spinal cord lamina neurones following electrical stimulation of the CG and NRM, and anatomical evidence shows the existence of projections from the midbrain to the dorsal horn of the spinal cord, both direct from the CG and also via the NRM and DLF (Figure 6.1.). Taken together these data suggest the existence of a pathway that plays a part in the transmission of nociceptive information. Furthermore other
evidence described above indicates that this pathway utilises the inhibitory neurotransmitter 5-HT and that stimulation of this pathway with opiate agonists such as morphine functions to inhibit the transmission of noxious information and promote analgesia.

6.2. CNF and nociception
Although early studies concentrated on the CG as the main locus in the midbrain involved in nociception, more recent studies have investigated the role of the CNF. Several studies have presented evidence for a direct spinal projection from the CNF (Castiglioni et al. 1978; Carlton et al. 1983). An electrophysiological study by McMahon and Wall (1985) has also identified a direct projection from neurones in lamina I of the dorsal horn of the spinal cord to the CNF. This input could constitute part of the ascending antinociceptive pathway (Figure 6.2.). However other anatomical studies argue that spinal inputs do not innervate the CNF directly but instead connect sites bordering the CNF (Bernard et al. 1989). However the CNF projects to both the NRM and the nucleus reticularis magnocellularis (NMC) (for references see chapter 3). As described above, these two structures form part of the ventral medulla which gives rise to a major descending pain inhibitory system. Since the CNF provides a major afferent innervation to these structures, it could influence pain transmission through this projection (Figure 6.1.).

There is some evidence that would support this hypothesis. For example both the CG and the CNF afferents to the NRM-NMC are excitatory (Behbehani and Fields 1979; Behbehani and Zemlan 1986). The influence of the CG connection to the NRM-NMC has been extensively studied but recent evidence suggests that the CNF projection to the NRM-NMC plays a greater part in the control of pain transmission to the spinal neurones (Zemlan and Behbehani 1988). For example 75% of NRM-NMC neurones are excited by CNF stimulation, and following a retrograde tracing study using HRP injected into the NRM-NMC it was found that twice as many CNF neurones than CG neurones were retrogradely labelled (Behbehani and Zemlan 1986). This would suggest that the CNF plays an important role in modulating the NRM-NMC neurones which in turn inhibit spinal pain transmission.

Behavioural and electrophysiological studies also suggest a role for the CNF in nociception. Stimulus-produced analgesia elicited from the lateral reticular
formation, which lies lateral to the CG and incorporates the CNF, was shown to be more effective in suppressing spinal cord neural responses to noxious thermal stimulation than stimulus-produced analgesia elicited from the CG (Carstens et al. 1981; Carstens and Watkins 1986). Gray and Dostrovsky (1983) reported similar results, showing that stimulation in the CNF was more effective at inhibiting dorsal horn spinal cord neurones than stimulation in CG. They suggested that the preferential effects of CNF stimulation could be due to its larger projection to the NRM. Dostrovsky et al. (1982) conducted a mapping study which examined the effects of electrical stimulation in the CG, NRM and adjacent regions in the mesencephalon and medulla. They studied the nociceptive jaw-opening reflex, which can be elicited by electrical stimulation of the infraorbital nerve or tooth pulp. They then investigated whether electrical stimulation in various brain areas was effective in suppressing this jaw-opening reflex, which is taken to be indicative of analgesia. They found that stimulation in several areas of the mesencephalon and medulla resulted in the inhibition of the jaw-opening reflex. They reported that within the mesencephalon, two areas were most effective in suppressing the jaw-opening reflex: stimulation of the CNF was equally as effective as stimulation in the CG, while in the medulla, both the NRM and NMC inhibited this reflex. The CNF has also been shown to have a role in stimulus-produced analgesia (Zemlan and Behbehani 1988). The latency to make a nociceptive tail-flick response in response to noxious thermal stimulation was measured both during and following electrical stimulation of the CNF. It was found that stimulation of the CNF increased tail-flick response latency both during and up to 5 mins following stimulation, after which tail-flick response latencies returned to control levels. Pre-treatment with naloxone blocked the stimulus-produced analgesia elicited from the CNF (Zemlan and Behbehani 1988).

Thus anatomical and behavioural data combine to suggest that the CNF plays an important role in the modulation of spinal pain transmission either via connections to the NRM-NMC or as part of a ‘network’ acting in conjunction with the CG (Behbehani and Zemlan 1986). That the CNF could form part of a network of influence over NRM neurones is suggested by the fact that it lies immediately adjacent to the CG, that the CNF and CG have reciprocal connections and that concurrent stimulation of both these sites produces a greater magnitude of excitation.
in the NRM than stimulation of either structure alone (Behbehani and Zemlan 1986; Zemlan and Behbehani 1984, 1988).

6.3. Summary
There is more than one pathway through which noxious stimuli gain access to descending pain pathways regulating transmission of nociceptive information. That these pathways can be inhibited to promote analgesia, is suggested by both neurochemical and behavioural studies. The CG projects to both NRM and directly to spinal dorsal horn neurones, and experiments described above suggest it has a role in nociception. However lesions of the CG do not affect the transmission of nociceptive messages indicating that this is not the only pathway in existence (Liebman et al. 1970). The CNF also projects to NRM and evidence indicates that it too has a role in nociception. Other studies have shown that naloxone blocks analgesia stimulated from the central inferior raphe nucleus in cats (Oliveras et al. 1977b), while stimulation of other raphe nuclei results in stimulus-produced analgesia (Oliveras et al. 1974). Thus the serotonergic raphe nuclei which are located in the mesencephalon also appear to have a role in the mediation of nociception. Stimulus-produced analgesia can also be elicited from the lateral hypothalamus and some thalamic nuclei (Fields and Basbaum 1978), while a role for the PPTg and SC in the transmission of nociceptive information has also been hypothesised (Iwamoto 1989, 1991; Mitchell et al. 1988). There are therefore multiple pathways that have some role in the transmission of nociceptive information. A more detailed and specific behavioural investigation of exactly what other behaviours these various nuclei mediate, might help clarify their precise role in the mediation of nociception. A possible role for the CNF in the integration of nociceptive information with sensory and motor information, which results in the production of appropriately directed motor behaviours will be discussed in chapter 15.
Figure 6.1. Simplified diagram of the connections of the descending pathway for the transmission of nociceptive messages. CG = central gray, NRM = nucleus raphe magnus, DLF = dorsolateral funiculus (spinal cord). For fuller description see text.
Transmission of Nociception: Descending Pathway

CG → NRM → DLF → Dorsal Horn of the Spinal Cord Lamina I & Lamina V → Nociceptive Output

CNF → NRM → DLF → Dorsal Horn of the Spinal Cord Lamina I & Lamina V → Nociceptive Output
Figure 6.2. Simplified diagram of the connections of the ascending pathway for the transmission of noxious/painful information. For fuller description see text.
Transmission of Noxious Information: Ascending Pathway

- CG
- CNF

Gigantocellular Reticular Formation

Dorsal Horn of the Spinal Cord Lamina I & Lamina V

Noxious Input
Part III. Investigation of the PPTg and CNF as the possible loci of the mesencephalic locomotor region
Chapter 7. The mesencephalic locomotor region, the pedunculopontine tegmental nucleus and the cuneiform nucleus.

7.1. The mesencephalic locomotor region (MLR)
The MLR is a region within the mesencephalon from which locomotor activity can be stimulated in decerebrate rats and cats suspended over a moving treadmill (Shik et al. 1966; Garcia-Rill et al. 1983a; Nicolopoulos-Stournaras and Iles 1984; Garcia-Rill et al. 1985). Electrical stimulation of the MLR initially results in a postural-righting response, followed by co-ordinated locomotion, the speed of which is positively linked to the intensity of the stimulation (Nicolopoulos-Stournaras and Iles 1984; Coles et al. 1989). The MLR has been identified by mapping out the areas in the CNS which when stimulated result in controlled locomotion on a treadmill; spontaneous locomotion does not occur in the decerebrate preparation (Nicolopoulos-Stournaras and Iles 1984). Thus the precise anatomical location of the MLR is still in doubt because it can only be identified, using electrical or pharmacological stimulation.

7.2. PPTg and MLR
The PPTg has many reciprocal connections with the basal ganglia and receives striatal outflow, via the GP, STN, SNr, and VP region (Jackson and Crossman 1983; Moon-Edley and Graybiel 1983; Swanson et al. 1984; Rye et al. 1987; Steininger et al. 1992). Their input to the PPTg is inhibitory and primarily influences the non-cholinergic neurones (Rye et al. 1987; Spann and Grofova 1991) though there is basal ganglia innervation of the cholinergic PPTg (Semba and Fibiger 1992; Steininger et al. 1992). Both the non-cholinergic and cholinergic PPTg project to sites within the pons and medulla (Rye et al. 1988; Spann and Grofova 1991). Because of these connections parallels have been drawn between both the cholinergic and non-cholinergic PPTg and the MLR.

It has been claimed by several authors that the PPTg is the major component of the MLR (Nicolopoulos-Stournaras and Iles 1984; Skinner and Garcia-Rill 1984; Garcia-Rill et al. 1986; Garcia-Rill 1991). For example from an investigation of the projections of the MLR Garcia-Rill et al. (1986, p38) reported that "the extent of our studies overlapped more strictly with the distribution of midbrain cholinergic neurones, termed by some the Ch5-group". However, Rye et al. (1987) claim that the
anatomical and electrophysiological evidence suggests that the midbrain extrapyramidal area (MEA; non-cholinergic PPTg) could in fact be the MLR. They have also drawn attention to the fact that the connections of the MLR are homologous to the non-cholinergic PPTg, and speculate that the non-cholinergic PPTg may effect locomotion either directly via the spinal cord or indirectly via the reticulospinal medulla. Others believe that the PPTg lies close to the MLR and that it may serve as a connectional interface between the basal ganglia (the "higher" motor structures) and the "lower" motor system (Jackson and Crossman 1983).

There is some evidence that relates the MLR to both the cholinergic and non-cholinergic components of the PPTg. Locomotion stimulated from the MLR can be blocked by injections of atropine or GABA into the medioventral medulla suggesting both cholinergic and non-cholinergic innervation of this area influences locomotion (Garcia-Rill and Skinner 1987a). That this is indeed the case has been shown by a subsequent tracing study (Skinner et al. 1990b). However regardless of which neurochemical population in the PPTg is activated there is a general consensus that electrical or chemical stimulation of the PPTg will elicit locomotion on a treadmill in decerebrate rats and cats (Garcia-Rill et al. 1985, 1986). The MLR can be activated by injection of GABAergic antagonists into this region while locomotor activity is inhibited by administration of GABAergic agonists (Garcia-Rill et al. 1985; Garcia-Rill et al. 1990). Evidence for a direct spinal projection from the PPTg is controversial (Rye et al. 1988; Skinner et al. 1990a; Spann and Grofova 1991) and there is no evidence of a direct spinal projection from the MLR in cats (Kuypers and Maisky 1975; Tohyama et al. 1979). However a number of the medullary neurones involved in the stimulation of locomotion (Garcia-Rill and Skinner 1987a) were shown to project directly to the spinal cord in the cat which further supports a role for the MLR in the generation of locomotor activity (Garcia-Rill and Skinner 1987b). The fact that the MLR lacks a precise anatomical designation and has in turn been described as corresponding to the cholinergic PPTg, non-cholinergic PPTg, CNF and subcuneiform area increases the confusion surrounding the non-cholinergic PPTg's functional role.
7.2.1. *PPTg* and nucleus accumbens-stimulated locomotion

Research has shown that the NAcc influences locomotion by communication with the GP and VP region (Mogenson et al. 1980, Swerdlow and Koob 1984). It is hypothesized that a decrease in GABA activity in the GP and VP region (in particular the SI) might stimulate the locomotor activity seen following DAergic stimulation of the NAcc. Injections of picrotoxin, a GABA antagonist, into the VP region combined with intra-accumbens amphetamine produces a robust locomotor response (Mogenson and Nielson 1983; Mogenson et al. 1985). It has been suggested that this locomotor response is mediated through pallidal innervation of the PPTg (Bechara and Van der Kooy 1989, Mogenson et al. 1980). Mogenson and Wu (1988) investigated the role of the PPTg and dorsomedial nucleus of the thalamus (DMT) in the mediation of locomotor activity stimulated from the SI (a component of the VP region) using picrotoxin. They reported that injections of procaine (a local anaesthetic that reversibly blocks or slows the conduction of electrical impulses down the axon) into the PPTg attenuated the locomotor response to picrotoxin while procaine in the DMT had no effect on locomotion.

Others have disagreed with this hypothesis and there is an accumulating body of evidence which supports the argument that the PPTg plays no direct part in the mediation of locomotor outflow (Swerdlow and Koob 1987; Inglis et al. 1994a, 1994b; Olmstead and Franklin 1994). For example Swerdlow and Koob (1987) investigated three major output pathways of the VP region; the PPTg, DMT and the medial prefrontal cortex (MPT) to discover which structure translated the effects of NAcc DA activity into locomotion. The effect of lesioning these areas on the "supersensitive" locomotor response to intra-accumbens apomorphine in rats with 6-OHDA NAcc lesions was investigated. They found that the DMT but not the PPTg or MPT, disrupted this "supersensitive" locomotor response to apomorphine. Furthermore in a second experiment, lesions of the DMT also attenuated locomotor activity produced by injections of picrotoxin into the VP region. These results directly contradict those reported by Mogenson and Wu (1988). However that experiment used procaine, a reversible anaesthetic, the spread of which is impossible to trace, while lesions are much easier to locate anatomically. From the data of Swerdlow and Koob (1987) it appears that a projection to or through the DMT is crucial for the activation of locomotion stimulated from the accumbens-pallidal circuitry.
In a series of experiments conducted in this laboratory (Dunbar et al. 1992; Inglis et al. 1994a, 1994b) the effect of bilateral excitotoxic PPTg lesions on spontaneous and amphetamine-induced locomotion was investigated. Amphetamine was administered either systemically or microinjected directly into the NAcc. Neither spontaneous nor amphetamine-induced locomotor activity were affected by the lesion in any of the experiments conducted (Dunbar et al. 1992; Inglis et al. 1994a, 1994b). Olmstead and Franklin (1994) also reported that lesions of the PPTg did not affect locomotor activity stimulated by peripheral injections of amphetamine. Recent evidence would therefore question the role of the PPTg in the mediation of basal ganglia outflow as it relates to locomotor stimulation.

However bilateral ibotenate lesions of the PPTg have been reported to attenuate conditioned locomotion (Bechara and Van der Kooy 1992c). Rats were given 3 daily injections of high doses of amphetamine or morphine and their locomotor activity recorded for 2 minutes prior to, and 2 minutes following the systemic injections. Locomotor activity recorded in the 2 minutes prior to injection was taken as a measure of the conditioned locomotor effects of the drugs. In agreement with conditioned increases in locomotion reported by Mucha et al. (1981), sham-lesioned rats showed increases in conditioned locomotor activity prior to amphetamine or morphine injections while PPTg lesioned rats did not. Thus an intact PPTg appears to be required to mediate aspects of motor activity as they pertain to motivational stimuli (Bechara and Van der Kooy 1992c). These data support the data of Inglis et al. (1994b), who reported reward-related motor disruption in PPTg-lesioned rats on a conditioned reinforcement paradigm, and further suggest that the PPTg's role is one of the integration of reward-related, motivational stimuli with motor responses.

7.3. CNF and MLR
While there is a school of thought that identifies the PPTg as being the primary neural substrate of the MLR, the efferent connections of the CNF suggest the possibility that it could effect sites of motor control within the pons and medulla. Recent evidence has indicated that the CNF may constitute a major substrate of the mesencephalic locomotor region (MLR) (Steeves and Jordan 1984; Mori et al. 1977). For example, the MLR has been identified as lying ventral to the IC in the dorsal part of the mesencephalon (Steeves and Jordan 1984, Mori et al. 1977). Although the CNF
receives only sparse innervation from the ventral pallidal region, one of the primary outflow sites of the nucleus accumbens (NAcc) (Swanson et al. 1984), it does receive a substantial innervation from the SC (Bernard et al. 1989; Redgrave et al. 1988). Thus NAcc outflow to the CNF could be mediated though a NAcc-SN-SC circuit. Furthermore direct connections with the spinal cord have been identified in primates (Castiglioni et al. 1978) and the CNF innervates sites of motor control within the pons and medulla which in turn project to parts of the spinal cord (Zemlan et al. 1984).

The CNF has also been identified as the area responsible for eliciting controlled stepping in the acute precollicular-postmammillary decerebrate cat (Coles et al. 1989; Garcia-Rill et al. 1981; Steeves and Jordan 1984). Garcia-Rill et al. (1981) showed that sites within the medial CNF elicited locomotion at the lowest thresholds of electrical stimulation. Brudzynski and Mogenson (1985) injected procaine into the area of the CNF and PPTg to investigate its effects on locomotion stimulated by intra-accumbens amphetamine. They reported that procaine injected into the region of the CNF attenuated accumbens-stimulated locomotion whereas injections of procaine into the PPTg had no effect. Further evidence that the CNF is involved in the mediation of treadmill locomotion in the decerebrate rat is provided by Shojania et al. (1992). They reported that locomotor activity on a treadmill induces the production of a protein called c-fos in the mesencephalon. They localised c-fos production to the CNF. Bilateral electrolytic lesions of the CNF have also been shown to attenuate locomotor activity measured in the open field (Mager et al. 1983).

7.4 Summary
While the evidence presented above suggests that the PPTg does not constitute a major component of the MLR, and has no role to play in either spontaneous or amphetamine-induced locomotor activity, the data suggests that the CNF could well be the major neural locus of the MLR. In the following series of experiments, the CNF’s role in spontaneous and accumbens-stimulated locomotion was investigated.

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1. *c-fos* is an immediate early gene whose presence in a neurone is activated by various types of stimulation, for example neurotransmitters, and agents that allow the influx of CA\(^{2+}\) into a neurone. It is increasingly being used as an histochemical marker in place of the metabolic mapping technique which utilises 2-deoxyglucose.
The first experiment was a pilot study which was conducted to investigate whether it was possible to make bilateral excitotoxic lesions of the CNF which would not encroach upon the PPTg or result in high levels of mortality. The second experiment examined the effects of bilateral CNF lesions on both spontaneous and amphetamine-induced locomotion. The third experiment was designed to investigate the role of the PPTg and CNF in conditioned locomotion. While neither the PPTg nor the CNF appear to mediate the effects of spontaneous or drug-induced locomotion, an interaction between motor activity and the mediation of motivated behaviours has been suggested (Inglis et al. 1994b; Bechara and Van der Kooy 1992c). This final experiment was carried out to investigate whether lesions of the PPTg or CNF could disrupt conditioned locomotor activity elicited as a direct consequence of responding to rewarding stimuli.

This pilot study was conducted to determine whether it is possible to make discrete excitotoxic lesions in the CNF. Two issues needed to be addressed: first, is it possible to make bilateral ibotenate lesions of the CNF during one surgical procedure without producing the high fatality rate which occurs when making bilateral PPTg lesions? Second, can CNF lesions be made which do not encroach upon the PPTg? Unilateral and bilateral lesions of the CNF nucleus were attempted with different volumes of toxin to investigate which produced the most efficient lesion. The rats were also run through some basic locomotor testing to give an initial impression of how CNF lesions would affect both spontaneous and drug-induced locomotion.

8.1. Methods

14 male hooded Lister rats were individually housed under a 12 h light/dark cycle (lights on 08:00 h). They were maintained ad lib. on SDS maintenance diet no.1 chow pellets and tap water. Mean body weight at the time of surgery was 309.5 g (SD=11.9).

Surgery

Rats were anaesthetised with 60 mg/kg sodium pentobarbitone (Sagatal; RMB Animal Health Ltd) and placed in a stereotaxic frame with the skull level. All injections were made with a 1.0 µl syringe mounted on the stereotaxic frame, care being taken to ensure that the needle bevel was pointing forwards. Ibotenic acid (Cambridge Research Biochemicals) was prepared as 0.12 M solution in phosphate buffer (pH 7.4); the final pH of the ibotenate solution was adjusted with 2M NaOH to 7.2. Rats received either unilateral or bilateral CNF lesions using either 0.1 µl (12 nmol) or 0.2 µl (24 nmol) 0.12M ibotenate (one rat accidentally received a volume of 0.14µl (16.8 nmol) bilaterally). Rats acting as controls received vehicle alone, also delivered unilaterally or bilaterally in volumes of either 0.1 µl or 0.2 µl. All injections were made at the following stereotaxic co-ordinates: 0.2 mm anterior to the interaural line, ± 1.8 mm from the midline and 6.0 mm below the skull surface. All infusions were made at a rate of 0.02 µl steps at 10 s intervals and the needle was left in situ for 300 s to allow diffusion of the toxin away from the needle tip. Different volumes
of excitotoxin were used to determine which would produce the most discrete lesion without damaging other structures. Rats were allowed at least 7 days to recover from surgery before behavioural testing began.

Pre- and post-operative measures
For 6 days prior to and 7 days following surgery body weight, spillage (collected on foil trays placed underneath the food hopper), food and water intake were recorded. This was done in order to monitor post-operative recovery and ensure that the lesioned group had no gross motor deficits. Once food and water intake had recovered to pre-operative levels and remained stable for a few days, locomotor testing began.

Spontaneous and drug-induced locomotion
Both spontaneous and drug-induced locomotion were investigated. Rats were divided into 4 groups care being taken to ensure that the same cages were used for rats from both the lesioned and sham-lesioned control groups. Each rat was placed in a wire locomotor cage (38 cm X 24 cm X 19 cm), through which passed two infra-red light beams. Each time a beam was interrupted a count was registered by a computer ("Spider" Paul Fray Ltd). The beams had to be broken sequentially to avoid misrepresenting behaviour directed towards one beam only (e.g. sniffing) as locomotion. The testing sessions lasted 60 min and were conducted under blue light illumination. (This allowed the rats to be observed without the infra-red light beams registering false counts from the overhead lights). Spontaneous locomotion was measured for 4 consecutive days immediately followed by 4 days of drug-induced locomotion. Each rat received two doses of d-amphetamine sulphate (1.5 mg/ml and 5.0 mg/ml dissolved in 0.9% sterile saline) separated by two doses of vehicle alone. The amphetamine was delivered systemically via i.p. injection, and the order of administration was counterbalanced across the rats.

Histological procedures and lesion assessment
Rats were sacrificed between 24 and 26 days after surgery. Rats were deeply anaesthetised with an i.p. injection of 1.5 ml "Euthatal" (sodium pentobarbitone, 200 mg·ml\(^{-1}\); May and Baker) and were then perfused transcardially with 0.1 M
phosphate buffered saline (PBS) at 37°C, followed by at least 300 ml fixative (4% paraformaldehyde in 0.1 M phosphate buffer) at a rate of 20 ml·min⁻¹. The brains were removed and post-fixed in 4% paraformaldehyde for 60-120 min at room temperature. 50 μm coronal sections were cut for NADPH-diaphorase histochemistry and 25 μm coronal sections cut for nissl staining on a freezing microtome. Adjacent sections were stained for Nissl using cresyl violet and NADPH-diaphorase using a modification of the method of Vincent and colleagues (Vincent et al. 1983b; Vincent et al. 1992; Inglis et al. 1993).

All sections were inspected using a Leitz Diaplan microscope fitted with a Sony DXC-3000P video camera for visualisation of sections on a high resolution colour monitor. Lesions were identified in cresyl violet stained sections by the presence of gliosis and degenerating neuronal somata. Estimates of neuronal loss in the CNF and the surrounding structures were made on a scale of 0 to 4 as described by Inglis et al. (1994a), and direct cell counts from NADPH-diaphorase sections allowed the assessment of damage to PPTg cholinergic neurones.

Statistics

All regulatory and locomotor data were analysed parametrically using ANOVA and post-hoc testing was done using Tukey's method of multiple comparisons. Locomotor data were square-root transformed to reduce the heterogeneity of variance (Winer 1971).

8.2. Results

Analysis showed that there were no differences between rats with unilateral and bilateral lesions at either of the different volumes so their data was merged together, as was the data from the control-lesioned rats. This left two groups: CNF-lesioned (N=5) and sham-lesioned controls (N=4).

Histological analysis

Five rats did not have lesions in the appropriate area and were dropped from the analysis, leaving a total N=9. For the assessment of the lesion volumes and calculation of % NADPH-diaphorase cell loss, the rats with bilateral lesions had each lesioned side considered independently of the other. Lesion volumes were assessed using
cresyl violet nissl stain and the average cholinergic cell loss from the PPTg was calculated using cell counts from the NADPH-diaphorase histochemistry for each volume of toxin infused. Tables 8.1, 8.2 and 8.3 show the general damage summary, average lesion volume and % NADPH-diaphorase cell loss for each volume of ibotenate infused. Figures 8.1, 8.2 and 8.3 illustrate the largest and smallest lesion at each dose. At all three doses of ibotenate, more than 90% cell loss was seen in the CNF with varying degrees of damage to adjoining structures (although one rat showed only 30-60% damage to the CNF following the 0.1 $\mu$l dose). Damage to the PPTg was very slight in each case constituting less than 10% of the structure. The damage seen in the PPTg was confined to the pars compacta. Although the damage summaries do not show much difference in the amount of damage to other structures, there was more damage following administration of 0.2 $\mu$l ibotenate as compared with 0.14 $\mu$l and 0.1 $\mu$l, and with 0.14 $\mu$l compared with 0.1 $\mu$l.

Regulatory measures

Figure 8.4 shows the average daily body weight, spillage, food and water intake for the 6 days prior to and 7 days following surgery. Analysis of pre-operative data showed no significant effects of group for body weight ($F_{1,7}=0.68$), spillage ($F_{1,7}=2.77$) food intake ($F_{1,7}=0.02$) or water intake ($F_{1,7}=1.73$). A significant days effect was found for body weight ($F_{5,35}=63.69\ P<0.01$), spillage ($F_{5,35}=3.21\ P<0.05$), food intake ($F_{5,35}=6.66\ P<0.01$) and water intake ($F_{5,35}=4.28\ P<0.01$). This reflects the day-to-day variation in spillage, food and water intake and the steady daily increase in weight which occurs in all rats. Analysis also revealed no group by days interactions for body weight ($F_{5,35}=1.12$), spillage ($F_{5,35}=1.75$), food intake ($F_{5,35}=0.93$) or water intake ($F_{5,35}=1.90$).

Analysis of post-operative body weight revealed no significant effects of group ($F_{1,7}=0.03$) or a group by day interaction ($F_{6,42}=1.29$). A significant effect of days was found ($F_{6,42}=170.50\ P<0.01$) which reflected the steady daily weight gain of all the rats. There were no differences found between the groups in the amount of food they spilled following surgery ($F_{1,7}=0.02$) nor was there a significant days effect ($F_{6,42}=3.28$) or group by days interaction ($F_{6,42}=0.60$). Analysis of post-operative food intake failed to identify a significant main effect for group ($F_{1,7}=1.32$) but a significant days effect was found ($F_{6,42}=13.01\ P<0.01$) as was a significant group by
days interaction (F_{6,42}=2.70 \ P<0.05) Post hoc analysis revealed that the differences lay in the reduced food intake of both groups on the first day following surgery compared to all the other post-operative days (P<0.01) and that the CNF-lesioned group ate less food than the sham-lesioned controls on the second post-operative day (P<0.01). Analysis of water intake following surgery revealed no differences between the amount of water both groups drank in the post-operative period (F_{1,7}=0.02) nor a significant group by days interaction (F_{6,42}=0.60). A significant days effect was found (F_{6,42}=3.28 \ P<0.01) and post hoc analysis showed that this was due to both groups drinking more water on days 2, 3 and 4 compared with the first day following surgery (P<0.05).

*Spontaneous locomotion*

Figure 8.5 shows the mean (± SEM) locomotor activity for each group for the 4 sessions of spontaneous locomotor activity. ANOVA revealed a significant days effect (F_{3,21}=3.56 \ P<0.05) which post hoc analysis identified to be the result of both groups engaging in less locomotor activity on day 4 in comparison to day 1 (P<0.05). This fits with the small trend showing a decrease in locomotor activity over the 4 days and is probably the effect of habituation to the apparatus and procedure. There were no significant differences found between the groups (F_{1,7}=1.26) nor was there a group by days interaction (F_{3,21}=0.81).

*Amphetamine-induced locomotion*

Figure 8.6 shows the average locomotor activity for each group on the 4 different drug days. ANOVA revealed no significant effect of group (F_{1,7}=0.56) nor a group by days interaction (F_{3,21}=1.67). There was a significant effect of dose (F_{3,21}=1.67 \ P<0.01) and post hoc analysis revealed that the rats were significantly more active at 1.5 mg/kg compared with both of the saline days (P<0.01) and also compared with 5.0 mg/kg (P<0.01).

8.3. Discussion

It is clear from the histological results and the damage summary tables that it is possible to make discrete excitotoxic lesions of the CNF. Damage to the CNF was successful following all three doses that were investigated, with varying degrees of
damage to other structures. None of the lesions encroached upon the PPTg. The greatest loss of NADPH-diaphorase positive cells was following 0.2 µl (24 nmol) ibotenate, and even then the mean loss only amounted to 8.95%. Although the numbers in this experiment are small, inspection of the raw data suggests that 16.8 nmol is the best volume of toxin to use.

The behavioural analysis is also of interest since it gives some preliminary indications of what can be expected from subsequent larger studies. Lesions of the CNF did not affect the rats ability to regulate their body weight, spillage, food or water intake, and they recovered in line with their sham-lesioned controls. This suggests that rats bearing lesions to the CNF do not have any gross or long-lasting motor impairments. The investigation of locomotion also clearly demonstrated no deficits in baseline or amphetamine-induced locomotor activity. The CNF lesioned rats did not display reduced locomotor activity at any time in comparison to controls and they showed a normal pattern of habituation and normal response to amphetamine.

It is difficult to draw firm conclusions from these data because of the small sample sizes and different lesion volumes used. However these preliminary findings are of interest and provide enough evidence to suggest that a more extensive study would be of value.
Table 8.1. 0.1 μl ibotenate: summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Lesion placement:</th>
<th>CNF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Damage assessment:</strong></td>
<td></td>
</tr>
<tr>
<td>(modal scores)</td>
<td></td>
</tr>
<tr>
<td>Central gray</td>
<td>x</td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>xxxx</td>
</tr>
<tr>
<td>Dorsal tegmental bundle</td>
<td>x</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>x</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>ND</td>
</tr>
<tr>
<td>Parabrachial nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Retrolemniscal nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Ventral Spinocerebellar nucleus</td>
<td>x</td>
</tr>
</tbody>
</table>

Lesion Volume (mm$^3$): 1.59
(Mean ± SEM values) ± 0.756

% NADPH-diaphorase Cell Loss: 7.10
(Mean ± SEM values) ± 6.45

Table 8.1. A summary showing structural damage, mean (±SEM) lesion volume (mm$^3$) calculated from cresyl violet sections and % cell loss calculated from NADPH-diaphorase cell counts. Damage was identified by glial infiltration and degeneration of neuronal somata, and a summary of affected structures for every animal estimated. The modal score in the group was then taken for each structure. The key is as follows: ND No damage; x < 30%; xx 30 - 60%; xxx 60 - 90%; xxxx > 90%.
Table 8.2. 0.14 μl ibotenate: summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Lesion placement:</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Damage assessment:</td>
<td></td>
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<tr>
<td>(modal scores)</td>
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</tr>
<tr>
<td>Central gray</td>
<td>x</td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>xxxx</td>
</tr>
<tr>
<td>Dorsal tegmental bundle</td>
<td>x</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>x</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>x</td>
</tr>
<tr>
<td>Parabraclial nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Retrolemniscal nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Ventral Spinocerebellar nucleus</td>
<td>x</td>
</tr>
</tbody>
</table>

| Lesion Volume (mm³):                          | 1.94|
| (Mean ± SEM values)                           | ± 0.139|
| % NADPH-diaphorase Cell Loss:                 | 4.70|
| (Mean ± SEM values)                           | ± 4.49|

Table 8.2. A summary showing structural damage, mean (±SEM) lesion volume (mm³) calculated from cresyl violet sections and % cell loss calculated from NADPH-diaphorase cell counts. See legend in Table 8.1. for fuller description.
Table 8.3. 0.2 µl ibotenate: summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Damage assessment: (modal scores)</th>
<th>CNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central gray</td>
<td>x</td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>xxxx</td>
</tr>
<tr>
<td>Dorsal tegmental bundle</td>
<td>x</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>x</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>x</td>
</tr>
<tr>
<td>Parabrachial nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Retrolemniscal nucleus</td>
<td>xxx</td>
</tr>
<tr>
<td>Ventral Spinocerebellar nucleus</td>
<td>x</td>
</tr>
</tbody>
</table>

| Lesion Volume (mm³): (Mean ± SEM values) | 2.85 ± 0.257 |
| % NADPH-diaphorase Cell Loss: (Mean ± SEM values) | 8.95 ± 3.83 |

Table 8.3. A summary showing structural damage, mean (±SEM) lesion volume (mm³) calculated from cresyl violet sections and % cell loss calculated from NADPH-diaphorase cell counts. See legend in Table 8.1. for fuller description.
Figure 8.1., 8.2., and 8.3. Representative sections through the CNF showing lesion volumes. Shading represents the largest and smallest lesion following ibotenate infused into the CNF; sections redrawn from the atlas of Paxinos and Watson (1986).
IAL: -0.30 mm

Smallest Lesion

IAL: 1.36 mm

Largest Lesion

IBOTENATE 0.14 μl
Figure 8.4. Mean body weight, food intake, water intake and spillage for CNF-lesioned rats and sham-lesioned rats for the 6 days before and 7 days following surgery. For statistical analysis see text.
Figure 8.5. Mean (± SEM) locomotor counts for each group during four sessions recording of spontaneous locomotion. Data was square-root transformed to reduce the variance. For statistical analysis see text.
Figure 8.6. Mean (± SEM) locomotor counts for each group during the four sessions recording of d-amphetamine-induced locomotion. Data was square-root transformed to reduce the variance. For statistical analysis see text.
AMPHETAMINE-INDUCED LOCOMOTION

SQUARE-ROOT TRANSFORMED

SAL 1  SAL 2  1.5 mg/kg  5.0 mg/kg
Chapter 9. Does the cuneiform nucleus constitute the major substrate of the mesencephalic locomotor region? Evidence from excitotoxic lesions of the cuneiform nucleus and their effect on spontaneous and accumbens-stimulated locomotion.

The purpose of this study was to investigate whether the CNF does in fact constitute a major component of the MLR. The results from the pilot study described in the previous chapter show that it is possible to make discrete excitotoxic lesions of the CNF. In the present experiment bilateral ibotenate lesions of the CNF were combined with bilateral cannulation of the NAcc and the effect of the lesions on spontaneous and accumbens-induced locomotor activity investigated. In view of the belief of some researchers that the PPTg corresponds more closely to the MLR, care was taken to confine the lesion to the CNF without impinging on the PPTg, in order to prevent confusion in the interpretation of the results.

9.1. Methods

24 male hooded Lister rats were individually housed under a 12 h light/dark cycle (lights on 08:00 h). They were maintained ad lib. on SDS maintenance diet no.1 chow pellets and tap water. Mean body weight at the time of surgery was 287.4 g (SD=12.4).

Surgery
Refer to chapter 8 for a full description of the surgical procedures for the CNF lesion. Rats received either bilateral CNF lesions using 0.12 M ibotenate or control injections of vehicle alone. 0.14 μl (16.8 nmol) of ibotenate was infused at a rate of 0.02 μl/10 s at the following co-ordinates: 0.2 mm anterior to the interaural line, ±1.8 mm from the midline and 6.0 mm below the skull surface. The needle was left in situ for 300 s to allow diffusion of the toxin. During the same surgical procedure, 4 skull screws were fixed in place (care being taken not to penetrate the cerebral cortex) and guide cannulae (made from 11.0 mm long 23 ga hypodermic needles) were implanted bilaterally, aimed at the NAcc: 2.0 mm anterior to bregma, ± 1.5 mm from the midline and 5.5 mm from the skull surface. The cannulae (roughened slightly at the top to
improve adhesion) were fixed in place by dental cement which was packed round them and the skull screws. The cannulae were occluded using 30 ga stainless steel wire stylets. Rats had at least 10 days to recover from surgery before testing began.

**Pre- and post-operative measures**

Body weight, food spillage, food and water intake were recorded for 7 days prior to and 10 days following surgery. This was done to monitor post-operative recovery and ensure that the ibotenate-lesioned rats had no gross motor impairments following surgery.

**Measurement of locomotion**

On the 11th day following surgery rats were divided into 4 groups with approximately equal numbers of sham- and ibotenate-lesioned rats in each group. Rats were placed in individual locomotor cages (chapter 8 for full description). Test sessions lasted 3 h, were separated by 48 h and took place under blue light illumination. Rats received one 3 h habituation trial and then spontaneous locomotion was measured for 3 days. Immediately afterwards rats were tested following bilateral microinjection of amphetamine into the NAcc. Three doses of \(d\)-amphetamine sulphate (10.0 \(\mu\)g, 20.0 \(\mu\)g and 30.0 \(\mu\)g dissolved in 2.0 \(\mu\)l sterile 0.9% saline), and an additional saline-only injection which acted as a control, were administered in a counterbalanced order to each rat. All microinjections were made via 30 ga stainless steel cannulae (12.5 mm long) which terminated 7.0 mm below the skull surface. These cannulae were connected by polyethylene tubing to two SGE 10 \(\mu\)l syringes mounted on an infusion pump (Harvard Pump 22). Simultaneous bilateral injections into the NAcc were made and the volume injected was 2.0 \(\mu\)l infused at a rate of 0.5 \(\mu\)l/60 s. Cannulae were left in place for a further 120 s to allow for diffusion of the drug away from the cannulae.

**Histological procedures and lesion assessment**

On completion of testing rats were deeply anaesthetised and perfused transcardially. Brains were removed, post-fixed and then cut into 50 \(\mu\)m sections on a freezing microtome. Adjacent sections were stained for nissl substance using cresyl violet and damage to the PPTg was also assessed by NADPH-diaphorase histochemistry (see chapter 8 for full description).
Cannula placements in the NAcc were mapped onto sections of the Paxinos and Watson atlas (1986), and direct cell profile counts were made of the NADPH-diaphorase stained PPTg neurones, in order to assess any damage. Lesions to the CNF were identified from the cresyl violet sections by the presence of gliosis and degenerating neural bodies. Estimates of neuronal loss were made on a scale of 0-4 as described previously (Inglis et al. 1994).

Statistics
All regulatory and locomotor data were analysed parametrically using ANOVA and post-hoc testing was done where necessary using Tukey's method of multiple comparisons. Locomotor data were square-root transformed to reduce the heterogeneity of variance (Winer 1971).

9.2. Results

Histological analysis
One rat died in the immediate post-op period, and the data for another was lost during histological processing. Of the others, one rat was discarded from the analysis for having the cannulae placed bilaterally in the anterior commissure, and a further three rats discarded for having less than 30% bilateral damage to the CNF. This left both lesion and sham control groups with an N=9. Figure 9.1 shows the largest and smallest CNF lesions and Table 9.1 gives a summary of the damage to the CNF and adjacent structures, mean (± SEM) lesion volume (mm$^3$) and mean % (± SEM) diaphorase loss in the PPTg. $\frac{4}{9}$ of the ibotenate-lesioned rats showed bilateral CNF loss >90%. Of the other 5 lesioned rats, 4 showed unilateral CNF loss of approximately 90%, with loss on the other side varying from 45-65% and 1 rat had bilateral CNF loss of approximately 85%. All 9 lesioned rats had very little damage to adjacent structures and none showed any major loss of PPTg neurones as indicated by the small % loss of diaphorase stained neurones, for which ibotenate is known to be a potent excitotoxin (Inglis et al. 1993). (Figure 9.3. shows two representative nissl sections of an intact and lesioned CNF and a third section shows NADPH-diaphorase stained neurones from a lesioned rat). $\frac{6}{9}$ of the ibotenate-lesioned and $\frac{6}{9}$ of the sham-lesioned rats had bilateral placements in the NAcc, the remaining 3 rats from each group had a unilateral placement in the NAcc. These rats were included in the
analysis, since unilateral stimulation of the NAcc is sufficient to generate a locomotor response. Cannulae placements are illustrated in Figure 9.2.

**Body weight, spillage, food and water intake**

Figure 9.4. illustrates the daily means for each group for body weight, food and water intake and food spillage during the 7 days prior to and 10 days following surgery. Analysis of pre-operative data revealed no significant main effects of group on body weight (F1,16=0.62), food intake (F1,16=0.02), water intake (F1,16=1.18) or food spillage (F1,16=0.27). There were also no group by days interactions. Analysis revealed a significant days effect for body weight (F6,96=255.82 P<0.01) which reflects the daily increase in body weight shown by all the rats. There was also a significant days effect for food intake (F6,96=2.31 P<0.01) and water intake (F6,96=3.28 P<0.01). Post hoc testing revealed that both groups ate on average more food on the day prior to surgery than they did 4, 5 and 6 days before surgery (P<0.01 for days 4 and 6 and P<0.05 for day 5). Post hoc testing also revealed that both groups drank more water the day prior to surgery compared to 6 days before surgery (P<0.01). However in both cases the numbers involved are small, no more than 3 g or 3 ml respectively.

Over the 10 days following surgery analysis revealed that there were no significant differences in body weight between the two groups (F1,16=0.25) although there was a significant days effect (F9,144=226.92 P<0.01) and a group by days interaction (F9,144=7.79 P<0.01). Post hoc analysis revealed that the ibotenate-lesioned group weighed less than the sham-lesioned group on the first and second day following surgery (P<0.01, and P<0.05 respectively) after which the lesion group recovered their body weight and gained weight in line with the sham-lesioned controls. Analysis of food intake revealed a significant days effect (F9,144=60.49 P<0.01) but no group effect (F1,16=1.18) nor group by days interaction (F9,144=1.40). Post hoc testing revealed that the differences were due mainly to a reduction in food intake during the immediate post-operative period. Both groups ate significantly less on the first day following surgery compared to all the other days (P<0.01), and on the second day after surgery compared with days 5-10 (P<0.01 for all except day 7 P<0.05). Other daily variations in food intake are more random and reflect small variations in the amount of food eaten which never exceeded 4 g and are
therefore not reported here. Analysis of water intake revealed a similar pattern of
results to that of food intake. There was a significant days effect (F9,144=24.92
\( P<0.01 \)) which \textit{post hoc} testing revealed to be the reduction in water intake of both
groups on the first day after surgery compared with all the other post-operative days
(\( P<0.01 \)). However there was no group effect (F1,16=1.42) or a group by days
interaction (F9,144=1.03). Analysis of post-operative spillage revealed exactly the
same pattern of results, with no group effect (F1,16=0.00) or group by days
interaction (F9,144=1.06) but a significant days effect (F9,144=16.83 \( P<0.01 \)) which
\textit{post hoc} testing showed to be the result of the reduction in spillage on the first post-
operative day compared with all the other days (\( P<0.01 \)).

\textit{Spontaneous locomotion}

Figure 9.5. shows the mean total number of counts for each group during the 3 h test
sessions for spontaneous locomotion. Analysis of the 3 days of spontaneous
locomotion revealed no significant differences between the groups (F1,16=0.14). Nor
was there an effect of days (F2,32=2.23) or group X days interaction (F2,32=0.08).
In order to ascertain whether the two groups showed the same \textit{pattern} of response
over the sessions, the first two hours for each locomotor day was broken down into
10 min time bins, and analysed. The final 60 min were not treated in this way, since by
this time more than 50\% of the rats from both groups scored zero on activity levels,
and as such analysis was deemed unnecessary. The rats did not perform differently
over the 120 min time course on any of the three spontaneous locomotor sessions,
analysis revealing no significant effects of group or a group by time bin interaction.
There was an effect of time on locomotor activity (day1: F11,176=21.96 \( P<0.001 \);
day2: F11,176=37.95 P<0.001; day3: : F11,176=25.86 P<0.001) which is the result
of decreasing locomotor activity over time as the animal habituated to the cage
(Figure 9.6.).

\textit{Amphetamine-induced locomotion}

Figure 9.7. shows the mean total number of counts for each group following intra-
accumbens \textit{d}-amphetamine. ANOVA revealed no significant differences between the	wo groups (F1,16=0.03) in the level of locomotor activity they engaged in, nor was
there any interaction dose (F3,48=2.03). There was a significant effect of dose
(F3,48=59.06 P<0.01) and post hoc testing showed that both groups engaged in higher levels of locomotor activity following all doses of d-amphetamine compared to saline (P<0.01), and following 30.0 µg compared with 10.0 µg and 20.0 µg (P<0.05). When the first 120 min of each session were analysed separately, the same pattern of results reported for spontaneous locomotion was found. There were no significant effects of group nor any interaction of group with dose following saline or any of the doses of d-amphetamine. Again there was a significant effect of time on locomotor activity which as Figure 9.8. shows is also the result of a steady decrease in activity levels as the amphetamine wore off and the rat habituated to the cage (saline: F11,176=47.45 P<0.001; dose 10.0 µg: F11,176=116.40 P<0.001; dose 20.0 µg: F11,176=95.65 P<0.001; dose 30.0 µg: F11,176=87.98 P<0.001).

9.3. Discussion
From the histological analysis described above it is clear that it is possible to make discrete excitotoxic lesions of the CNF without encroaching upon the PPTg. The average % NADPH-diaphorase loss was only 5.54%, which is a small amount. Ibotenate destroys both cholinergic and non-cholinergic PPTg neurones equally well (Rugg et al. 1992; Inglis et al. 1993, 1994a, 1994b), therefore there is no reason to believe that non-cholinergic neurones would survive ibotenate infusion if cholinergic neurones had not. Conversely when using this toxin, little damage to cholinergic PPTg neurones would also reflect trivial damage to the non-cholinergic neurones. Therefore NADPH-diaphorase was used to assess any loss to PPTg neurones in the present experiment. Following lesions of the CNF, rats had no difficulty regulating their body weight, or food and water intake in comparison to their sham-lesioned controls. This indicates that they had no gross or long-lasting motor deficits following surgery. The lack of a locomotor deficit was also clearly demonstrated during both baseline and amphetamine-induced locomotor testing. The CNF lesioned rats did not display reduced locomotor activity at any time in comparison to controls; they showed the same pattern of habituation and a normal response to amphetamine. This more detailed experiment supports and extends the data described in the previous chapter and suggests that the CNF does not constitute a major component of the MLR.
Table 9.1. Summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Lesion placement:</th>
<th>CNF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Damage assessment:</strong></td>
<td></td>
</tr>
<tr>
<td>(modal scores)</td>
<td></td>
</tr>
<tr>
<td>Central gray</td>
<td>ND</td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>xxx</td>
</tr>
<tr>
<td>Deep mesencephalic nucleus</td>
<td>xxx</td>
</tr>
<tr>
<td>Dorsal tegmental bundle</td>
<td>ND</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>x</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>x</td>
</tr>
<tr>
<td>Parabrachial nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Paracollicular tegmentum</td>
<td>x</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Retrolemniscal nucleus</td>
<td>xx</td>
</tr>
<tr>
<td>Ventral spinocerebellar tract</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Lesion Volume (mm(^3))</strong>:</td>
<td>2.55</td>
</tr>
<tr>
<td>(Mean ± SEM values)</td>
<td>± 0.236</td>
</tr>
<tr>
<td><strong>% NADPH-diaphorase Cell Loss:</strong></td>
<td>5.54</td>
</tr>
<tr>
<td>(Mean ± SEM values)</td>
<td>± 1.59</td>
</tr>
</tbody>
</table>

**Table 9.1.** A summary describing the structural damage, average (± SEM) lesion volume (mm\(^3\)) and mean % (± SE) NADPH-diaphorase loss from the PPTg following lesions of the CNF. Damage was estimated from cresyl violet sections and the modal score for each group taken. Key: ND - no damage; x - >30% damage; xx - 30-60% damage; xxx - 60-90% damage; xxxx - >90% damage.
Figure 9.1. Representative sections through the CNF showing lesion volumes. Shading depicts the largest and smallest lesion volumes resulting from ibotenate infused into the CNF. Sections were re-drawn from Paxinos and Watson (1986).
Figure 9.2. Representative sections though the nucleus accumbens showing cannulae placements. The circles represent the ibotenate-lesioned group and the triangles the sham-lesioned group. Sections were re-drawn from Paxinos and Watson (1986).
Figure 9.3. Photomicrographs showing cresyl violet sections in the area of the CNF and PPTg.

A. A section from a sham-lesioned rat showing neurones of the CNF depicted by the dotted line. PPTg neurones lie between the superior cerebellar peduncle (scp) and lemniscal fibres. Scale bar = 0.25 mm.

B. A section from an ibotenate-lesioned rat. The dotted line again indicates the CNF which shows few intact neurones and an increased amount of reactive gliosis. Scale bar = 0.25 mm.

C. A section stained for NADPH-diaphorase from an ibotenate-lesioned rat. There is no loss of diaphorase-positive neurones in the PPTg. The dotted line indicates the CNF. Scale bar = 0.25 mm.
Figure 9.4. Graphs show the mean body weight, food and water intake and food spillage for each group over the 7 days prior to surgery and 10 days following surgery. For statistical analysis see text.
Figure 9.5. Mean (± SEM) locomotor counts for each group during the three sessions recording spontaneous locomotion. Data was square-root transformed to reduce the variance.
SPONTANEOUS LOCOMOTION
3 HOUR SESSIONS

SQUARE-ROOT TRANSFORMED

- IBOTENATE
- SHAM

DAY 1   DAY 2   DAY 3
Figure 9.6. Mean (± SEM) locomotor counts for each group for the level of activity over 120 min for the three days spontaneous locomotion. Data was square-root transformed to reduce the variance.
Figure 9.7. Mean (± SEM) locomotor counts for each group during the four sessions drug-induced locomotion following intra-accumbens d-amphetamine. Data was square-root transformed to reduce the variance.
AMPHETAMINE-INDUCED LOCOMOTION
3 HOUR SESSIONS

SQUARE-ROOT TRANSFORMED

IBOTENATE  SHAM

SALINE  10.0 ug  20.0 ug  30.0 ug
Figure 9.8. Mean (± SEM) locomotor counts for each group for the level of activity over 120 min following intra-accumbens saline and d-amphetamine. Data was square-root transformed to reduce the variance.
Chapter 10. Investigation into a possible role for the pedunculopontine tegmental nucleus and cuneiform nucleus in conditioned locomotion.

Previous experiments have failed to reveal a role for the PPTg in either spontaneous or accumbens-stimulated locomotion (Swerdlow and Koob 1987; Inglis et al. 1994a, 1994b, Olmstead and Franklin 1994). However a role for the PPTg in the mediation of reward-related behaviours has been suggested (Bechara and Van der Kooy 1989, 1992a, 1992b; Inglis et al. 1994b), and it has been reported that PPTg lesions attenuate conditioned locomotor activity prior to delivery of morphine or amphetamine reward (Bechara and Van der Kooy 1992c). The present experiment was intended to investigate whether lesions of the PPTg would attenuate conditioned locomotion in water deprived rats prior to being given access to water. The results from the previous chapter show that the CNF, like the PPTg, is not involved in the mediation of spontaneous or accumbens-stimulated locomotion, though it also has connections with sites of motor control in the pons and medulla. It was therefore decided to investigate whether the CNF could also be involved in the mediation of more subtle locomotor behaviours, such as conditioned locomotion.

10.1. Methods

25 male hooded Lister rats were individually housed under a 12 h light/dark cycle (lights on 08:00 h). They were maintained ad lib. on SDS maintenance diet no.1 chow pellets and tap water. Mean body weight at the time of surgery was 395.36 g (SD=18.33).

Surgery

The rats were split into 4 groups. One group received bilateral PPTg lesions (N=8) and another bilateral CNF lesions (N=6); the toxin in both cases was 0.1 M ibotenic acid. The remaining two groups received sham lesions of vehicle alone delivered into either the PPTg (N=5) or CNF (N=6). Rats were anaesthetised with sodium pentobarbitone, and placed in a stereotaxic frame with the skull level. PPTg sham and lesioned rats received injection volumes of 0.2 μl in both anterior and posterior PPTg.
while CNF sham and lesioned rats received 0.14 μl. In all cases the injection was delivered in 0.02 μl/10 s step-downs and the needle left in situ for 300 s. Due to the large size of the rats adjustments had to be made to the stereotaxic co-ordinates previously used for the CNF lesions and also for the co-ordinates normally used for PPTg lesions (see chapters 8 and 13 respectively). For the CNF groups, rats that weighed <380 g received injections at the following co-ordinates: 1.2 mm anterior to the interaural line, ± 1.8 mm from the midline and 6.5 mm from the skull surface. The following co-ordinates were used for rats weighing >380 g: 0.7 mm anterior to the interaural line, ± 1.8 mm from the midline and 6.3 mm from the skull surface. Rats weighing >340 g in the PPTg groups were injected as follows: 1.3 mm anterior to the interaural line, ± 2.1 mm from the midline and 7.3 mm below the skull surface (posterior PPTg) and 2.0 mm anterior to the interaural line, ± 2.2 mm from the midline and 8.1 mm below the skull surface (anterior PPTg). For full descriptions of CNF and PPTg surgery please refer to chapters 8 and 13 respectively. Rats were allowed at least 4 weeks to recover.

**Measurement of conditioned locomotion**

On recovery from surgery the rats were split into 5 groups and placed into individual locomotor cages (see chapter 8). Conditioned locomotion was tested according to the protocol of Bechara and Van der Kooy (1992c) who measured locomotor activity for 2 min prior to injecting the rats with amphetamine or morphine. In the present experiment the rats initially received 9 daily habituation sessions each lasting 2 min to enable them to habituate to the apparatus and test procedure. This was continued until a stable baseline was reached. On the day of the last habituation session, the rats' drinking water was removed from the home cage and the rats were water deprived for 23.5 h prior to the next session. Water deprivation continued throughout the next testing phase. During these 6 daily sessions, the rats were placed in the locomotor cages for 2 min without water present and were then given 30 min unrestricted access to water from water bottles placed in the locomotor cages. Activity in the 2 min prior to water delivery was recorded. On the final day of testing the rats were again placed in the locomotor cages for 2 min. However after the 2 min had elapsed, the water bottles were put into the cage but the rats received no water. Locomotor activity in
the following 30 min was measured before the rats were returned to their home cages and given free access to water.

**Histological procedures and lesion assessment**

On completion of testing rats were deeply anaesthetised and perfused transcardially. Brains were removed, post-fixed and then cut into 50 μm sections on a freezing microtome. Adjacent sections were stained for nissl substance using cresyl violet and damage to the PPTg was also assessed by NADPH-diaphorase histochemistry (see chapter 8 for full description).

Lesions to the CNF and PPTg were identified from the cresyl violet sections by the presence of gliosis and degenerating neural bodies. Direct cell profile counts were made of the NADPH-diaphorase stained PPTg neurones, in order to further assess any damage.

**Statistics**

All locomotor data were analysed parametrically using ANOVA and post-hoc testing was done where necessary using Tukey's method of multiple comparisons. Locomotor data were square-root transformed to reduce the heterogeneity of variance (Winer 1971).

**10.2. Results**

**Histological analysis**

Microscopic assessment of the cresyl violet sections uncharacteristically revealed very little nissl loss in both the CNF and PPTg lesioned groups. Nissl sparing in both these groups was so pronounced as to make it impossible to calculate lesion volumes or draw out representative lesions accurately. 3/8 PPTg-lesioned rats showed no neuronal damage at all, while 6/6 of the CNF-lesioned rats had little or no damage to the CNF. It was therefore decided to drop all the CNF-lesioned and sham-lesioned rats, and also to discard the 3 PPTg-lesioned rats which showed no damage. Despite the difficulty in interpreting the cresyl violet sections in the PPTg-lesioned rats, more detailed assessment of the extent of their lesions was possible due to the NADPH-diaphorase histochemistry, which meant cell profile counts could be made. Of the remaining 5 PPTg lesioned rats, 2 had only unilateral loss of the right PPTg, while the
other 3 had varying degrees of bilateral loss. Table 10.1 shows the % diaphorase loss for each rat in the PPTg lesioned group. By discarding the two CNF groups and the 3 non-PPTg-lesioned rats only 2 groups remained; PPTg-lesioned group (N=5) and PPTg sham-lesioned group (N=5).

**Conditioned locomotion**

Analysis of the locomotor data proved difficult due to the small sample sizes. However, by taking all the PPTg-lesioned animals as one group regardless of the size or side of the lesion, there were enough animals in each group to run ANOVA. Analysis of the 9 habituation sessions did not reveal any effect of group (F\(_1,8=1.60\)) or group by session interaction (F\(_8,64=0.56\)). There was a significant effect of days (F\(_8,64=3.51, P<0.01\)) and post hoc testing showed that both groups of rats were less active on session 2 compared to sessions 4, 5, 6, 7, 8, and 9 (all \(P<0.001\)). This shows that by the end of the 9th habituation session a stable baseline had been reached (Figure 10.1). There was also no difference between the two groups over the conditioned locomotor testing session, as Figure 10.2 shows. Analysis revealed no significant effects of group (F\(_1,8=0.972\)), session (F\(_5,40=1.03\)) or group by session interaction (F\(_5,40=0.93\)). The PPTg-lesioned rats did appear to show less locomotor activity in the 30 min period when no water was delivered (Figure 10.3). However one-way ANOVA failed to show any significant differences between the groups (F\(_1,8=3.45\)). Figure 10.4 shows the raw data for the locomotor counts for the unilateral PPTg-lesioned rats (N=2) and bilateral lesioned rats (N=3). This shows that rats with bilateral PPTg lesions did show a depression in locomotion during the 30 min period when water was absent, but since the N=3, no final analysis was made.

**10.3. Discussion**

The disappointing effect of the lesions reported in this study could be explained by a number of confounding factors. It is possible that the preparation of ibotenate (from a new sample) or the advanced age and large size of the rats used in this study could have affected the potency of the ibotenate.

While it is impossible to say anything about the CNF’s role in conditioned locomotion, the PPTg-lesioned data does provide some food for thought. Although there were no statistically significant differences between the PPTg-lesioned and
sham-lesioned groups, a number of other factors could have influenced the data. For example, the lack of effect found in the 6 conditioned locomotion sessions may be due to the fact that 2 min session are not long enough to show-up any differences in locomotor activity measured in this way (Bechara and Van der Kooy (1992c) measured locomotor activity by quadrant crossing). It is therefore interesting that the 3 bilaterally PPTg-lesioned rats showed the lowest levels of activity during the final session, in the 30 min period when water was not provided. Although the numbers were too small to perform any kind of statistical manipulation, the raw data does suggest that bilateral PPTg lesions may attenuate conditioned locomotion. This is in keeping with the data of Bechara and Van der Kooy (1992c) who also reported attenuation of conditioned locomotion to amphetamine and morphine, and lends further support to the hypothesis that while the PPTg does not mediate locomotor activity per se, it may be involved in locomotion as it relates to more complex functions, for example in the mediation of motor activity in relation to reward-related behaviours. Better lesions and a larger sample size would show whether this effect was more than a coincidence in the data.
Table 10.1. % NADPH-diaphorase loss per rat in the PPTg-lesioned group.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Left Side</th>
<th>Right Side</th>
</tr>
</thead>
<tbody>
<tr>
<td>94/181</td>
<td>59.98</td>
<td>0.00</td>
</tr>
<tr>
<td>94/177</td>
<td>74.49</td>
<td>0.00</td>
</tr>
<tr>
<td>94/173</td>
<td>59.15</td>
<td>52.06</td>
</tr>
<tr>
<td>94/188</td>
<td>41.64</td>
<td>75.82</td>
</tr>
<tr>
<td>94/189</td>
<td>68.74</td>
<td>79.99</td>
</tr>
</tbody>
</table>

Table 10.1. Raw data showing the % NADPH-diaphorase loss for 5 remaining rats following Ibotenate PPTg lesions. % NADPH-diaphorase loss was calculated by looking at the % diaphorase sparing for each rat in comparison to the mean diaphorase count for the sham-lesioned group, and then subtracting the % diaphorase sparing from 100% to give an estimation of % diaphorase loss.
Figure 10.1. Mean (± SEM) locomotor counts for each group for the nine habituation sessions each lasting 2 min. Data was square-root transformed to reduce the variance. For statistical analysis see text.
CONDITIONED LOCOMOTION
HABITUATION SESSIONS

SQUARE-ROOT TRANSFORMED

IBOTENATE
SHAM

SESSIONS
Figure 10.2. Mean (± SEM) locomotor counts for each group for the six conditioned locomotion sessions each lasting 2 min. Data was square-root transformed to reduce the variance. For statistical analysis see text.
CONDITIONED LOCOMOTION

SQUARE-ROOT TRANSFORMED

IBOTENATE  SHAM

DAY1  DAY2  DAY3  DAY4  DAY5  DAY6

SESSIONS
Figure 10.3. Mean (± SEM) locomotor counts for each group for conditioned locomotor activity in the 30 min session when no water was present. Data was square-root transformed to reduce the variance. For statistical analysis see text.
CONDITIONED LOCOMOTION
30 MIN SESSION WITHOUT WATER

SQUARE-ROOT TRANSFORMED

16
14
12
10
8
6
4
2
0

SHAM

LESION (N=5)
Figure 10.4. Raw data showing locomotor counts for each rat for the 30 min locomotor session without water. Sham rats (N=5), unilateral PPTg-lesioned rats (N=2) and bilateral PPTg-lesioned rats (N=3). Data was square-root transformed to reduce the variance.
CONDITIONED LOCOMOTION
30 MIN SESSION WITHOUT WATER

SQUARE-ROOT TRANSFORMED

RAW DATA

<table>
<thead>
<tr>
<th>Group</th>
<th>SQUARE-ROOT TRANSFORMED</th>
<th>RAW DATA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>UNILAT</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>BILAT</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

(N=5) (N=2) (N=3)
Part IV. Investigation into a role for the PPTg in orofacial behaviours
Chapter 11. The pedunculopontine tegmental nucleus and oral motor behaviours.

The PPTg receives innervation from the majority of basal ganglia structures and has descending outflow to sites in the medulla, caudal pons and spinal cord (see Chapter 2). A functional role in the mediation of striatal outflow has been hypothesised for the PPTg on the basis of its connections, and the PPTg's part in influencing behaviours stimulated from the nucleus accumbens (NAcc) has been investigated. Although there have been suggestions that the PPTg mediates locomotion stimulated from the NAcc there is increasing evidence that an intact PPTg is not required for spontaneous or drug-induced locomotion (see chapter 7). The PPTg does however appear to be involved in reward-related behaviours stimulated from the NAcc, such as conditioned place preference, responding for conditioned reinforcement, and conditioned locomotion (see chapter 7).

11.1. Interactions between the PPTg and ventrolateral caudate putamen

The PPTg's role in mediating behaviours stimulated from the CP is also receiving attention. From the evidence reviewed in section 4.3.4, chapter 4, it is clear that the lateral CP is involved in oral behaviours. Pisa (1988) has shown that lesions of the lateral striatum cause deficits in tongue reaching, and single neurones which respond either selectively to licking or more generally to oral motor behaviours have been identified in ventral areas of the lateral striatum (Mittler et al. 1994). Kelley and her colleagues (Kelley et al. 1988, 1989; Delfs and Kelley 1990) have demonstrated that intense orofacial activation, including biting and licking, can be induced by microinjection of a number of substances including d-amphetamine, dopamine receptor agonists and cholinergic agonists into a specific subregion of the CP, the ventrolateral caudate-putamen (VLCP). These intense orofacial stereotypies are not normally observed after systemic injection of low doses of amphetamine. However an experiment conducted in this laboratory has shown that while lesions of the PPTg had no effect on drug induced locomotion following administration of DAergic stimulants, they did affect the expression of oral stereotypies (Inglis et al. 1994a). Intense orofacial stereotypies were observed at low systemic doses of d-amphetamine (3.0 - 5.0 mg·kg⁻¹) in rats with bilateral ibotenate PPTg lesions. The results of this study
suggest that the PPTg is in receipt of and in some way influences the expression of outflow from the VLCP.

Orofacial behaviours are mediated by the VLCP and lesions of the PPTg appear to alter striatal outflow allowing this part of the striatum to have a greater impact on behavioural expression, which results in the appearance of orofacial stereotypies. Lyon and Robbins (1975) put forward a hypothesis of striatal competition whereby the NAcc and CP outflow competed with each other for behavioural expression. Thus, following low doses of systemic amphetamine, locomotor activity is the result and the NAcc ‘wins’ the competition for behavioural expression while at higher doses the CP has more influence and stereotyped behaviours such as sniffing, licking and biting appear. The PPTg receives outflow from both the NAcc and CP and it may be one site at which this competition for behavioural expression is gated. The data of Inglis et al. (1994a) suggest that the PPTg mediates striatal outflow and that lesions in this area have disrupted the balancing of this striatal competition and allowed the VLCP to have a greater impact on behavioural expression than normal. Furthermore the fact that lesions lead to an increase in the incidence of oral stereotypies at lower doses than reported in non-PPTg lesioned rats suggests that the PPTg’s role could be one of inhibition.

11.2. Possible anatomical pathways through which the VLCP could gain access to the PPTg

There are several neural pathways through which VLCP outflow could reach the PPTg, though the exact route or routes are still unknown. Groenewegen et al. (1993) have indicated the presence of a direct connection from the VLCP to the PPTg, while indirect connections have been recognised for several years. Neurones in the VLCP project to both the SNr and GP, using the inhibitory neurotransmitter GABA (Von Krosigk et al. 1992) and both the GP and the SNr project to the PPTg (Rye et al. 1987; Spann and Grofova 1991; Steininger et al. 1992). Thus outflow from the VLCP could be channelled to the PPTg directly, or via the GP or SNr, or through a more indirect route, such as GP-SNr-PPTg. An additional possibility is that DAergic input from the SNc to the subthalamic nucleus (STN) is involved (Lavoie et al. 1989), which then reaches the PPTg via a projection from the STN. Injections of apomorphine into the STN elicits orofacial stereotypies (Parry et al. 1994). However,
the orofacial stereotypies generated by stimulation of this area are different in character to those seen following administration of amphetamine in the VLCP. In contrast to the intense directed licking and biting seen following stimulation of the VLCP, apomorphine injected into the STN of rats elicited non-directed, irregular bouts of orofacial activity, characterised by tongue protrusion (Parry et al. 1994). Furthermore this effect could be attenuated by prior injection of D1 DA receptor antagonists, while Delfs and Kelley (1990) reported that orofacial stereotypies generated from the VLCP require concurrent stimulation of both D1 and D2 DA receptors. Thus it would appear that the types of orofacial stereotypy elicited from these sites could constitute different mechanisms of orofacial control. There is also some disagreement about the existence of an afferent input into the non-cholinergic PPTg which could arise from the STN (Steininger et al. 1992; Moon Edley and Graybiel 1983). It would seem unlikely therefore, that VLCP outflow could reach the PPTg via the SNc and STN.

11.3. Summary

The results of Inglis et al. (1993) indicate that the PPTg may play a part in the mediation of oral motor behaviours stimulated from the VLCP. However in that study, systemic injections of amphetamine were given and the behaviours observed could therefore be the result of general DAergic excitation. Thus the following series of experiments were conducted with the aim of clarifying a number of questions. First, a dose-response study was conducted to confirm that oral motor stereotypies can be stimulated directly from the VLCP and to identify a suitable dose range for subsequent experiments. A second experiment sought to confirm that PPTg lesions can affect oral motor behaviour stimulated from the VLCP, and to determine what form such changes took. While suggesting that excitotoxic lesions of the PPTg should increase the incidence of such behaviour after stimulation of the VLCP, Inglis et al. (1994a) did not specify the nature of the orofacial stereotypies they witnessed. Are the oral stereotypies characterised by changes in the latency, duration, frequency or form of oral motor behaviour? The final study was conducted to further extend the knowledge gained from the second experiment, and again combined bilateral PPTg lesions with direct \textit{d}-amphetamine stimulation from the VLCP.

A dose response experiment was undertaken for two reasons; first to confirm Kelley's co-ordinates for the VLCP (1988) and second, to define a dose response curve to different doses of amphetamine microinjected directly into the VLCP.

12.1. Methods

12 male hooded Lister rats were individually housed and kept under a 12 h light/dark cycle (lights on 08:00 h). They were maintained ad lib on SDS maintenance diet no.1 chow pellets and tap water. Mean body weight at the time of surgery was 307.0 g (SD=15.4).

Surgery

Rats were anaesthetised with 7 mg/kg xylazine ("Rompun" 2% solution; Bayer UK Ltd) and 100 mg/kg ketamine ("Velatar"; Parke Davis and Company)1. Rats were placed in a stereotaxic frame with the skull in the orientation of De Groot (nose bar 5 mm above the interaural line) and 4 skull screws were fixed in place (care was taken not to penetrate the cerebral cortex). 23 ga stainless steel cannulae (10 mm long and constructed from hypodermic needles roughened slightly at the top to improve adherence of the dental cement) were implanted at the following co-ordinates: 2.5 mm anterior to bregma, ± 4.0 mm from the midline and 4.5 mm below the skull surface. Dental cement was packed around these and the cannulae, which were normally occluded using 30 ga stainless steel wire stylets. Rats were allowed at least 14 days to recover from surgery before testing began.

Intracranial Drug Administration

All microinjections were made via 30 ga stainless steel cannulae terminating 7.5 mm below the skull surface and connected by polyethylene tubing to SGE 10 μl syringes

1Ketamine is a dissociative anaesthetic characterised by analgesia and normal skeletal tone. Therefore it is used in conjunction with xylazine, a sedative which is also characterised by analgesia but more importantly has muscle relaxant properties (Compendium of Data Sheets for Veterinary Products 1988-89. National Office of Animal Health; Datapharm Publications Ltd, London; 27 & 225.)
mounted on an infusion pump (Harvard Pump 22). Simultaneous bilateral injections were made into right and left VLCP. The injection volume in all instances was 0.5 µl, infused at 0.25 µl / 60 s, with cannulae left in place for a further 60 s to allow for drug diffusion away from the tips. Three different doses of d-amphetamine sulphate (5.0, 10.0 and 20.0 µg dissolved in 0.5 µl 0.9% saline) were administered and an additional saline-only injection acted as a control. Amphetamine and saline were administered in an individually randomised order; 48 h separated each injection.

**Measurement of oral motor behaviour**

Prior to drug testing each rat was habituated to the test procedure over 2 days. Following microinjection, rats were tested in cages (38 cm X 21 cm X 24 cm) which had smooth sides and a wire floor. A camera (RS Components) suitable for low light conditions and with an infra-red response was mounted underneath the cages and attached to a Sony monochrome monitor and VHS video recorder. Test sessions lasted 45 min, took place under red light illumination and were recorded onto videotape. The tapes were then analysed by an observer who was blind with respect to the drug condition. Behavioural ratings were recorded every 5 min using a modified version of the Creese-Iversen scale (Kelly et al. 1975). The scores were as follows:

- 0  Still/Asleep.
- 1  Generally Active.
- 2  Active with bursts of stereotyped sniffing and rearing.
- 3  Stereotyped sniffing and rearing over a wide area.
- 4  Stereotyped behaviour in one place.
- 5  Bursts of stereotyped licking or gnawing.
- 6  Continual licking or gnawing.

**Histological procedures and microscopic assessment**

Rats were sacrificed between 34 and 35 days after surgery. Rats were deeply anaesthetised with an i.p. injection of 1.5 ml "Euthatal" (sodium pentobarbitone, 200 mg·ml⁻¹; May and Baker) and were then perfused transcardially with 0.1M phosphate buffered saline (PBS) at 37°C, followed by at least 300 ml fixative (4% formalin in 0.1 M phosphate buffer) at a rate of 20 ml·min⁻¹. The brains were removed and post-
fixed in 4% formalin. 25 μm coronal sections were cut on a freezing microtome. For verification of cannulae placements sections were collected every 200 μm through the visible tract sites in the caudate-putamen and stained with cresyl violet.

All sections were inspected using a Leitz Diaplan microscope fitted with a Sony DXC-3000P video camera for visualisation of sections on a high resolution colour monitor. Cannulae placements were mapped onto schematic drawings taken from the atlas of Paxinos and Watson (1986).

Statistical analysis
The raw data of Creese-Iversen ratings were analysed using $\chi^2$ (Siegel 1956).

12.2. Results

Histological Analysis
Cannulae placements were verified post-mortem and Figure 12.1 maps out the placements on representative sections taken from the atlas of Paxinos and Watson (1986). One rat had placements which did not lie within the VLCP and was discarded from the analysis. Three rats each had one cannula misplaced but were included in analysis, since behavioural results indicated that the response to amphetamine could be elicited unilaterally from the VLCP.

Analysis of Creese-Iversen data
Table 12.1 shows the % scores on the Creese-Iversen for all the rats at each dose of amphetamine, and also for the saline dose. Representative group scores were calculated by taking the frequency of scores for each behavioural category at a given dose, dividing this by the total number of possible scores for all the rats and multiplying by 100.

For analysis, data were collapsed into three categories: 0-1 (no stereotypy), 2-4 (sniffing and rearing stereotypies) and 5-6 (licking and biting stereotypies). $\chi^2$ revealed a significant difference between saline and 5.0 μg d-amphetamine ($\chi^2=64.28$, df=2, P<0.001), with the rats scoring more ratings of 0 and 1 following saline and more scores of 2-4 and 5 and 6 following 5.0 μg d-amphetamine. Comparison of 5.0 μg and 10.0 μg d-amphetamine also revealed significant differences ($\chi^2=10.47$, df=2, P<0.01) with the rats scoring more 0 and 1 and 2-4 ratings following 5.0 μg d-
amphetamine, and more 2-4 and 5 and 6 ratings following 10.0 μg d-amphetamine. The final comparison between 10.0 μg and 20.0 μg d-amphetamine did not yield a significant result ($\chi^2=0.59$, df=2, P<0.8).

12.3. Discussion

The cannulae placements in this experiment were all placed within the confines of the VLCP at sites similar to those described by Kelley et al. (1988). Analysis of the categorical data provided by the Creese-Iversen checklist has proved problematical. Fray et al. (1980) made some attempt to address this issue, but the techniques described in that paper concerned analysis of independent measures and are therefore not relevant to the repeated measures design used in this experiment. While $\chi^2$ has been used in this experiment there is still a problem when using a repeated measures design. There is the possibility that the data values collected from the rats at different doses are not independent but may be predictable across the doses. If this is the case then there is a risk that the significant results yielded have been inflated. Therefore caution should be used when interpreting the data, and the results yielded here should be regarded as showing a trend towards a dose dependent increase in stereotyped licking and biting, which is supported by the observational data. For example, following saline only two rats registered a score of 5 and this score featured only once in the 12 X 5 min recording bins. Injection of 5.0 μg d-amphetamine produced some incidents of licking and biting of the cage floor which were relatively short-lived. This increased in duration and intensity following doses of 10.0 μg and 20.0 μg amphetamine. The majority of rats directed their licking or biting behaviour towards the cage floor, though some rats (N=4) also licked and gnawed at their paws and nails. This behaviour was not so extreme that the rats injured themselves but resembled more a form of intensified stereotyped grooming. This experiment suggests that there is a subregion within the CP where catecholaminergic stimulation can produce oral motor stereotypies which are dose dependent.
Table 12.1. % Creese-Iversen scores following drug treatments

<table>
<thead>
<tr>
<th>Dose</th>
<th>No stereotypy</th>
<th>Sniffing-Rearing</th>
<th>Biting-Licking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1</td>
<td>2 3 4</td>
<td>5 6</td>
</tr>
<tr>
<td>Saline</td>
<td>42.4 38.4</td>
<td>10.1 0.0 6.1</td>
<td>3.0 0.0</td>
</tr>
<tr>
<td>5.0µg</td>
<td>13.1 11.1</td>
<td>23.2 5.1 26.3</td>
<td>20.2 1.0</td>
</tr>
<tr>
<td>10.0µg</td>
<td>5.1 6.1</td>
<td>18.2 4.0 27.3</td>
<td>39.4 0.0</td>
</tr>
<tr>
<td>20.0µg</td>
<td>3.0 5.1</td>
<td>15.2 8.1 26.3</td>
<td>42.4 0.0</td>
</tr>
</tbody>
</table>

Table 12.1. Creese-Iversen scores. Percentages were calculated by taking the frequency of each score for all the rats in the group, dividing this by the total number of scores per group and then multiplying by 100.
Figure 12.1. Representative sections through the VLCP showing cannulae placements for the dose response study. Sections redrawn from the atlas of Paxinos and Watson (1986).
Chapter 13. The pedunculopontine tegmental nucleus mediates orofacial behaviours stimulated by microinjections of $d$-amphetamine into rat ventrolateral caudate-putamen.

This experiment was conducted in order to investigate whether lesions of the PPTg affect the licking and biting response to central injections of $d$-amphetamine as the findings of Inglis et al. (1994a) suggest. Injections of $d$-amphetamine were made directly into the VLCP, the site identified by Kelley et al. (1988) as underlying the oral motor response generated by such catecholaminergic stimulation. It was decided that the nature of the licking and biting response also required further investigation. The use of the Creese-Iversen check-list is not an ideal method of scoring since it fails to record the rats' behaviour over a continuous period of time resulting in loss of information. Furthermore, the categories used to record the behaviour of the rats cannot be related as there is no linear relationship between the numbers assigned to the various behaviours recorded. This makes analysis difficult. Therefore two methods of behavioural analysis were investigated, the Creese-Iversen checklist which consists of scoring behaviour into categories; and parametric analysis of behaviour over time, allowed quantitative assessment of behavioural categories. It was hoped that the more detailed time-based analysis would indicate whether the increase in oral motor activity shown by rats with PPTg lesions (Inglis et al. 1994a) would be in the form of changes in the latency, duration, frequency or form of oral motor behaviour. The present study sought therefore to confirm that PPTg lesions would affect oral motor behaviour stimulated from the VLCP, and to determine what form such changes took.

13.1. Methods

17 male hooded Lister rats (Olac) were individually housed and kept under a 12 h light/dark cycle (lights on 08:00 h). They were maintained ad lib. on SDS

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1 Originally 25 rats were housed, 8 of which formed a group who received bilateral quinolinate lesions. However this group had to be discarded from the experiment when it was discovered that the quinolinate had not made an effective lesion. It is not clear why the excitotoxin did not work, though it may have been confounded by the depth of anaesthesia, or the age of the quinolinate may have affected its potency.
maintenance diet no.1 chow pellets and tap water. Mean body weight at the time of surgery was 295.5 g (SD=12.4).

**Surgery**

Rats were divided into two groups: PPTg ibotenate (n=9) and phosphate buffer control (n=8). Ibotenic acid (Cambridge Research Biochemicals) was prepared as 0.12 M solution in phosphate buffer (pH 7.4); the final pH of the ibotenate solution was adjusted with 2M NaOH to 7.2. Rats were anaesthetised with 10 ml·kg⁻¹ Avertin 2 (10 g tribromoethanol/5 g tertiary amyl-alcohol; 10 ml of this concentrate was then dissolved in 450 ml 0.9% saline and 40 ml absolute alcohol) and placed in a stereotaxic frame with the skull level. Injections of excitotoxin were made using a 1µl SGE syringe mounted on a stereotaxic frame, with the needle bevel pointing forwards. 2 injections were made in each hemisphere at the following co-ordinates: 0.8 mm anterior to the interaural line, ± 1.6 mm from midline and 7.0 mm below skull surface (posterior PPTg); and 1.5 mm anterior to the interaural line, ± 1.7 mm from midline and 7.8 mm below skull surface (anterior PPTg). Ibotenate or phosphate buffer was delivered in a volume of 0.2 µl (24 nmol) to each site in 0.02 µl steps at 10 s intervals, and the needle left in situ for 300 s to allow for diffusion from the needle tip. The rats had 2 unilateral operations 24 h apart as previous experience has shown that ibotenate infused bilaterally into these regions during one surgical procedure is often fatal. During the second unilateral operation rats also received bilateral cannulation of the VLCP. Cannulae were implanted as described in Chapter 12, at the following co-ordinates: 2.5 mm anterior to bregma, ± 3.5 mm from the midline 3 and 4.5 mm below the skull surface. The rats were allowed at least 21 days to recover from surgery before testing began.

**Regulatory Measures**

Body weight, food spillage (collected on foil trays beneath the cages) food and water intake were recorded for each rat for 7 days prior to surgery and for 21 days

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2 Avertin was used as the anaesthetic of choice since it is a non-barbiturate anaesthetic that does not affect the toxicity of quinolinic acid which, like barbiturates, binds at NMDA receptor subtypes. There is a risk of post-operative gastric pathology when using avertin, and to reduce this risk, each rat received an injection of 5 mls 6% glucose dissolved in sterile 0.9% saline (w/v).

3 An adjustment was made to the lateral co-ordinate to allow for the smaller body size of the rats in this experiment.
afterwards in order to monitor post-operative recovery and ensure that the PPTg-lesioned group were able to maintain themselves properly. Drug testing and analysis of oral motor behaviour began once food and water intake had returned to preoperative levels and had remained stable for several days.

**Intracranial Drug Administration**

Simultaneous bilateral injections were made into right and left VLCP via 30ga stainless steel cannulae terminating 7.5 mm below the skull surface. Three different doses of *d*-amphetamine sulphate (5.0, 10.0 and 20.0 µg dissolved in 0.5 µl 0.9% saline) were administered and an additional saline-only injection acted as a control. Amphetamine and saline were administered in an individually randomised order; 48 h separated each injection. For a full description of the procedure refer to Chapter 12.

**Measurement of oral motor behaviour**

Prior to drug testing each rat was habituated to the test procedure over 2 days. The rats were tested in the same apparatus and following the same protocol described in Chapter 12. Test sessions lasted 45 min, took place under red light illumination and were recorded onto videotape. The tapes were then analysed by an observer who was blind with respect to the drug condition. Behavioural ratings were recorded in two ways. First, behaviour was scored every 5 min using a modified version of the Creese-Iversen scale (Kelly et al. 1975) (see Chapter 12).

Second, all the sessions for each rat were observed on videotape and the incidence of oral motor behaviours exhibited by each rat was timed. Oral motor behaviour fell into four categories: (i) grooming; (ii) licking or biting of the body; (iii) licking or gnawing the cage; (iv) vacuous mouth movements. For each category the latency to begin the behaviour (sec), the number of bouts and the duration of each bout (sec) was recorded. A bout was defined as the occurrence of a target activity which lasted a minimum of 3 sec. This detailed analysis was conducted to evaluate the categorical data obtained using the Creese-Iversen scale by providing a quantitative measurement of oral motor behaviour for analysis and to investigate further any

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4 I carried out both the drug preparation and observation. To ensure that I did not know which dose was being given to which animal, someone else assigned labels of A, B, C and D to the vials of drug used. On completion of the video analysis, I discovered which drug dose had been assigned which label.
differences in frequency, or direction of the oral motor behaviour shown by the lesioned and sham-lesioned rats.

**Histological procedures and lesion assessment**

Rats were sacrificed between 36 and 45 days following surgery; sacrifices from each group were spread evenly over this period. Rats were deeply anaesthetised and perfused transcardially with 0.1 M PBS at 37°C, followed by at least 300 ml fixative (4% paraformaldehyde in 0.1 M phosphate buffer). The brains were post-fixed for 60-120 min in 4% paraformaldehyde at room temperature. 50 μm coronal sections were cut on a freezing microtome. For lesion assessment sections were collected from the anterior portion of the cerebellum through to the posterior substantia nigra. Adjacent sections were stained for Nissl using cresyl violet and NADPH-diaphorase using a modification of the method of Vincent and colleagues (Vincent et al. 1983b; Vincent et al. 1992; Inglis et al. 1993). Another set of sections were cut through the CP and stained with cresyl violet for verification of cannulae placements.

All sections were inspected using a Leitz Diaplan microscope fitted with a Sony DXC-3000P video camera for visualisation of sections on a high resolution colour monitor. Lesions were identified in cresyl violet stained sections by the presence of gliosis and degenerating neuronal somata. Estimates of neuronal loss in the PPTg and the surrounding structures were made on a scale of 0 to 4 as described by Inglis et al. (1994a), and direct cell counts from NADPH-diaphorase sections allowed the assessment of damage to PPTg cholinergic neurones. Cannulae placements were mapped onto schematic drawings redrawn from the atlas of Paxinos and Watson (1986).

**Statistical Analysis**

The duration of body licking and biting data was analysed using Fisher Exact Probability Test (Siegel 1956), while all other video analysis and regulatory measures were analysed parametrically using ANOVA and post hoc tests were carried out where necessary using Tukey's method for multiple comparisons. Time-based data were log transformed to reduce skewness before being analysed using ANOVA. The Creese-Iversen ratings were analysed using $\chi^2$ (Siegel 1956).
13.2. Results

Histological Analysis

Fig. 13.1 shows the VLCP cannulae placements for all rats. 8/9 PPTg lesioned and 6/8 control rats had cannulae placed in the VLCP bilaterally; the other three had unilateral placements. All were included in the following analyses because results from a previous experiment (Chapter 12) showed that a unilateral placement was sufficient to generate biting in response to d-amphetamine as great as that generated by bilateral injection. Fig. 13.2 depicts the largest and smallest lesion for the group; Fig. 13.3 shows diaphorase-positive neurones in the control and PPTg-lesion groups; Table 13.1 presents a summary of the average lesion volume and per-cent NADPH-diaphorase cell loss for the PPTg-lesioned rats. The lesions were generally slightly larger than those reported previously by Inglis et al. (1994a) as is clear from Fig. 13.2 and Table 13.1. The table does not list the occasional damage seen in the ventral spinocerebellar tract (4 rats), or the Kolliker-Fuse nucleus (5 rats) because the damage in these places was always unilateral and amounted to less than 30% of the volume of the structure concerned. The lesions of PPTg were relatively uniform: 8 rats showed almost complete destruction of the PPTg, as is clear from Fig. 13.2 and from the fact that the average loss of diaphorase-positive neurones was 76% (SEM=4.44). Seven of these 8 also had damage in the adjoining retrorubral nucleus. The cuneiform nucleus was also damaged in 4 rats while the others showed lesser degrees of loss. Six rats had 30-60% damage to the parabrachial nucleus; the other 3 had less than 30%. All the rats had sustained some damage to the superior cerebellar peduncle. The amount of damage caused by the ibotenate to other structures was negligible. What have previously been identified as calcium-rich deposits (Winn 1991) often identified the borders of the lesion.

Regulatory Measures

Fig. 13.4 shows the average body weight, food intake, water intake and food spillage per group for the 7 days before and 21 days after surgery. Analysis of pre-operative data revealed no significant differences between the groups. (ANOVA group by days interactions: body weight $F_{6,90}=2.14$; spillage $F_{6,90}=2.1$; food intake $F_{6,90}=0.29$; water intake $F_{6,90}=0.73$.) Of the main effects, only that for body weight over days
was significant \((F_{6,90}=119.7, P<0.01)\) reflecting the steady daily increase in weight which occurs in all rats.

After surgery, ANOVA of group body weights revealed neither a significant effect of group \((F_{1,15}=0.05)\) nor a group by days interaction \((F_{20,300}=0.38)\). The significant days effect \((F_{20,300}=171.42, P<0.01)\) found reflects the drop in weight seen immediately after surgery and then the steady daily increase in weight but this was common to all rats. A similar pattern of results was found for food spillage: no significant effect of group \((F_{1,15}=1.06)\) or group by days interaction \((F_{20,300}=0.98)\) but a significant days effect \((F_{20,300}=2.28, P<0.01)\), reflecting the variation in spillage in the days immediately following surgery. For food intake, analysis produced no significant main effect of group \((F_{1,15}=0.19)\) but there was a significant effect of days \((F_{20,300}=41.75, P<0.01)\) and a group by days interaction \((F_{20,300}=2.29, P<0.01)\). Post hoc analysis revealed that the PPTg-lesioned group ate significantly less food for the first three days following surgery \((P<0.01)\), but as they recovered from surgery their food intake returned to the level of sham-lesioned controls. Water intake was more systematically affected: ANOVA revealed significant main effects of group \((F_{1,15}=6.09, P<0.05)\) and days \((F_{20,300}=15.21, P<0.01)\) and a group by days interaction \((F_{20,300}=2.49, P<0.01)\). Post hoc testing indicated that over the 21 days following surgery the PPTg-lesioned rats drank significantly less than sham-lesioned controls \((P<0.01)\). Post hoc analysis identified statistically significant differences on days 1, 2, 3, 5 \((P<0.01)\), 6, 7, 9, 10, 11, 15, 16, 17, 18, 19, 20 \((P<0.05)\). It is important to note however that the reduction in water intake was never large \((< 5 \text{ ml})\) and did not affect body weight in the way significant dehydration would have done.

**Analysis of Creese-Iversen Data**

Table 13.2 shows the Creese-Iversen scores for PPTg-lesioned and sham-lesioned groups. Representative group scores were calculated by taking the frequency of each score for all the rats in each group at a given dose, dividing this by the number of scores/group and multiplying by 100. For analysis by \(\chi^2\) data were collapsed into three categories: 0-1 (no stereotypy), 2-4 (sniffing and rearing stereotypies) and 5-6 (licking and biting stereotypies). The behaviour of the two groups was similar following administration of saline; scores of 0 and 1 predominated in both groups, showing that PPTg lesions had no effect on baseline scores. At 5.0 \(\mu\)g and 10.0 \(\mu\)g d-
amphetamine there were no differences in the frequency of scores of 2-4. PPTg-lesioned rats however scored 0 or 1 less frequently than controls and 5 or 6 more frequently following 5.0 µg (χ²=8.2, df=2, P<0.02) and 10.0 µg d-amphetamine (χ²=22.7, df=2, P<0.001). At 20.0 µg d-amphetamine the proportion of scores of 0 and 1 did not differ between the groups, however PPTg-lesioned rats were rated 5 or 6 more frequently and 2-4 less frequently than sham-lesioned controls (χ²=14.5, df=2, P<0.001). In summary these data show that PPTg-lesioned rats scored 5-6 on the Creese-Iversen scale more frequently than sham-lesioned controls and, while sham-lesioned rats did display biting and licking in response to intra-VLCP d-amphetamine as expected, the PPTg-lesioned rats showed a marked shift to the left in the doseresponse curve.

Parametric analysis of oral motor behaviours

Fig. 13.5 shows the time spent by rats in each group in grooming, biting/licking their cages and in vacuous mouth movements. Grooming was affected by PPTg lesions (F₁,₁₅=5.94, P<0.05) and there was a significant main effect of dose (F₃,₄₅=15.56, P<0.01), and group by dose interaction (F₃,₄₅=6.75, P<0.01). Post hoc analysis revealed that PPTg-lesioned rats groomed less than controls following 20.0 µg and that both groups groomed much less following 20.0 µg amphetamine in comparison to 5.0 µg, 10.0 µg and saline (all P<0.01). The PPTg-lesioned rats showed a longer duration of vacuous mouth movements (main effect of group F₁,₁₅=15.23, P<0.01) but this was not dose-dependent (main effect of dose F₃,₄₅=1.45; group by dose interaction F₃,₄₅=0.43). The greatest difference between the two groups was in the time spent licking or gnawing the cage for which ANOVA revealed significant main effects of group (F₁,₁₅=4.70, P<0.05) and dose (F₃,₄₅=30.03, P<0.01) and a group by dose interaction (F₃,₄₅=3.31, P<0.05). Post hoc analysis revealed that both groups showed an increase in the duration of cage licking/gnawing at all doses compared with saline (all P<0.01) with the PPTg-lesioned group spending significantly longer periods engaged in this activity in comparison to sham-lesioned rats at all three doses (P<0.01).

In contrast to the cage licking/gnawing data, an ANOVA could not be computed using the body licking/biting data because of the high variance and the
failure of some rats to display this type of behaviour at all. The Fisher exact probability test was used to assess the probability that the behaviour would occur. Following saline injections, none of the animals exhibited this type of oral motor behaviour. There were no significant differences between the groups in the probability of body licking/biting occurring after 5.0 μg \((P=0.102)\) or 10.0 μg \((P=0.290)\) \(d\)-amphetamine. Only after 20.0 μg \(d\)-amphetamine did the PPTg-lesioned rats show a greater probability of body licking/biting \((P=0.041)\) than the sham-lesioned group. Thus the PPTg-lesioned group exhibited more bouts of body licking/biting only at the highest dose of \(d\)-amphetamine.

Analyses of the latency to display grooming and the latency to display abnormal oral behaviours were conducted and mean values are shown in Fig. 13.6. Abnormal oral behaviours were considered to be a combination of the cage licking and biting, body licking and biting and vacuous mouth movements categories. There were no differences in the two groups in the latency to exhibit grooming \((F_{1,15}=0.00)\) or abnormal oral behaviours \((F_{1,15}=4.27)\). There was an effect of dose on the latency to display grooming \((F_{3,45}=2.94, P<0.05)\), though there was no group by dose interaction \((F_{3,45}=1.54)\). Post hoc analysis revealed that both groups engaged in grooming much sooner following saline in comparison to 10.0 μg \((P<0.05)\). However, the dose of \(d\)-amphetamine had no effect on the latency to exhibit abnormal oral behaviours \((F_{3,45}=1.41)\), nor was there a group by dose interaction \((F_{3,45}=0.33)\).

When looking at the number of bouts of grooming (Fig. 13.6), there were no differences between the groups \((F_{1,15}=3.08)\), nor was there any interaction with dose. This indicates that there were no differences between the two groups in the number of times that they engaged in normal oral behaviours. The dose of \(d\)-amphetamine did have an effect on the number of grooming bouts displayed \((F_{3,45}=3.53, P<0.05)\), with both groups displaying fewer bouts of grooming following 20.0 μg in comparison to saline \((P<0.05)\). However, when looking at the number of bouts of abnormal oral motor activity engaged in, there was a significant main effect of group \((F_{1,15}=6.13, P<0.05)\), dose \((F_{3,45}=21.93, P<0.01)\) and group by dose interaction \((F_{3,45}=3.57, P<0.01)\). Although both groups showed an increase in the number of bouts of abnormal oral motor activity at 5.0 μg, 10.0 μg and 20.0 μg (all \(P<0.05)\) compared with saline, the PPTg-lesioned group engaged in more bouts
of abnormal oral behaviour compared with the sham-lesioned controls at all doses
($P<0.01$).

13.3. Discussion

These data confirm those of Kelley et al. (1988), in that microinjections of $d$-amphetamine into the VLCP clearly increased the incidence of abnormal oral motor activities. They also show that ibotenate-induced lesions of the PPTg affected this response. These data extend the findings of Inglis et al. (1994a), which were concerned with the effects of PPTg lesions on responses to systemic $d$-amphetamine and apomorphine, and strengthen the hypothesis that the PPTg is involved in the mediation of oral motor behaviours controlled by the VLCP. The rats showed no long-lasting regulatory deficits following surgery, indicating that ordinarily they had no problem in co-ordinating their oral musculature for the purpose of feeding and drinking. The PPTg-lesioned group did however show reduced daily water consumption in the home cage. This was never more than 5 ml a day, and was not statistically significant every day and although formal tests have never been conducted, there is no reason to believe that this represents a regulatory deficit. There were also no measurable differences between the PPTg-lesioned and control rats following microinjection of saline into the VLCP, demonstrating that the PPTg-lesioned group displayed no spontaneous changes in oral motor behaviour.

Analysis of the Creese-Iversen data showed that bilateral lesions of the PPTg caused a shift in the dose response curve to $d$-amphetamine, as judged by the increased frequency with which scores of 5 and 6 were given to PPTg-lesioned compared to control-lesioned rats. In both groups the licking and biting was predominantly directed towards the bars of the cage floor, though licking and biting of the paws was also observed. The parametric analysis added to this analysis by showing where the differences lie in the structure of behaviour. Compared to controls, PPTg lesioned rats showed an increased number of bouts of abnormal motor behaviour and an increase in the duration of vacuous mouth movements. They also spent more time cage licking and biting; and, at the highest dose of amphetamine an increased frequency of body licking and/or biting. However in comparison to sham-lesioned controls they showed no changes in the duration of normal oral motor
activity associated with grooming and no changes in the latency to initiate either normal or abnormal oral motor behaviours.

Thus the behavioural evidence presented here supports the data reported by Inglis et al. (1994a) which showed a shift in the dose response curve to amphetamine following bilateral excitotoxic lesions of the PPTg. However in that study the amphetamine was injected systemically, and the licking and biting effects found could have been the result of general activation of DA (and NA) systems. This experiment combined bilateral lesions of the PPTg with microinjections of amphetamine into the VLCP. This approach meant that the stimulation was specific to the VLCP, an area that has been reported to mediate the oral stereotypies shown in response to high doses of amphetamine (Kelley et al. 1988). In this experiment it has been shown that bilateral lesions of the PPTg cause a shift in the dose response curve, intensifying this oral motor response which appears at lower doses.
Table 13.1. Summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Lesion placement:</th>
<th>PPTg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Damage assessment:</strong> (modal scores)</td>
<td></td>
</tr>
<tr>
<td>Basal Cerebral Peduncle</td>
<td>x</td>
</tr>
<tr>
<td>Central gray</td>
<td>ND</td>
</tr>
<tr>
<td>Central tegmental tract</td>
<td>x</td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>xx</td>
</tr>
<tr>
<td>Deep mesencephalic nucleus</td>
<td>xxxx</td>
</tr>
<tr>
<td>Dorsal sub coeruleus nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Dorsal tegmental bundle</td>
<td>x</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>x</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>x</td>
</tr>
<tr>
<td>Microcellular tegmental nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Oral pontine reticular nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Parabrachial nucleus</td>
<td>xx</td>
</tr>
<tr>
<td>Paralemniscal nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Paratrochlear nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>xxxx</td>
</tr>
<tr>
<td>Retrorubral field</td>
<td>x</td>
</tr>
<tr>
<td>Retrorubral nucleus</td>
<td>xxxx</td>
</tr>
<tr>
<td>Rubrospinal tract</td>
<td>x</td>
</tr>
<tr>
<td>Subpeduncular tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Substantia nigra pars compacta</td>
<td>x</td>
</tr>
<tr>
<td>Substantia nigra zona reticulata</td>
<td>x</td>
</tr>
<tr>
<td>Superior cerebellar peduncle</td>
<td>x</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>x</td>
</tr>
<tr>
<td>Supra trigeminal nucleus</td>
<td>x</td>
</tr>
</tbody>
</table>

| Lesion Volume (mm$^3$):                             | 4.96  |
| (Mean ± SEM values)                                 | ± 0.405 |
| % NADPH-diaphorase Cell Loss:                       | 75.93 |
| (Mean ± SEM values)                                 | ± 4.44 |

Table 13.1. A summary showing structural damage, mean (±SEM) lesion volume (mm$^3$) calculated from cresyl violet sections and % cell loss calculated from NADPH-diaphorase cell counts. Damage was identified by glial infiltration and degeneration of neuronal somata, and a summary of affected structures for every animal estimated. The modal score in the group was then taken for each structure. The key is as follows: ND No damage; x < 30%; xx 30 - 60%; xxx 60 - 90%; xxxx > 90%.
Table 13.2. % Creese-Iversen scores following drug treatments

<table>
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<tr>
<th>Dose Licking</th>
<th>Lesion Condition</th>
<th>No stereotypy</th>
<th>Sniffing-Rearing</th>
<th>Biting-Licking</th>
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<td></td>
<td>0 1 2 3 4 5 6</td>
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<tr>
<td>Saline</td>
<td>PPTg-lesioned</td>
<td>35.7 59.3 2.5 0.0 2.5 0.0 0.0</td>
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<tr>
<td></td>
<td>Sham-lesioned</td>
<td>37.5 54.1 6.9 0.0 1.4 0.0 0.0</td>
<td></td>
<td></td>
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<tr>
<td>5.0µg</td>
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<td>1.2 8.6 17.4 7.4 27.2 37.0 1.2</td>
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<tr>
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<tr>
<td>10.0µg</td>
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<td>1.2 0.0 4.9 14.8 30.9 43.3 4.9</td>
<td></td>
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<tr>
<td></td>
<td>Sham-lesioned</td>
<td>15.3 6.9 11.1 12.5 33.4 20.8 0.9</td>
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<tr>
<td>20.0µg</td>
<td>PPTg-lesioned</td>
<td>0.0 7.4 2.5 4.9 17.3 51.9 16.0</td>
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<tr>
<td></td>
<td>Sham-lesioned</td>
<td>6.9 4.2 13.9 18.1 19.4 34.7 2.8</td>
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Table 13.2. Creese-Iversen scores. Percentages were calculated by taking the frequency of each score for all the rats in the group, dividing this by the total number of scores per group and then multiplying by 100.
Figure 13.1. Representative sections through the VLCP showing cannulae placements for the PPTg-lesioned and control rats: picture A represents the PPTg-lesioned rats, and picture B the sham-lesioned controls. Sections redrawn from the atlas of Paxinos and Watson (1986).
Figure 13.2. Representative sections through the PPTg showing lesion volumes. Shading represents the largest and smallest lesion following ibotenate infused into the PPTg; sections redrawn from the atlas of Paxinos and Watson (1986).
Figure 13.3 Photomicrographs showing NADPH-diaphorase stained neurones in the PPTg.

A. A section from a sham-lesioned rat showing LDTg neurones embedded in the central gray, SPTg neurones are beneath the scp and PPTg neurones between the scp and lemniscal fibres. Scale bar = 0.25 mm

B. A section from an ibotenate-lesioned rat. The LDTg and SPTg are clearly intact, but the majority of NADPH-diaphorase positive neurones in the PPTg have been lost. Scale bar = 0.25 mm.

C. Section B at higher magnification. Calcium rich-deposits can be seen in the region of the PPTg. Scale bar = 0.1 mm.
Figure 13.4. Mean body weight, spillage, food and water intake for PPTg-lesioned and control rats for the 7 days before and 21 days after surgery. For statistical analysis see text.
Figure 13.5. Mean (±SEM) duration (sec) of oral motor behaviours for both PPTg-lesioned and control rats following intra-VLCP microinjection of \(d\)-amphetamine. The duration of abnormal oral motor behaviours (i.e. all oral motor activity minus grooming), grooming, vacuous mouth movements, cage licking/gnawing are all shown. For statistical analysis see text.
DURATION OF GROOMING

SEC (LOG TRANSFORMED)

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<tr>
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DURATION OF VACUOUS MOUTH MOVEMENTS

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<tr>
<td>20.0ug</td>
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DURATION OF CAGE LICKING/GNAWING

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<tr>
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<td>2.0</td>
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<td>1.5</td>
</tr>
<tr>
<td>20.0ug</td>
<td>2.0</td>
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</table>
Figure 13.6 Mean (±SEM) latency (sec) to engage in grooming and abnormal oral motor behaviour and mean (±SEM) number of bouts of grooming and abnormal oral behaviour for PPTg-lesioned and control rats. For statistical analysis see text.
LATENCY TO DISPLAY GROOMING
SEC (LOG TRANSFORMED)

LATENCY TO DISPLAY ORAL BEHAVIOURS
SEC (LOG TRANSFORMED)

NUMBER OF BOUTS OF GROOMING

NUMBER OF BOUTS OF ORAL BEHAVIOUR

D-AMPHETAMINE SULPHATE
Chapter 14. Investigation of oral motor behaviours, spontaneous locomotion, orienting, and freezing to overhead threat following bilateral lesions or knife cut lesions of the pedunculopontine tegmental nucleus and bilateral cannulations of the ventrolateral caudate-putamen.

Following the results reported in Chapter 13 it was decided to repeat and extend the experiment. The quinolinate lesion was repeated since an effective quinolinate lesion might allow the investigation of whether the cholinergic or non-cholinergic PPTg underlies the oral motor stereotypies, if the results of Rugg et al. (1992) and Dunbar et al. (1992) could be replicated. A further experimental group of rats who received undercuts of the PPTg were also added to investigate whether the descending outflow fibres of the PPTg could be severed, and if this would affect the oral motor response. The knife cut lesions were performed by Dr Peter Redgrave. Three additional behavioural tests were also carried out; spontaneous locomotion, orienting and freezing to overhead threat to investigate whether the lesions or knife cuts had any effect on these behaviours. Freeing to overhead threat and the test of orientating are tests which can be used to investigate collicular dysfunction. Previous research has implicated the PPTg in the acoustic startle response (Jordan and Leaton 1983; Koch et al. 1993), and the PPTg receives afferent innervation from the SC (Steininger et al. 1992), which is involved in orienting to sudden moving stimuli (Redgrave et al. 1992).

14.1. Methods

60 male hooded Lister rats (Olac) were individually housed and kept under a 12 h light/dark cycle (lights on 08:00 h). They were maintained *ad lib.* on SDS maintenance diet no.1 chow pellets and tap water. Mean body weight at the time of surgery was 322.41 g (SD= 19.15).

*Surgery*

Rats were divided into four groups: ibotenate-, quinolinate-, knife cut- and sham-lesioned controls. Ibotenic acid was prepared as described in Chapter 12. Quinolinic
acid (Sigma) was prepared as a 0.12 M solution using the same method. Rats in the quinolinate group were anaesthetised with 10 ml·kg⁻¹ Avertin (see Chapter 13). All other rats were anaesthetised using 60 mg/kg sodium pentobarbitone (Sagatal; RMB Animal Health Ltd) which was diluted 50:50 with sterile water. The rats were placed in a stereotaxic frame with the skull level. Ibosinate, quinolinate and sham lesions were made using the same protocol described in Chapter 13. Bilateral knife cut lesions were made during one surgical procedure at the following co-ordinates: 2.5 mm anterior to the interaural line, ±1.0 mm from the midline and 7.8 mm from the skull surface. A retractable knife encased in a 27 ga stainless steel sheath was inserted and the blade, made of tungsten wire, extended at the appropriate site. The blade was then turned through an arc in the mediolateral plane, retracted and removed.

During the second unilateral operation (or immediately following the knife cut lesions) rats also received bilateral cannulation of the VLCP. Cannulae were implanted following the same protocol described in Chapters 12 & 13, at the following co-ordinates: 2.5 mm anterior to bregma, ±3.5 mm from the midline and 4.5 mm below the skull surface. The rats were allowed at least 15 days to recover from surgery before testing began.

**Intracranial Drug Administration**

All microinjections were made via 30 ga stainless steel cannulae terminating 7.5 mm below the skull surface. Three different doses of d-amphetamine sulphate (5.0 µg, 10.0 µg and 20.0 µg dissolved in 0.5 µl 0.9% saline) were administered and an additional saline-only injection acted as a control. Amphetamine and saline were administered in a counterbalanced order; 48 h separated each injection. For full description of the procedure refer to Chapter 12.

---

1 Sagatal was used as the anaesthetic of choice for the ibosinate-lesioned, sham-lesioned and knife cut-lesioned groups because there is no risk of post-operative gastric pathology as there is with Avertin and barbiturate anaesthetic does not affect the efficacy of ibosinate. Avertin was used for the quinolinate-lesioned group because barbiturates affect the potency of quinolinate. These rats also received glucose injections as described in footnote 2 chapter 13.

2 If the rat weighed >350g at the time of surgery, the PPTg co-ordinates were modified to account for this larger body size. The co-ordinates were as follows: 1.3 mm anterior to the interaural line, ±2.1 mm from the midline and 7.3 mm below the skull surface (posterior PPTg); and 2.0 mm anterior to the interaural line, ±2.2 mm from the midline and 8.1 mm below the skull surface (anterior PPTg). These co-ordinates were used for 3 rats.
Measurement of oral motor behaviour

Prior to drug testing each rat was habituated to the test procedure over 2 days. Test sessions lasted 45 min, took place under red light illumination and were recorded onto videotape. The tapes were then analysed by an observer who was blind with respect to the drug condition. For full description of procedure and apparatus refer to Chapter 12. All the sessions for each rat were observed on videotape and the incidence of oral motor behaviours exhibited by each rat was timed. Oral motor behaviour fell into four categories: (i) grooming; (ii) licking or biting of the body; (iii) licking or gnawing the cage; (iv) vacuous mouth movements. For each category the latency to begin the behaviour (sec), the number of bouts and the duration of each bout (sec) was recorded. A bout was defined as the occurrence of a target activity which lasted a minimum of 3 sec. This detailed analysis provided a quantitative measurement of oral motor behaviour for statistical analysis.

Locomotor Activity

Spontaneous locomotor activity was measured for three consecutive days, each session lasting 60 min. Rats were placed into individual locomotor cages (38 cm X 24 cm X 19 cm) through which passed two infra-red light beams. Each time the beams were interrupted a count was registered by a computer ("Spider" Paul Fray Ltd). The beams had to be broken sequentially to avoid misrepresenting behaviour directed towards one beam only (e.g. sniffing) as locomotion. Testing took place under blue light illumination to prevent the infra-red light beams registering false counts from the lights.

Orienting Behaviour

Each rat was placed in turn in an open-topped cage, with a sawdust floor under blue light illumination. A piece of dry spaghetti was used to touch the rat. (Spaghetti of uniform length was used, because it gives a fair indication of the force of the stimulation). The rats were touched hard enough for the piece of spaghetti to bend, but not break. Each rat was stimulated 8 times: left hind flank (X 2), right hind flank (X 2), left fore flank (X 2) and right fore flank (X 2). The rats received this in a counterbalanced order and were tested for 5 consecutive days. The rats' reactions were scored according to a scale developed by Dunnett et al. (1985) as follows: 0 =
no response; 1 = weak head turning response; 2 = strong head turning response. Thus the maximum possible score for each session was 16, the scores from each session were added together to give each rat a final score out of 80.

**Freezing to Overhead Threat**

Each rat was placed in turn into a cage with a sawdust floor and a perspex lid placed over the top. Rats were left for 30 sec and then a heavy bunch of keys was dropped from a height of 25 cm onto the perspex where it remained for a further 60 sec. The length of time the rat remained motionless was measured in seconds. Each rat was examined using this procedure once only.

**Histological procedures and lesion assessment**

Rats were sacrificed between 34 and 57 days after surgery; sacrifices from all groups were spread evenly over this period. Rats were deeply anaesthetised and then perfused transcardially as described in Chapter 13. The brains were removed and post-fixed for 60-120 and then 50 μm coronal sections were cut on a freezing microtome. For lesion assessment sections were collected from the anterior portion of the cerebellum through to the posterior substantia nigra. Adjacent sections were stained for Nissl using cresyl violet and NADPH-diaphorase using a modification of the method of Vincent and colleagues (Vincent et al. 1983b; Vincent et al. 1992; Inglis et al. 1993). Another set of sections 25 μm thick were cut through the CP and stained with cresyl violet for verification of cannulae placements.

All sections were inspected using a Leitz Diaplan microscope fitted with a Sony DXC-3000P video camera for visualisation of sections on a high resolution colour monitor. Lesions were identified in cresyl violet stained sections by the presence of gliosis and degenerating neuronal somata. Estimates of neuronal loss in the PPTg and the surrounding structures, and direct cell counts from NADPH-diaphorase sections were made as described in Chapter 13. Nissl body counts were also made; 3 representative sections that corresponded most closely to 0.7 mm, 1.00 mm, and 1.70 mm anterior to the interaural line (sections taken from the atlas of Paxinos and Watson 1986) were selected. Under a magnification of X16 nissl bodies in the area most closely corresponding to the PPTg were counted with the aid of a
gridded graticule. Cannulae placements were mapped onto schematic drawings taken from the atlas of Paxinos and Watson (1986).

**Statistical Analysis**

All video analysis and regulatory measures were analysed parametrically using ANOVA and *post hoc* tests were carried out where necessary using Tukey’s method for multiple comparisons. Time-based data were log transformed to reduce skewedness, while locomotor counts were square-root transformed to reduce the homogeneity of variance before being analysed using ANOVA.

14.2. Results

**Histological Analysis**

Of the initial 60 rats, 11 died either during or immediately following surgery, 7 were discarded due to poor lesions or misplaced cannulae, 3 lost headcaps before the end of the microinjection study and 1 was euthanised following a suspected stroke. This left a total of 38 rats, with group content as follows: ibotenate group (N=12), quinolinate group (N=9), knife cut group (N=6) and sham-lesioned controls (N=11). Figs. 14.1-14.4 show the VLCP cannulae placements for all rats in the four groups. One rat in each of the ibotenate-, quinolinate- and sham-lesioned groups had a unilateral placement, all other rats had bilateral placements within the VLCP. The 3 rats with unilateral placements were included in the analyses because results reported in Chapter 12 showed that a unilateral placement was sufficient to generate biting in response to *d*-amphetamine.

Figs. 14.5-14.7 depict the largest and smallest lesion for each group. Tables 14.1-14.3 present summaries of the average lesion volume and per-cent NADPH-diaphorase cell and nissl stained cell loss for the 3 groups of lesioned rats. Fig. 14.8 shows the means (± SEM) for the diaphorase-positive cell counts and nissl counts for each group. Different excitotoxins have been used in the PPTg in an attempt to make relatively selective lesions, of either cholinergic or non-cholinergic neurones (Rugg et al. 1992; Dunbar et al. 1992). In this experiment the number of cholinergic neurones is indicated by NADPH-diaphorase histochemistry (see Chapter 15). Nissl staining displays all neurones, so the numbers of non-cholinergic neurones can be estimated as
the number of nissl stained neurones minus the number of diaphorase-positive neurones. In the following text therefore, "cholinergic neurones" are those stained by NADPH-diaphorase, and "non-cholinergic neurones" are the number of nissl stained neurones minus the diaphorase positive neurones. Analysis to investigate whether there were any differences between the groups revealed a significant effect of group for both diaphorase-positive cell counts \((F_{3,72}=100.26, P<0.001)\) and nissl counts \((F_{3,70}=163.63, P<0.001)\). Post hoc testing showed that the ibotenate-lesioned group had significantly less diaphorase-positive cells than all the other groups \((P<0.001)\), and that the quinolinate-lesioned rats also had significantly less diaphorase profile counts than the knife cut group \((P<0.001)\) and the shams \((P<0.05)\). For the nissl counts post hoc testing revealed exactly the same pattern of results \((all \ P<0.001, except\ the \ quinolinate-lesioned \ group \ also \ had \ less \ nissl \ than \ the \ knife \ cut \ group \ P<0.05)\). Previous studies have indicated that quinolinate is more selective for cholinergic neurones while ibotenate destroys both cholinergic and non-cholinergic neurones equally well (Rugg et al. 1992; Dunbar et al. 1992). It was decided to investigate whether there were any differences between the ibotenate-lesioned and quinolinate lesioned rats in the proportion of non-cholinergic to cholinergic loss in comparison to the sham-lesioned controls. The cell profile count for the non-cholinergic neurones for each rat was divided by its corresponding cholinergic count and a one-way ANOVA executed. ANOVA revealed no significant effects between the groups \((F_{2,61}=0.502)\) which indicates that the proportion of cholinergic to non-cholinergic loss does not differ between the groups and therefore that ibotenate and quinolinate both destroy equal proportions of cholinergic and non-cholinergic neurones (Figure 14.9.). The knife cut-lesioned group were excluded from this analysis since it was concerned with comparisons of excitotoxins.

**Ibotenate-lesioned rats:** The lesions of the PPT\(g\) were consistent, with 7 of the rats showing almost total destruction of the PPT\(g\) and the other 5 showing damage of between 70-90%. The average diaphorase positive cell loss was 82.67\% \((SEM=2.92)\), and nissl loss averaged 94.81\% \((SEM=0.98)\). All the ibotenate-lesioned rats also had damage to the cuneiform nucleus. In 8 rats this was fairly extensive (between 60-90\%) but the remaining 3 had much less damage to this structure. The retrorubral nucleus was the only other structure that was frequently damaged by the ibotenate lesion. Three rats had almost total damage of the retrorubral nucleus.
 (>90%), 5 rats had extensive damage (60-90%) and 4 rats had lesser damage (30-60%). Only one rat showed any damage to the laterodorsal tegmental nucleus, the locus of the Ch6 neurones, and this damage was less than 30%. Similarly only one rat had damage to the subpeduncular tegmental nucleus that fell within the 30-60% damage assessment, while 7 rats displayed less than 30% damage and 4 had no damage at all. There was an even split of rats with damage to the parabrachial nuclei, with 6 rats showing damage of less than 30% and 6 with damage ranging from 30-60%. All the rats sustained minor damage to the superior cerebellar peduncle and the amount of damage to other structures was small. The borders of the lesion were often identified by the presence of calcification (Winn 1991).

**Quinolinate-lesioned rats**: Damage to the PPTg in the quinolinate-lesioned rats was much more variable, and much harder to assess from nissl stained sections. The average loss of diaphorase-positive neurones was small (24.58% SEM=5.32), as was the average loss of cell bodies stained for nissl (37.43% SEM=7.09). The lesions were identified and drawn (Figure 14.6) using the presence of gliosis as a guide, although there were often intact neurones within the area shaded. Very large amounts of calcium-rich deposits were seen round the borders of the lesion and these deposits often stretched a considerable distance.

**Knife cut-lesioned rats**: Assessment of both diaphorase-positive cells and nissl staining showed that there was no damage within the PPTg. There was an apparent mean increase in diaphorase positive cell bodies of 16.37% (SEM=5.6), although the diaphorase figures lie within the range of those of the sham-lesioned controls and was not statistically significant compared to controls. Therefore it can be concluded that there was no significant effect on the diaphorase-positive cells. From the nissl counts it was found that there was again a non-significant loss; 18.68% (SEM=4.91). The knife cut lesions caused very little structural damage, only 2 rats showed any damage to the PPTg, and in both cases this was less than 30%, all the rats also sustained damage to the ventrolateral tegmental nucleus of between 30-60%.

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3 One rat in this group sustained damage to the nissl sections during histological processing and was excluded from all nissl analysis and damage profile assessment, reducing the group size to (N=8). However from what could be seen on the nissl sections, the good quality diaphorase sections and behavioural data, it was decided to keep this rat for all other analyses.
Parametric analysis of oral motor behaviours

Figures 14.10-14.12 show the mean (± SEM) duration of grooming, vacuous mouth movements and cage and body licking and biting for each group. Grooming was affected, ANOVA revealing a significant effect of group ($F_{3,34}=4.38, P<0.01$) and a main effect of dose ($F_{3,102}=12.02, P<0.01$), but no group by dose interaction ($F_{9,102}=0.34$). Post hoc analysis revealed that ibotenate-lesioned rats groomed less than both the sham-lesioned controls and the knife cut lesioned group ($P<0.05$) at all doses, and all groups of rats groomed less following 10.0 μg and 20.0 μg amphetamine compared to saline ($P<0.05$ and $P<0.01$ respectively). They all also groomed less following 20.0 μg compared to doses 5.0 μg and 10.0 μg amphetamine ($P<0.01$ and $P<0.05$ respectively).

Although there was no overall effect of dose on the duration of vacuous movements ($F_{3,102}=0.37$), there was a significant main effect of group ($F_{3,34}=6.34, P<0.01$) and group by dose interaction ($F_{9,102}=2.31, P<0.05$). Post hoc analysis showed that the ibotenate-lesioned group was engaged in making vacuous mouth movements for longer periods of time compared to all the other groups following 5.0 μg amphetamine ($P<0.05$ compared to the sham-lesioned controls and quinolinate-lesioned group, and $P<0.01$ compared with the knife cut-lesioned group). Following 10.0 μg amphetamine the knife cut-lesioned group showed shorter durations of vacuous mouth movements compared to all the other groups ($P<0.05$ compared to the sham-lesioned controls, and $P<0.01$ compared with the ibotenate-lesioned and quinolinate-lesioned group). Following 20.0 μg amphetamine the ibotenate-lesioned group spent longer engaged in vacuous mouth movements than either the knife cut-lesioned ($P<0.01$) or the quinolinate-lesioned group ($P<0.05$).

For analysis of licking and biting the categories for the duration of licking and biting of the body and licking and biting of the cage floor were added together to give a single measure of licking and biting in contrast to the separate analyses described in Chapter 13. This was done following the observation that the rats in this experiment locked into either licking and biting of the cage floor or body at the higher doses and did not alternate between them. Thus the data were combined since separate analysis would have weakened the differences between the groups which observation indicated were very strongly apparent. Analysis revealed significant main effects for group ($F_{3,34}=3.89, P<0.01$), dose ($F_{3,102}=119.38, P<0.01$) and a group by dose
interaction \( (F_{9,102}=3.34, P<0.01) \). Post hoc analysis showed that the ibotenate-lesioned rats spent longer periods of time engaged in cage and body licking and biting compared to all the other groups following all three doses of amphetamine \( (P<0.01 \) in all instances). Following 10.0 \( \mu \)g the sham-lesioned controls also spent longer licking and biting in comparison to the quinolinate-lesioned group \( (P<0.01) \). All the groups spent longer licking and biting following all three doses of amphetamine compared to saline \( (P<0.01) \) and following doses 10.0 \( \mu \)g and 20.0 \( \mu \)g compared to 5.0 \( \mu \)g \( (P<0.01) \).

Figure 14.13 shows the mean values \( (\pm\text{ SEM}) \) for the latency to begin grooming and the latency to display abnormal oral motor behaviours (all oral motor activity minus grooming). The dose of amphetamine had no effect on the latency to begin grooming \( (F_{3,102}=1.08) \) and there was also no group by dose interaction \( (F_{9,102}=0.64) \) though an overall group effect was identified \( (F_{3,34}=5.12, P<0.01) \) which revealed that the ibotenate-lesioned rats showed an increased latency to begin grooming in comparison to the sham-lesioned controls \( (P<0.01) \). The same pattern of results was produced from analysis of the latency to display abnormal oral motor behaviours: the dose of amphetamine had no effect \( (F_{3,102}=1.10) \), and there was no group by dose interaction \( (F_{9,102}=1.89) \). Again there was a main effect of group \( (F_{3,34}=7.54, P<0.01) \) which this time revealed that the ibotenate-lesioned rats showed a decreased latency to engage in abnormal oral behaviours in comparison to both knife cut-lesioned group and sham-lesioned controls \( (both\ P<0.01) \) but not the quinolinate-lesioned group.

Analysis of the number of bouts of grooming showed there were no differences between the groups \( (F_{3,34}=2.52) \), nor was there any interaction with dose \( (F_{9,102}=1.45) \). This indicates that there were no differences between the groups in the number of times that they engaged in normal oral behaviours (Figure 14.13). The dose of \( d \)-amphetamine did have an effect on the number of grooming bouts displayed \( (F_{3,102}=6.38, P<0.01) \), with all groups displaying fewer bouts of grooming following 10.0 \( \mu \)g and 20.0 \( \mu \)g in comparison to saline \( (P<0.05 \) and \( P<0.01 \) respectively). There was also a significant dose effect \( (F_{3,102}=49.56, P<0.01) \), and group by dose interaction \( (F_{9,102}=3.74, P<0.01) \), though no group effect \( (F_{3,34}=2.72) \), when looking at the number of bouts of abnormal oral motor behaviours shown. Following doses 5.0 \( \mu \)g and 10.0 \( \mu \)g amphetamine the ibotenate-lesioned group showed more
bouts of abnormal oral behaviours than all the other groups (P<0.01 in all cases). Following the highest dose of amphetamine, the quinolinate-lesioned group showed fewer bouts of abnormal oral behaviours compared to both the ibotenate-lesioned group (P<0.05) and knife cut-lesioned group (P<0.01). All the groups showed an increased number of bouts of abnormal oral motor behaviour following 5.0 μg, 10.0 μg and 20.0 μg amphetamine compared with saline, and following 20.0 μg compared with 5.0 μg amphetamine (P<0.01, in all instances) (Figure 14.13).

Spontaneous locomotion

Figure 14.14 illustrates the mean (±SEM) locomotor counts for each group. ANOVA for the 3 days spontaneous locomotor activity revealed no significant main effect of group (F_{3,34}=2.76) or group by days interaction (F_{6,68}=0.42). A significant days effect was found (F_{6,68}=4.09, P<0.05) this was due to all the rats being less active on day 3 compared with day 1 (P<0.01), and is probably the result of habituation to the cages and procedure.

Orienting

Figure 14.15 shows the mean score (±SEM) for each group on the orienting test. A one-way ANOVA identified a significant group effect (F_{3,34}=11.40, P<0.001). Post hoc testing showed that the sham group was significantly more responsive to tactile stimuli than the knife cut and quinolinate-lesioned groups (P<0.01), and that the ibotenate-lesioned group were also more responsive than the knife cut group (P<0.05).

Freezing to overhead threat

A one-way ANOVA identified a significant group effect (F_{3,34}=3.85, P<0.05), but post hoc testing revealed no significant values, although there were a number of P values approaching significance. This seemed surprising when looking at the graph of the group means and standard error values (Figure 14.16). Although the standard deviations did show large variation in the data, examination of the raw data suggested that there might be significant differences between the groups (see Table 14.4. which plots out the raw data values for each group). Therefore non-parametric analysis using frequency counts was performed. Since the minimum value was 0 s and the
maximum value 22 s, the raw data were segregated into two frequency groups, those that fell between 0-11, and those >11. Analysis using the Fisher exact probability test failed to identify any significant differences between the sham group compared to any of the lesion groups.

14.3. Discussion

The ibotenate-lesioned rats showed consistent loss of both the NADPH-diaphorase positive neurones and nissl stained neurones, with relatively little damage to other structures. Although a number of rats sustained extensive damage to the CNF, this structure can be ruled out as having any effect on the oral motor behaviours seen since none of the rats with bilateral CNF lesions in the pilot study described in Chapter 8 showed any evidence of altered oral motor behaviour following administration of systemic amphetamine. The damage to the retrorubral nucleus can also be discounted as having any major impact on the behaviours described below, since a previous study showed that damage to this structure was as extensive following lesions of the deep mesencephalic nucleus (DpMe) as that seen in rats with PPTg lesions, but the DpMe-lesioned rats did not show any orofacial stereotypies to peripheral \(d\)-amphetamine stimulation (Inglis et al. 1994a). Furthermore The RRF has been shown to project via the striatonigral pathway to the caudate nucleus in the cat (Vandermaelen et al. 1978) and has also cytological and electrophysiological properties similar to the SN neurones (Rye et al. 1987; Preston et al. 1981). Thus it would not be unreasonable to expect general motor impairments following destruction of the retrorubral nucleus and it is evident from the locomotor data that the ibotenate-lesioned rats showed no deficits in this test.

Assessment of the quinolinate-lesioned rats again confirm previous studies about the difficulty in making this lesion work in the PPTg. NADPH-diaphorase cell loss and nissl loss were both small, and the extent of the lesion in the PPTg was highly variable. There is a problem with the use of anaesthetic in this area (see Chapter 15) and this may cause the quinolinic acid to be much less effective. Although previous evidence has suggested that the quinolinate is relatively selective for cholinergic neurones in this area (Rugg et al. 1992) the results of the more systematic analyses done on the diaphorase and nissl counts suggests that this is not the case. The average loss for both types of neurone within the PPTg were similar, and analysis indicates
that they destroy equal proportions of both cholinergic and non-cholinergic neurones. Instead of being more selective for cholinergic neurones, quinolinate could be viewed as a smaller ibotenate lesion. Both toxins destroy equal proportions of cholinergic and non-cholinergic neurones. The average lesion volume for the quinolinate-lesioned rats was also smaller than the ibotenate-lesioned rats and within the lesion boundaries there was considerable nissl-sparing. This suggests that in comparison to the ibotenate-lesioned rats, the quinolinate-lesioned rats had less extensive lesions which were also less complete. However, small as the lesion is, there did appear to be some behavioural consequences resulting from this lesion that were different to the sham-lesioned group.

Damage following the knife cut lesions was assessed to identify any extensive damage to structures while undercutting the PPTg and to see if there was any loss of neurones in the PPTg itself which could be due to severing it’s efferent connections. Assessment of the knife cut lesions showed that there was very little damage to nissl stained neurones in the PPTg and no difference in the diaphorase positive neurones compared to the sham-lesioned controls. The knife cuts themselves were discrete, and caused little structural damage. One possible explanation for the lack of any damage to the PPTg could be that the knife cuts were simply made in the wrong place and that a knife cut placed more caudally would have been more effective in severing descending outflow. However Semba et al. (1990) have reported that many of the descending PPTg neurones are collateralised and have ascending innervation of the thalamus. It is possible therefore that severing the descending axons of these collaterals would not cause neural degeneration in the PPTg since the ascending axons would remain intact. The lack of a behavioural effect from these lesions means that it is impossible to conclude that the altered orofacial behaviours reported in the ibotenate group are due to disrupting descending outflow. In order to answer this question it would be necessary to combine knife-cut lesions which undercut PPTg descending fibres with injections of retrograde tracer into the pons and medulla. It is stressed however that these lesions were speculative and since not all descending fibres are collateralised, some degeneration in the PPTg would be expected if the knife cuts were correctly placed.

Analysis of the oral motor behaviours confirm and extend the results reported in the previous chapter and by Kelley et al. (1988). The lack of differences between
the groups following saline injections into the VLCP indicate that none of the lesion groups showed any spontaneous changes in oral motor behaviours. The ibotenate-lesioned rats showed a shift in the dose response curve to d-amphetamine spending much longer engaged in licking and biting of the cage and body than any of the other groups following all three doses of d-amphetamine. They also displayed an increase in the duration of vacuous mouth movements following 5.0 µg and 20.0 µg d-amphetamine compared to the other groups and a related decrease in the duration of grooming at all doses in comparison to the sham- and knife cut-lesioned groups. Ibotenate-lesioned rats were also slower to initiate grooming, but quicker to initiate abnormal oral motor behaviours than the sham-lesioned rats, and following 5.0 µg and 10.0 µg they engaged in more bouts of abnormal oral behaviours than any of the other groups. Neither the quinolinate-lesioned nor the knife cut-lesioned rats showed any notable differences to the sham-lesioned controls in the generation of oral motor behaviours. All three groups showed an increase in the duration of cage and body licking and biting, and a decrease in the duration of grooming following all doses of d-amphetamine. They also displayed fewer bouts of grooming following 10.0 µg and 20.0 µg d-amphetamine and an increased number of bouts of abnormal oral motor behaviours following all doses.

The results from the locomotor test further confirm the lack of effect that the PPTg lesion has on locomotor activity, with none of the lesion groups showing any differences in the generation of locomotor activity. There was also no effect of lesion on freezing to overhead threat though the results from the orientation experiment are more interesting. Both the knife cut-lesioned and quinolinate-lesioned rats showed a reduced orientation response to tactile stimulation compared to the sham-lesioned group.

It is clear that while both the quinolinate- and knife cut-lesioned rats could be considered as extra control groups for the investigation of oral motor behaviours, due to the lack of effect that these lesions had, the results from the orientation experiment suggest that the lesions did have some effect. It is possible that the knife cut lesions severed the outflow pathway of some structure other than the PPTg and one could speculate that perhaps the decrease in orientation towards a stimulus could suggest that the efferent connections of the SC had been disrupted, since this structure has an important role in the generation of such behaviours. However it is perfectly possible
that damage to a different site within the brainstem could have resulted in these effects. The lack of a significant difference between the quinolinate-lesioned group and the ibotenate-lesioned group on the locomotor task and orientation task suggests that there is no difference between the two excitotoxins in their ability to make discriminably different lesions. This is borne out by the histological analysis which shows that the quinolinate-lesioned rats do not have a disproportionate ratio of NADPH-diaphorase loss compared with non-cholinergic nissl loss. That the ibotenate-lesioned rats show differences in oral motor behaviours compared to the quinolinate-lesioned rats suggests that the quinolinate lesion is just a smaller version of the ibotenate lesion, which is not extensive enough to produce the increases in oral motor activity that are observed following ibotenate lesions of the PPTg. Whether this is due to different parts of the PPTg mediating different functional processes is unclear. The extent of the lesions in the quinolinate-group were smaller on average compared with the ibotenate-group, but there was a lot of variability in the quinolinate-lesioned group which could suggest that different parts of the PPTg do not subserve different behaviours. An alternative explanation is that damage caused by the quinolinate lesions, while not completely disrupting the PPTg's functional behavioural role does unbalance the normal input and output patterns of the PPTg, causing deficits in some behaviours but not others.
Table 14.1. Ibotenate-Lesioned Group: Summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Lesion placement:</th>
<th>PPTg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage assessment: (modal scores)</td>
<td></td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>xxx</td>
</tr>
<tr>
<td>Deep mesencephalic nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Dorsal tegmental bundle</td>
<td>x</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>ND</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>x</td>
</tr>
<tr>
<td>Laterodorsal tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Microcellular tegmental nucleus</td>
<td>xx</td>
</tr>
<tr>
<td>Oral pontine reticular nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Parabrachial nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Paralemniscal nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>xxxx</td>
</tr>
<tr>
<td>Retrorubral field</td>
<td>xx</td>
</tr>
<tr>
<td>Retrorubral nucleus</td>
<td>xxx</td>
</tr>
<tr>
<td>Rubrospinal tract</td>
<td>ND</td>
</tr>
<tr>
<td>Subpeduncular tegmental nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Substantia nigra pars compacta</td>
<td>ND</td>
</tr>
<tr>
<td>Substantia nigra zona reticulata</td>
<td>ND</td>
</tr>
<tr>
<td>Superior cerebellar peduncle</td>
<td>x</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lesion Volume (mm$^3$): (Mean ± SEM values)</th>
<th>6.45 ± 0.368</th>
</tr>
</thead>
<tbody>
<tr>
<td>% NADPH-diaphorase Cell Loss: (Mean ± SEM values)</td>
<td>82.67 ± 2.92</td>
</tr>
<tr>
<td>% Nissl Loss (Mean ± SEM values)</td>
<td>94.81 ± 0.98</td>
</tr>
</tbody>
</table>

Table 14.1. A summary showing structural damage, mean (±SEM) lesion volume (mm$^3$) calculated from cresyl violet sections and % cell loss calculated from NADPH-diaphorase cell counts and nissl body counts. Damage was identified by presence of gliosis and degeneration of neuronal somata, and a summary of affected structures for every animal estimated. The modal score in the group was then taken for each structure. The key is as follows: ND No damage; x < 30%; xx 30 - 60%; xxx 60 - 90%; xxxx > 90%.
Table 14.2. Quinolinate-Lesioned Group: Summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Lesion placement</th>
<th>PPTg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage assessment:</td>
<td></td>
</tr>
<tr>
<td>(modal scores)</td>
<td></td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Deep mesencephalic nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Dorsal tegmental bundle</td>
<td>ND</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>ND</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>x</td>
</tr>
<tr>
<td>Laterodorsal tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Microcellular tegmental nucleus</td>
<td>xx</td>
</tr>
<tr>
<td>Oral pontine reticular nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Parabrachial nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Paralemniscal nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>xx</td>
</tr>
<tr>
<td>Retrorubral field</td>
<td>x</td>
</tr>
<tr>
<td>Retrorubral nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Rubrospinal tract</td>
<td>ND</td>
</tr>
<tr>
<td>Subpeduncular tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Substantia nigra pars compacta</td>
<td>ND</td>
</tr>
<tr>
<td>Substantia nigra zona reticulata</td>
<td>ND</td>
</tr>
<tr>
<td>Superior cerebellar peduncle</td>
<td>x</td>
</tr>
</tbody>
</table>

| Lesion Volume (mm$^3$):                | 3.25 |
| (Mean ± SEM values)                    | ± 0.522 |

| % NADPH-diaphorase Cell Loss:          | 24.58 |
| (Mean ± SEM values)                    | ± 5.32 |

| % Nissl Loss                           | 37.43 |
| (Mean ± SEM values)                    | ± 7.09 |

Table 14.2. A summary showing structural damage, mean (±SEM) lesion volume (mm$^3$) calculated from cresyl violet sections and % cell loss calculated from NADPH-diaphorase cell counts and nissl body counts. See legend in Table 14.1 for fuller description.
Table 14.3. Knife Cut Lesioned Group: Summary of damage and lesion volumes

<table>
<thead>
<tr>
<th>Lesion placement</th>
<th>PPTg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Damage assessment:</strong></td>
<td></td>
</tr>
<tr>
<td>(modal scores)</td>
<td></td>
</tr>
<tr>
<td>Cuneiform nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>ND</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>x</td>
</tr>
<tr>
<td>Laterodorsal tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Motor root trigeminal nerve</td>
<td>x</td>
</tr>
<tr>
<td>Motor trigeminal nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Oral pontine reticular nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Parabrachial nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Paralemniscal nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Pedunculopontine tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Peritrigeminal zone</td>
<td>x</td>
</tr>
<tr>
<td>Retrorubral field</td>
<td>x</td>
</tr>
<tr>
<td>Retrorubral nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Rubrospinal tract</td>
<td>x</td>
</tr>
<tr>
<td>Subpeduncular tegmental nucleus</td>
<td>ND</td>
</tr>
<tr>
<td>Superior cerebellar peduncle</td>
<td>ND</td>
</tr>
<tr>
<td>Ventral subcoeruleus nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Ventrolateral principle sensory</td>
<td></td>
</tr>
<tr>
<td>trigeminal nucleus</td>
<td>x</td>
</tr>
<tr>
<td>Ventrolateral tegmental nucleus</td>
<td>xx</td>
</tr>
</tbody>
</table>

Lesion Volume (mm³):
(Mean ± SEM values) 2.55 ± 0.450

% NADPH-diaphorase Cell Increase: 16.37 ± 5.65

% Nissl Loss
(Mean ± SEM values) 18.68 ± 4.91

Table 14.3. A summary showing structural damage, mean (±SEM) lesion volume (mm³) calculated from cresyl violet sections and % cell loss calculated from NADPH-diaphorase cell counts and nissl body counts. See legend in Table 14.1 for fuller description.
### Table 14.4. Raw Data Values for Freezing to Overhead Threat

<table>
<thead>
<tr>
<th>IBOTENATE</th>
<th>QUINOLINATE</th>
<th>KNIFE CUT</th>
<th>SHAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>7</td>
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</tr>
<tr>
<td>2</td>
<td>2</td>
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</tr>
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<td>3</td>
<td>12</td>
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<td>14</td>
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<td>16</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 14.4. Raw data values (in sec) for freezing to overhead threat. Data was divided into two frequency groups for non-parametric analysis using the Fisher Exact Probability Test (Siegel 1956). The scores that fell within 0-11 sec were placed within the first frequency group, while those that lay between 12-22 were placed in the second. The heavy black line in each column indicates the cut off for the two frequency groups for each lesion group.
Figure 14.1, 14.2, 14.3 and 14.4. Representative sections through the VLCP showing cannulae placements for the 3 lesioned groups and control rats: diamonds represent the ibotenate-lesioned rats, triangles the quinolinate-lesioned rats, circles the knife cut lesioned rats and squares the sham-lesioned controls. The symbols drawn do not correspond exactly to the number of rats in the group due to the large amount of overlap in the placements. Therefore one symbol may represent the cannulae sites for a number of rats. Sections redrawn from the atlas of Paxinos and Watson (1986).
Figure 14.5, 14.6 and 14.7. Representative sections through the PPTg showing lesion volumes. Shading represents the largest and smallest lesion following ibotenate or quinolinate infused into the PPTg or knife cuts undercutting the PPTg; sections redrawn from the atlas of Paxinos and Watson (1986).
IAL: 2.70 mm

IAL: 0.28 mm

Largest Lesion

Smallest Lesion

QUINOLINATE-LESIONED GROUP
IAL: 2.70 mm

IAL: 0.28 mm

Largest Lesion
Smallest Lesion

KNIFE CUT-LESIONED GROUP
Figure 14.8. Mean (±SEM) of NADPH-diaphorase positive cell counts and non-NADPH-diaphorase counts for each group. To give an indication of non-diaphorase neuronal loss, parallel sections of NADPH-diaphorase counts were subtracted from nissl counts, before the nissl counts were analysed (see text for fuller description). Data was square-root transformed to reduce variance. For statistical analysis see text.
NON-NADPH-DIAPHRASE AND NADPH-DIAPHRASE CELL PROFILE COUNTS

SQUARE-ROOT TRANSFORMED

- IBO
- QUIN
- KNIFE CUT
- SHAM

NON-DIAPHRASE  DIAPHRASE
Figure 14.9. Mean (±SEM) of non-diaphorase nissl counts divided by corresponding diaphorase counts. To give an indication of non-diaphorase neuronal loss, parallel sections of NADPH-diaphorase counts were subtracted from nissl counts, before the nissl counts were analysed (see text for fuller description). This shows the proportion of neuronal loss for each group. Data was square-root transformed to reduce variance. For statistical analysis see text.
COMPARISON OF NON-NADPH-DIAPHORASE TO NADPH-DIAPHORASE CELL PROFILE COUNTS

COUNTS (SQUARE-ROOT TRANSFORMED)

IBOTENATE  QUINOLINATE  SHAM

NON-DIAPHORASE/DIAPHORASE
Figure 14.10. Mean (±SEM) duration (sec) of grooming for both PPTg-lesioned and control rats following intra-VLCP microinjection of d-amphetamine. For statistical analysis see text.
DURATION OF GROOMING

LOG TRANSFORMED

- IBO
- QUIN
- KNIFE CUT
- SHAM

SALINE  5.0ug  10.0ug  20.0ug
Figure 14.11. Mean (±SEM) duration (sec) of vacuous mouth movements for both PPTg-lesioned and control rats following intra-VLCP microinjection of d-amphetamine. For statistical analysis see text.
DURATION OF VACUOUS MOUTH MOVEMENTS

LOG TRANSFORMED

- IBO
- QUIN
- KNIFE CUT
- SHAM

SALINE  5.0ug  10.0ug  20.0ug
Figure 14.12. Mean (±SEM) duration (sec) of cage and body licking and biting for both PPTg-lesioned and control rats following intra-VLCP microinjection of \textit{d}-amphetamine. For statistical analysis see text.
DURATION OF CAGE & BODY LICKING & BITING

LOG TRANSFORMED

- **IBO**
- **QUIN**
- **KNIFE CUT**
- **SHAM**

**SALINE**  5.0ug  10.0ug  20.0ug
Figure 14.13. Mean (±SEM) latency (sec) to engage in grooming and abnormal oral motor behaviour (all oral behaviours minus grooming) and mean (±SE) number of bouts of grooming and abnormal oral behaviour for PPTg-lesioned and control rats. For statistical analysis see text.
Figure 14.14. Mean (±SEM) locomotor counts for three days of spontaneous locomotor activity for each group. Data has been square-root transformed. For statistical analysis see text.
SPONTANEOUS LOCOMOTOR ACTIVITY

SQUARE-ROOT TRANSFORMED

DAY 1  DAY 2  DAY 3

IBO  QUIN  KNIFE CUT  SHAM
Figure 14.15. Mean (±SEM) for orientation scores for each group. For statistical analysis see text.
Figure 14.16. Mean (±SEM) for freezing behaviour (sec) for each group. For statistical analysis see text.
FREEZING BEHAVIOUR

SECONDS

IBO  QUIN  KNIFE CUT  SHAM

0  2  4  6  8  10  12
Part V. A role for the PPTg in striatal outflow.
Chapter 15. General Discussion.

15.1. Methodological considerations

15.1.1. Comments on the use of different anaesthetics

In this thesis three different anaesthetics were used. This was done for a number of reasons. The combined use of xylazine and ketamine as the anaesthetic of choice for the cannulations was because they have a rapid onset, a wide range of safe doses and very rapid recovery. Anaesthesia length is around 40 min which is ample time for this short surgical procedure. However this anaesthetic is unsuitable for food deprived animals (personal communication E. Wood) possibly because it is difficult to manipulate the two doses. It is also unsuitable for longer lasting surgery and especially for surgical procedures involving the use of some excitotoxins since ketamine is an NMDA channel blocker. Anaesthesia can affect the potency of excitotoxins and NMDA, quinolinate and to some extent ibotenate all work at NMDA receptor subtypes. Sagatal, a barbiturate anaesthetic, also should not be used when making quinolinate lesions. Inglis et al. (1993) have demonstrated that the efficacy of quinolinate lesions (but not ibotenate) are reduced when made using barbiturate anaesthesia in comparison to Avertin (10 g tribromoethanol/ 5 g tertiary amyl-alcohol; 10 ml of this concentrate is then dissolved in 450 ml 0.9 % saline and 40 ml absolute alcohol), a non-barbiturate anaesthetic.

Lesions using ibotenate are not affected by barbiturate, which is why Sagatal is the anaesthetic of choice for ibotenate lesions. Sagatal is a slow onset anaesthetic and has a slow recovery time. Anaesthesia lasts approximately 60 min, but additional injections to increase duration of anaesthesia are not recommended due to the risk of respiratory depression. Rats are also unable to regulate their body temperature during surgery and the immediate post-operative period of recovery from the anaesthetic. However the use of Sagatal despite its drawbacks is preferable to Avertin since it carries no risk of post-operative gastric pathology.

If quinolinate lesions are to be made, then Avertin is the anaesthetic of choice. It is a non-barbiturate anaesthetic which does not affect the toxicity of quinolinic acid. It has rapid onset and duration of anaesthesia is approximately 60 min. Recovery time is fairly rapid. The major drawback of Avertin is that it is an intraperitoneal irritant,
that can cause "bloat" in the days following surgery. Bloat is characterised by a marked swelling of the abdomen in spite of a decrease in food intake, weight loss and the adhesion of the intestine to the muscle wall. However, the incidence of post-operative bloat can be reduced by giving the rat a 5 ml injection of 6% glucose in saline (w/v) immediately post-op. It is possible that this could reduce the concentration of Avertin remaining in the intraperitoneal cavity thus reducing irritation.

15.1.2. Comments on the use of excitotoxins

Excitotoxins destroy cell bodies and spare fibres of passage through the injection site. Rugg et al. (1992) showed that ibotenate and quinolinate produced dose-dependent damage in the PPTg, and that they both destroyed equally well ~75% of Ch5 cholinergic neurones. However, they reported that ibotenate and quinolinate differed in their ability to destroy non-cholinergic neurones, ibotenate producing damage twice as extensive as that of quinolinate. It seems therefore that quinolinate is more selective than ibotenate, mostly destroying ChAT-positive neurones and causing very little general neuronal damage (Rugg et al. 1992). However, since the studies by Rugg et al. (1992) and Dunbar et al. (1992), repeated experiments using quinolinate in this laboratory have failed to elicit such an extensive lesion of the PPTg. In the majority of experiments including the ones reported here, quinolinate causes a maximum of ~30% neural damage, and the experiment reported in Chapter 14, has also shown that it was not selective for cholinergic neurones, but caused approximately equal damage to both cholinergic and non-cholinergic neurones.

The efficacy of ibotenate as an excitotoxin has also been questioned by Coffey et al. (1988, 1990), who found that when injected into the rat septum it led to the breakdown of the blood-brain barrier. Consequently, the lesion site was invaded by macrophages from the bloodstream which promoted demyelination and the collapse of tissue. Demyelination has also been observed following lesions in the rat lateral hypothalamus (LH) using ibotenate and NMDA (Stellar et al. 1991; Brace et al. 1992), in the hippocampus following administration of ibotenate (Erselius and Wree 1991) and in the thalamus following kainate lesions (Dusart et al. 1992). It is suspected that demyelination could be a generalised phenomenon following excitotoxic administration. However the tissue collapse found in the septum does not
occur in the LH nor in the PPTg and remyelination has been reported to occur over time in the LH following NMDA lesions (Brace et al. 1992) and in the thalamus following kainate lesions (Dusart et al. 1992). Thus the fibres of passage are not themselves damaged, but the myelin around them is temporarily lost leading presumably to impaired transmission for a period of time following the lesion. This would indicate that any sensory impairments observed directly following surgery may be the result of demyelination. Whether such damage occurs in the PPTg remains to be seen, though preliminary investigations in this laboratory suggest that demyelination in the PPTg is not extensive, presumably because of the closer packing of fibre bundles (Brace et al. in preparation).

15.1.3. Comments on the use of microinjections

The use of microinjections in experimental studies have become popular for a number of reasons. First by injecting directly into brain tissue, the problem of whether the drug can gain access to the brain across the blood-brain barrier is avoided. Second, it allows the localisation of the drug within specific structures of the brain giving greater control over its action, and allowing for more specific conclusions to be drawn from the study, since general activation or suppression can be ruled out. However, there is a problem concerning the distance of diffusion of the solution injected into the brain. For example Ott et al. (1974) injected 1 μl potassium chloride into the hippocampus and investigated the extent of its diffusion. They reported that two important factors to take into consideration were the backward flow of fluid up the outside of the chronically implanted cannula, and spread of the substance to other brain structures through the ventricular system. They also argued that these confounding factors were independent of the pharmacological properties of the chemical injected (Ott et al. 1974). However it was believed that the molecular weight of the chemical also affected the distance of diffusion and Myers (1966) later discovered that a number of factors including the pH, solubility and osmolarity of the chemical further affected the capacity for diffusion. The volume injected is also crucial. Myers (1966) studied this by injecting a number of volumes of dyes which had different molecular weights into the thalamus or hypothalamus of anaesthetised rats and recording the distance each one diffused. On the basis of his results he advised that a fluid volume of 0.5 μl injected into rat brain tissue was maximal if the fluid was to remain inside the borders
of a circumscribed area, a finding he confirmed in another study where \(^{14}\text{C}\)-dopamine was injected into the brain stem of the rat (Myers and Hoch 1978). Further studies have also shown that the small volumes of chemicals injected directly into the brain are unlikely to diffuse far provided the fluid does not have access to the ventricular system (Myers and Hoch 1978; Rice et al. 1985).

15.1.4. Comments on the use of NADPH-diaphorase

NADPH-diaphorase (nicotinamide adenine dinucleotide phosphate diaphorase) is a histochemical marker that stains selected populations of neurones throughout the brain (Vincent and Hope 1990). The enzyme responsible for the NADPH-diaphorase histochemical reaction is nitric oxide synthase. Nitric oxide synthase is the enzyme responsible for the synthesis of nitric oxide (NO) from L-arginine (Vincent and Hope 1990). NO has been identified as a novel messenger molecule and may even function as a new type of neurotransmitter (Snyder and Bredt 1992). It appears to increase levels of cyclic guanosine monophosphate (cGMP) which acts as a second messenger for neurotransmitters and hormones. NO is an extremely short-lived unstable chemical which is therefore very difficult to locate, so its presence is deduced from the presence of nitric oxide synthase which occurs almost entirely in neurones and not in glial cells (Snyder and Bredt 1992). NO synthase is only localised in discrete populations of neurones and although its precise function remains to be elucidated, the presence of NO synthase in different neurones has proved useful as a medium for neuronal identification. NADPH-diaphorase selectively stains groups of cholinergic neurones in the mesencephalon (Vincent and Kimura 1992). It has been demonstrated that this stain is almost equally good in its ability to stain the PPTg and LDTg cholinergic neurones as is ChAT. Neurones are regarded as cholinergic if they contain the enzyme that is responsible for the synthesis of acetylcholine, choline acetyltransferase (ChAT). In this laboratory Inglis et al. (1994a) studied adjacent brain sections which were stained for ChAT and NO, and found an extremely high correlation between cell profile counts, though 10% of NO neurones are not ChAT positive. ChAT is time-consuming and costly, whereas the advantage of NADPH-diaphorase staining is that it is relatively cheap and quick to do. Thus NADPH-diaphorase is the stain of choice in this thesis.
15.1.5. Comments on cell counting

There has been a great deal of controversy concerning what constitutes an accurate cell counting technique, since it appears that cell numbers do not always correspond directly with cell profiles (Coggershall 1992). When serial sections are being analysed, it is possible that the same cell profile will be counted more than once. Coggershall (1992) outlines the various kinds of mathematical correction that can be employed in order to assess the number of cells in a section. In these experiments however, a corrective technique was not used, the reason being that the sections counted are 25 μm or 50 μm thick and 200 μm apart. As the cholinergic neurones within the PPTg are, on average, 15-25 μm in diameter, there is little chance that the same cell profile is being counted more than once and estimates of total neuronal number can be made by extrapolation.

15.2. Possible confounding features of lesions to the PPTg

It is clear from Chapter 5 that the PPTg has been implicated in a wide range of behavioural functions. Therefore the possibility of there being alternative explanations to the data presented here should be considered. Perhaps the alterations in behaviour following PPTg lesions could be explained by deficits in other behavioural functions. For example the PPTg is implicated in the mediation of the sleep/wake cycle, and it is possible that alterations in arousal may affect behavioural performance of PPTg-lesioned rats. However this seems unlikely, since PPTg-lesioned rats displayed normal patterns of locomotor activity, which they would have failed to do if underaroused. They also showed no changes in temperament, or increased aggressiveness, which might be expected if their sleep patterns had been disrupted.

Could the failure to perform appropriately on responding for conditioned reinforcement (Inglis et al. 1994b) be explained by a deficit in attention? This again seems unlikely, when the pattern of responding is examined. The rats responded at the same level as sham-lesioned controls on the conditioned lever, suggesting that they were not deficient in paying attention to the procedure. The rats also made panel pushes in exactly the same manner as the sham-lesioned controls. The difference lay in the increase in responding of the unconditioned lever. This suggests that the rats were able to pay attention to the task, and also that they were not underaroused.
Their problem appears to be one of using the information provided to generate an appropriately directed behavioural response.

Finally, could the oral motor stereotypies described in this thesis be due to disruption of motor control caused by the lesion? Again this seems highly unlikely. The regulatory measures recorded in several experiments, show that following surgery the PPTg-lesioned rats were able to eat and drink in line with the sham-lesioned controls. They did not spill more food, or water suggesting that their motor control was unaffected. They did not show impairments on locomotor tests. Also the differences described in this thesis are of an increase in the incidence of oral motor stereotypies, which again would be unlikely if the rats had deficits in motor control.

15.3. The CNF and the MLR

Although several authors have claimed that the PPTg is homologous to the MLR (Garcia-Rill et al. 1986, Rye et al. 1987) recent evidence has tended to contradict this view. For example behavioural evidence shows that the PPTg has no part to play in spontaneous or in NAcc stimulated locomotion (Swerdlow and Koob 1987; Dunbar et al. 1992; Inglis et al. 1994a, 1994b; Olmstead and Franklin 1994). Furthermore there is a body of evidence suggesting that the most important part of the MLR is the CNF and not the PPTg (Steeves and Jordan 1984; Mori et al. 1987; Coles et al. 1989). Moon Edley and Graybiel (1983) described several characteristics that may be used to define the MLR and cited evidence showing that according to these definitions the CNF and not the PPTg was the more likely candidate. Two of these defining characteristics were that the MLR lies immediately ventral to the IC and that the MLR has either direct or indirect innervation of the spinal cord. The CNF, not the PPTg, is the structure which lies immediately ventral to the IC and, furthermore, in a tracing study using HRP, Castiglioni et al. (1978) identified projections from the spinal cord to the CNF but not the PPTg in primates. The CNF receives major innervation from the SC (Mitchell et al. 1988; Redgrave et al. 1988), which is involved in attention to sudden moving stimuli and the mediation of the rapid fight or flight response which such a stimulus may necessitate. Thus the SC must be connected directly to sites that stimulate locomotion, and the CNF is therefore a potentially good candidate for comprising a major part of the MLR. However, the results reported here have demonstrated that lesions to the CNF have had no effect on
baseline or amphetamine stimulated locomotion. These results throw up two questions; if the CNF is not homologous to the MLR then which structure is and what is the functional role of the CNF if it plays no part in the MLR?

15.3.1. The MLR: a redundant concept?

The MLR can only be identified physiologically and has been extremely difficult to locate anatomically. The term MLR has been applied to the CNF and PPTg but these are not the only sites from which controlled stepping on a moving treadmill can be elicited following electrical stimulation in a decerebrate preparation. Coles et al. (1989) studied a distribution of sites within the midbrain where low threshold electrical stimulation could elicit stepping, including the SC, substantia grisea centralis, central gray and the lateral lemniscus. Garcia-Rill and Skinner (1987) demonstrated that locomotion could be produced following stimulation of the dorsal horn or mesencephalic trigeminal nucleus, and stimulation of the pontomedullary locomotor strip, Probst's tract, parabrachial nucleus and periaqueductal gray all produce the same effects in the decerebrate preparation (Steeves and Jordan 1984). This suggests that stimulation of the majority of structures within the mesencephalon in a decerebrate animal results in locomotion which is thus not a specific but a generalised phenomenon.

In his review of the PPTg, Garcia-Rill (1991) talked about the recruitment of locomotion. He suggested that the PPTg could be the site responsible for this recruitment and dismissed other areas from which locomotor activity can be generated by stating "as far as other locomotion-inducing sites are concerned, it may be advisable to specify a clear neurological substrate rather than using the term 'MLR' to label any locomotion-inducing site" (Garcia-Rill 1991). In this thesis I would like to put forward this view for all locomotion-inducing sites including the PPTg and the CNF.

Behavioural evidence from studies of the PPTg and the CNF including the results reported in this thesis suggest that neither the PPTg nor the CNF have a role in the generation of locomotor activity per se. This hypothesis is supported by Depoortere and colleagues (1990a) who reported that while electrical stimulation of the CNF in lightly anaesthetised rats resulted in locomotion when the rats were suspended over a moving treadmill, they found different effects following stimulation
of the same area in intact and freely moving rats. In this condition the rats displayed aversive reactions typical of escape behaviours, including explosive running and jumping (Depoortere et al. 1990a). Thus the locomotor response found in the decerebrate animal could be due to severing the cortical and limbic connections to the pons, preventing communication which would normally shape behaviour in an intact animal. It is possible that the locomotor response is a default behaviour common to most mesencephalic structures which occurs in the absence of other sensory input and reveals nothing about their role in normal behaviour.

15.3.2. A functional role for the CNF

Excitotoxic lesions of the PPTg and CNF have failed to reveal any effect on NAcc-stimulated locomotion, which agrees with the work of Swerdlow and Koob (1987) who reported that locomotion was only attenuated by lesions of the DMT. Rather than the recruitment of locomotion per se both the PPTg and the CNF appear to be involved in motor activity related to the execution of higher order functions. For example although lesions of the PPTg do not affect locomotor activity stimulated by intra-accumbens amphetamine, lesions do affect responding for conditioned reinforcement. Behavioural evidence also suggests the CNF may be implicated in locomotion in relation to aversive reactions.

Ipsilateral projections from the SC are involved in the mediation of defensive behaviours (Redgrave et al. 1986; Sahibzada et al. 1986). Injections of glutamate into the CNF produce behaviours similar to those stimulated from the SC (i.e. rapid locomotion and freezing) and bilateral lesions of the CNF abolish escape behaviours stimulated from the SC by GABA antagonists (Mitchell et al. 1988; Dean et al. 1988). Furthermore electrical stimulation of the CNF has been shown to promote escape behaviours in alert, freely moving rats, the intensity of which increases as a function of increasing electrical stimulation (Depoortere et al. 1990b). By training the rats to press a bar which cancelled the electrical stimulation, the escape behaviours were abolished. The authors interpreted these results as indicative of a reaction to an underlying aversive state and not merely a simple stimulation-locomotor response; if the rats were prevented from pressing the bar, violent escape behaviours reappeared. Zemlan and Behbehani (1988) reported an increased latency to respond to noxious thermal stimulation following electrical stimulation of the CNF, which was also
associated with concurrent ipsilateral circling behaviour. Although this nociceptive behaviour may have been due to inhibition caused by the interference of other motor behaviours, the effects of the tail flick response to thermal stimulation could be reversed by naloxone and scopolamine suggesting that the CNF has some role in behavioural analgesia. Thus the CNF is implicated in the integration of sensory and motor information with regards to nociception.

15.4. PPTg and oral motor behaviours

In the experiments described above, it was shown that microinjection of different doses of amphetamine into the VLCP produced dose-dependent increases in stereotyped licking and biting. In addition those animals that had been given bilateral lesions of the PPTg showed a shift in this dose-response curve, exhibiting a higher incidence of these stereotypies at lower doses compared to controls. It is well-known that the behavioural effects of amphetamine injected into the striatum are mediated through discriminably different pathways, with the NAcc subserving the locomotor response seen at low doses of amphetamine and the VLCP mediating the oral motor stereotypies observed at higher doses. It is believed that these different outflow pathways act in competition with each other (Joyce and Iversen 1984). Such competition would explain the differences in behaviour found between microinjecting amphetamine directly into the VLCP (Delfs and Kelly 1990) or administering it systemically (Kelly et al. 1975). Systemic injection of amphetamine promotes DA release which will affect many if not all sites within the striatum, thereby eliciting locomotion which wins the "competition" for expression at low levels of stimulation. Direct stimulation of the VLCP prevents generalised DA release and allows expression of biting that would normally be inhibited. It is proposed that suppression of the oral motor nuclei is normally mediated by an inhibitory projection from the PPTg. Thus following lesions of the PPTg, the oral motor nuclei are disinhibited and striatal outflow is biased in favour of the VLCP, resulting in an increase in licking and biting.

However, the PPTg is not the only recipient of striatal outflow. The SNr also receives a substantial innervation from the striatum, both directly through the striatonigral pathway and indirectly via the GP and STN. The SNr projects to the superior colliculus (SC), PPTg and pontomedullary reticular formation, including the
parvicellular reticular formation (PcRt) (Von Krosigk and Smith 1990). Von Krosigk et al. (1992) have identified converging striatal and pallidal inputs onto SNr neurones which project predominantly to the PcRt. Further studies from this group have identified projections from the PcRt to the trigeminal motor nuclei, from where direct projections to the facial musculature have been identified (Mogoseanu et al. 1993; Travers and Norgen 1983). Thus the VLCP is only four synapses from the facial muscles. However these anatomical connections raise the question of whether the PPTg receives VLCP innervation which is then projected onto the PcRt since the VLCP projection could travel more directly to the PcRt via the SNr.

Other structures in receipt of VLCP outflow also influence oral motor activity. Biting dependent upon the nigroreticulotectal pathway has been elicited by intranigral muscimol (Childs and Gale 1983; Baumeister et al. 1987) while systemic apomorphine increases locomotion and attenuates licking and gnawing following lesions of the SC (Redgrave et al. 1980). Electrolytic lesions of the SC also attenuate the biting elicited by intranigral muscimol (Baumeister et al. 1987). It has been suggested that oral motor activity is channelled through the SC so that it can be integrated with information concerning the orientation of the head, possibly in preparation for aggressive or defensive biting (Redgrave et al. 1992). Thus the PcRt receives innervation directly from the PPTg, SNr and SC and it is hypothesised that these separate systems work and interact in concert. Damage to any one of these areas will alter striatal outflow and bias it towards the expression of one behaviour by overriding the other.

15.4.1. Clinical implications
The present data are not only of theoretical interest. Parkinson's disease (PD) is characterised by loss of DA-containing neurones from the substantia nigra pars compacta (SNc), resulting in dopamine (DA) depletion from the striatum. However it has been suggested that loss of nigrostriatal DA alone is not sufficient to generate all the symptoms of PD (Steriade et al. 1991). Further neuronal loss affecting serotonergic, noradrenergic and cholinergic systems have been identified in Parkinsonian brains. Loss of neurones from the PPTg in Parkinson's disease and supranuclear palsy has been identified (Hirsch et al. 1987; Jellinger 1988). In addition, chronic inhibition of the PPTg caused by overactive pallidal outflow has been
reported in primates made hemiparkinsonian by MPTP (Mitchell et al. 1989). Around 60-80% of Parkinsonian patients treated with L-DOPA develop abnormal involuntary movements (dyskinesias) which vary in expression, though among the most common is choreoathetoid movements characterised by compulsive chewing and gnawing (Crossman 1987). It is thought that chronic DA receptor supersensitivity in the denervated striatum underlies these oral dyskinesias. This receptor supersensitivity coupled with chronic inhibition of the PPTg in Parkinson's disease (Mitchell et al. 1989) may cause disinhibition of the oral motor nuclei which suggests that L-DOPA-induced oral dyskinesias could be mediated through the PPTg. Oral dyskinesias have also been described in schizophrenic patients, sufferers of Tourette's syndrome and in Wilson's disease (Walshe 1986) and it is therefore possible that the PPTg may play some role in all these basal ganglia disorders.

15.5. Future considerations

Some questions of the role of the PPTg in the mediation of oral behaviour generated from the VLCP remain. First, does the PPTg receive information about oral motor control directly from the VLCP, indirectly from the SNr, or does it not receive this information at all? The PPTg receives output directly from the VLCP, the SNr and GP (Groenewegen et al. 1993; Spann and Grofova 1991; Moriizumi and Hattori 1992). In addition the PPTg innervates the pontine reticular nuclei oralis and caudalis, the gigantocellular reticular nucleus, the medullary reticular nuclei and the PcRt (Grofova and Keane 1991; Rye et al. 1988). Thus there is an anatomical basis for both direct or indirect communication of the VLCP with the PPTg, which the PPTg could then transmit down to sites of oral motor control in the pons and medulla. But there is also the possibility that the PPTg does not receive information pertaining to oral motor control, but simply has descending inhibitory influence over the oral motor nuclei in the pons and medulla. The question of whether the outflow from the VLCP goes through the SNr or not would need to be addressed functionally. Can biting elicited from the SNr be affected by PPTg lesions? It will be extremely difficult to determine whether the PPTg actually receives information about oral motor activity from the VLCP or simply has descending inhibitory influence over the PcRt, without having VLCP outflow channelled through it. However a recent study carried out in this laboratory by Winn et al. (unpublished observations) showed that microinjection
of GABA agonists or antagonists directly into the PPTg does not result in orofacial stereotypies. This would argue in favour of the PPTg having a descending inhibitory influence over the oral motor nuclei, without actually receiving any direct information from the VLCP regarding oral motor control.

Another question which remains to be answered is which population of PPTg neurones are important in mediating oral motor activity. Since the quinolinate lesions were unsuccessful, it is impossible to make a dissociation between whether the cholinergic or non-cholinergic neurones underlie this response. It is unlikely that this response is mediated through ascending cholinergic innervation of basal ganglia sites since direct stimulation of the SN with cholinergic drugs does not result in the oral motor activity observed following stimulation of the VLCP (Winn et al. 1983; Parker et al. 1993). Existing evidence would also suggest that the non-cholinergic neurones are responsible for the mediation of orofacial activity. The non-cholinergic neurones of the PPTg have been identified as the neurones which are in receipt of the majority of basal ganglia outflow, and are the principal targets of SNr outflow to the PPTg (Spann and Grofova 1991). Furthermore descending non-cholinergic neurones innervate sites in the pons and medulla implicated in oral motor control (Rye et al. 1988). However until a method can be found for making a lesion which discriminates between the cholinergic and non-cholinergic neurones of the PPTg, it is impossible to conclude definitively that the non-cholinergic neurones mediate the orofacial activity stimulated from the VLCP.

15.6. A functional role for the PPTg
A role for the CNF in the integration of sensory and motor information with regards to nociception has been hypothesised and the PPTg’s role could also be described as an integrative one which co-ordinates higher order functions. Initially the PPTg was believed to mediate accumbens-induced locomotion (Mogenson et al. 1980, 1985, 1989; Brudzynski and Mogenson 1985). However a number of studies have provided evidence which shows that loss of the PPTg affects neither spontaneous nor drug-induced locomotion, stimulated either peripherally or centrally from the NAcc (Swerdlow and Koob 1987; Olmstead and Franklin 1994; Inglis et al. 1994a, 1994b). However while the PPTg does not directly mediate locomotion, it may mediate other behaviours stimulated from the NAcc. For example the role of the PPTg in the
mediation of reward-related behaviours has been investigated. Bechara and Van der Kooy (1989, 1992a, 1992b) showed that lesions of the PPTg affected the formation of place-preferences and lesions also affected the increased responding for conditioned reinforcement stimulated by microinjection of $d$-amphetamine into the NAcc (Inglis et al. 1994b). Loss of the PPTg has also been reported to attenuate conditioned locomotion (Bechara and Van der Kooy 1992c). Injection of the cholinergic agonist carbachol into the PPTg has also been shown to reduce the amount of bar pressing for rewarding brain stimulation stimulated from the lateral hypothalamus, whereas microinjection of the muscarinic antagonist, scopolamine into the PPTg increases sensitivity to rewarding brain stimulation (Yeomans et al. 1993). Thus the PPTg has a role in the mediation of reward-related behaviours particularly those mediated by the NAcc, while this thesis also identifies a role for the PPTg in the mediation of CP outflow. What is the functional purpose of the PPTg?

One possibility is that, just as the SC integrates oral motor control with orientation systems, so the PPTg integrates oral motor control with the reward-related output, which presumably acts normally to inhibit oral motor systems. Such a hypothesis parallels that outlined for the SC: when rats bite and lick, it is important that they understand not only where to bite but also what to bite. Evidence for an integrative role is also provided by other studies. In their study of the auditory startle response in the rat, Ebert and Ostwald (1991) identified a “startle correlated potential” which only fired in conjunction with a motor response (measured by electromyogram recordings of the temporal muscle), and not directly with the auditory startle response, which also occurs in the absence of a motoric reaction to auditory stimulus. They hypothesised that because of the long latency for this “startle correlated potential” to be evoked, it was not part of the primary startle pathway, but could constitute a secondary component to the startle response. The exact function of this secondary pathway can only be speculated upon, but they suggested that it could serve to disrupt ongoing motor behaviours in order to prepare the animal for sudden evasive or aggressive behaviours. They localised this “startle correlated potential” to the PPTg. This is interesting given that the PPTg receives innervation from the CP which mediates ongoing habitual behaviours, and also has reciprocal connections with the SC, which is involved in orienting to sudden visual stimuli and involved in the fight or flight response. While intact, the PPTg could function to interrupt the outflow
of habitual motor programmes in order to prepare the animal for switching its behaviour (Ebert and Ostwald 1991).

The CP is believed to mediate the formation of simple stimulus-response sequences, where a motor programme becomes so well learnt, it becomes habitual. Evidence for this has been shown in an elegant experiment conducted by McDonald and White (1993). They investigated the effects of bilateral NMDA lesions in the dorsal striatum on three different memory tasks. The apparatus and reinforcer in all three tasks was the same. Food-deprived rats were tested in an 8-arm radial maze with food pellets as the reinforcer. The lesioned rats did not show any impairments on the acquisition of a win-shift task, where they were required to visit each arm of the maze only once to receive food reward. McDonald and White (1993) argued that successful performance on this task required the rat to learn about the relationship between the location of each arm and a number of different stimuli. Performance could not be interpreted as associative learning since some of the visual cues used for identification of one arm of the maze overlapped with adjacent arms. The lesioned rats did not show any impairments on a conditioned cue preference task either. The protocol for this task is very similar to that for CPP, and involved only two arms of the maze, one of which was lit and the other which was dark. The rats received four pairings each of food/ lit arm, no food/dark arm (or vice versa) before being tested for the amount of time spent in each arm. Rats with lesions to the dorsal striatum successfully acquired this task and spent longer in the arm previously paired with food. This task involved the formation of a stimulus-reward association where a neutral stimulus was paired with a reinforcer. However rats with lesions in the dorsal striatum were impaired on the acquisition of a win/stay memory task. In this task, four of the arms of the maze were lit randomly. The rat received a food reward from the lit arms, each arm could be visited twice before the light was turned off and no more food placed in the well at the end of the arm. Successful performance on this task required the formation of a simple stimulus-response sequence, where the food itself acquires no special significance other than to reinforce the approach response to the lit arm (stimulus). This win/stay task is an example of a stimulus-response sequence which becomes habitual after several pairings. Thus rats with bilateral lesions of the dorsal striatum are impaired on the acquisition of stimulus-response habits (McDonald and White 1993).
The PPTg is one site where information from the two functionally different striatal components - CP and NAcc - are combined. The PPTg's function may be to inhibit on-going motor programmes generated by the CP, in order to integrate these with information about the motivational state of the animal, mediated by the NAcc. While separate neural systems mediating "habits" and "motivation" have been identified, no mechanism for integrating them has previously been described.
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