FUNCTIONAL ROLES OF THE RAT NUCLEUS ACCUMBENS: FURTHER INVESTIGATIONS USING MICROINJECTION, LESION AND ELECTROCHEMICAL TECHNIQUES

Ruth Weissenborn

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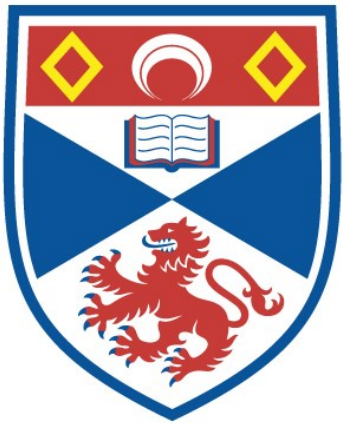

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FUNCTIONAL ROLES OF THE RAT NUCLEUS ACCUMBENS: FURTHER INVESTIGATIONS USING MICROINJECTION, LESION AND ELECTROCHEMICAL TECHNIQUES

Ph.D. Thesis

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October 1992

DECLARATION

(a) I, Ruth Weissenborn, hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

Signed Date 28/9/92

(b) I was admintted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12 in October 1989, and as a candidate for the degree of Ph.D. in October 1986.

Signed Date 28/9/92

(c) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the Degree of Ph.D.

Signature of Supervisor Date 28·9·92

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Als Dank an meine Eltern

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ABSTRACT

The nucleus accumbens (N.Acc.) has been ascribed an important role in mediating locomotor activity and exploration, as well as more complex processes such as reinforcement, reward and the acquisition of displacement activities. Previous investigations of N.Acc. functions have primarily been based on pharmacological manipulations of activity of one of the main neurotransmitters in the N.Acc., dopamine (DA), either through administration of dopaminergic agonists or antagonists or through depletion of DA terminal fields in the N.Acc.. In the present thesis, the functional role of the N.Acc. in a number of different forms of behaviour has been investigated further using specific, fibre-sparing excitotoxic lesions of intrinsic neurones, intra-accumbens injections of DA and *in vivo* electrochemical measurements of extracellular levels of DA in the N.Acc.. Excitotoxic lesions in the N.Acc. were found to enhance spontaneous locomotion and exploratory behaviours while leaving intact the locomotor-stimulating effects of an indirect dopaminergic agonist, displacement drinking in response to intermittent food-reinforcement (SIP) and amphetamine-induced conditioned place preference (CPP). Thus, fibre-sparing excitotoxic lesions induced a pattern of behaviour distinct from that observed following terminal depletion in the N.Acc.. Further, microinjection and *in vivo* electrochemical experiments showed no direct relationship between DA activity in the N.Acc. and SIP. Overall, these results are discussed in terms of a theoretical model proposing that the N.Acc. may function as an interface between sensory input and locomotor output and that inhibitory activity in the N.Acc. is needed to channel activity levels appropriately in response to cortical input about the direction of change. It is suggested that rather than viewing it as a unitary

structure with specific functions, the N.Acc. should be considered as a heterogeneous part of the striatal complex with a number of distinct subsystems that exist within a complex framework of interactive processes, where changes in one structure can only be understood by taking into account other, related structures.

ABBREVIATIONS IN THE TEXT

AA	-	Ascorbic acid
ADS	-	Antibody diluting solution
Ag/AgCl	-	Silver/Silver chloride
AMPA	-	Alpha-amino-3-hyroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	-	Analysis of variance
Ca^{++}	-	Calcium
cAMP	-	3,5-Cyclic adenosine monophosphate
CCS	-	Corticosterone
CPP	-	Conditioned place preference
DA	-	Dopamine
DAB	-	Diaminobenzidine
DMT	-	Dorsomedial nucleus of the thalamus
DOPAC	-	3,4-Dihydroxyphenylacetic acid
FI	-	Fixed interval schedule of reinforcement
GABA	-	Gamma-aminobutyrate
H_2O_2	-	Hydrogen peroxide
HPLC	-	High performance liquid chromatography
HRP	-	Horseradish peroxidase
5-HT	-	Serotonin
IgG	-	Immunoglobulin
i.p.	-	Intraperitoneal
K^+	-	Potassium
MAO	-	Monoamine oxidase
N.Acc.	-	Nucleus accumbens
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
NMDA	-	*N*-methyl-D-aspartate
6-OHDA	-	6-hydroxydopamine
PBS	-	Phosphate buffered saline
PE	-	Pulmonary edema
PPTg	-	Pedunculopontine tegmental nucleus
s.c.	-	Subcutaneous
SDL	-	State-dependent learning
TOH	-	Tyrosine hydroxylase
VTA	-	Ventral tegmental area

CONTENTS

CHAPTER I

INTRODUCTION

NEUROANATOMY OF THE VENTRAL STRIATUM

While the pathways arising from mesencephalic dopamine (DA)-containing cell groups were originally subdivided into a nigrostriatal system originating in A9 and a mesolimbic system originating in A10 (Ungerstedt, 1971a), recent work has identified several areas in which terminals from both systems overlap. In addition, parts of the limbic basal forebrain are now considered as ventral extensions of the neostriatum. Thus, this DA projection system is now referred to as the *mesotelencephalic system* with two main subsystems: the *mesostriatal system*, formed by projections from A9 and A10 to the entire striatum (caudate-putamen, nucleus accumbens, olfactory tubercle and bed nucleus of stria terminalis) and the *mesolimbocortical system*, formed by projections from A9 and A10 to limbic, allocortical and neocortical areas (Bjorklund and Lindvall, 1986). On the basis of afferent and efferent projections, two distinct but interactive striatal systems can be identified: a *nigrostriatal system* which consists of the *dorsal striatum* (most of the caudate-putamen) innervated primarily by the DA-containing cell group A9 (substantia nigra pars compacta) and in turn projecting to the dorsal pallidum (globus pallidus and entopeduncular nucleus). The *tegmentostriatal system* includes the *ventral striatum* - comprising the bed nucleus of stria terminalis, nucleus accumbens (N.Acc.) and olfactory tubercle, as well as the most ventral and medial parts of the caudate-putamen (Heimer et al, 1982) - which receives its dopaminergic input primarily from cell group A10 (ventral tegmental area - VTA) and projects

to the ventral pallidum (Bjorklund and Lindvall, 1986). The mesolimbocortical projection system forms part of the medial forebrain bundle connecting the brainstem reticular formation with diencephalic and telencephalic areas. It includes mesencephalic DA innervation of the lateral septum, amygdala, hippocampus, piriform, entorhinal and perirhinal cortices and the prefrontal cortex (Bjorklund and Lindvall, 1986).

Afferents and efferents of the ventral striatum

The majority of anatomical studies referred to in the following paragraphs have used a variety of tracing methods based on amino acid autoradiography or horseradish peroxidase (HRP) to obtain information about neuronal connections. Anterograde projections from a cell body to its terminals can be traced by injecting into brain radioactive amino acids which are then transported to the terminal fields in form of proteins and can be identified using photographic techniques. Retrograde connections are typically traced by infusing the enzyme HRP into the brain region of interest, where the enzyme is taken up by terminal fields and transported back to the cell bodies. HRP-positive staining techniques can then be used to mark the distribution of the enzyme. Fig. 1.1 represents the mesostriatal (A) and mesolimbocortical (B) DA projection systems. All ventral striatal areas receive extensive dopaminergic inputs originating primarily in the A10 cell group (Ungerstedt, 1971a). Further innervations arise from neurones located in the medial part of the substantia nigra pars compacta and, to a lesser extent, from cells in the A8 group in and around the retrorubal nucleus (Nauta et al, 1978). The majority of these projections are dopaminergic, with only 10-15% originating from nondopaminergic cells (e.g. serotonergic neurones in the dorsal raphe

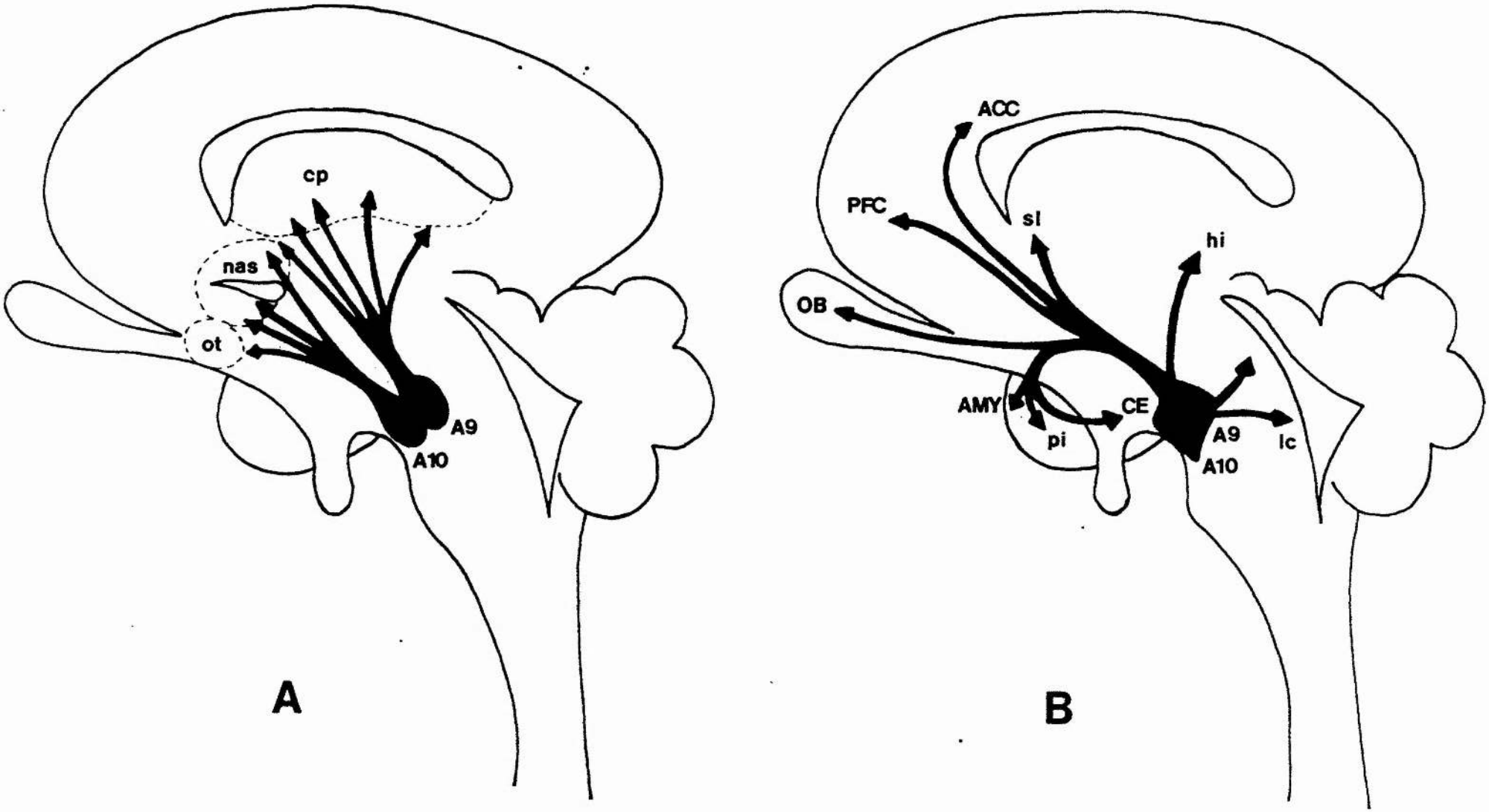


Fig. 1.1 Mesostriatal (A) and mesolimbocortical (B) dopamine projections. ACC, anterior commissure, AMY, amygdala, CE, entorhinal cortex; cp, caudate putamen, hi, hippocampus; OB, olfactory bulb; ot, olfactory tubercle; nas, nucleus accumbens; PFC, prefrontal cortex; pi, piriform cortex; sl, lateral septum.

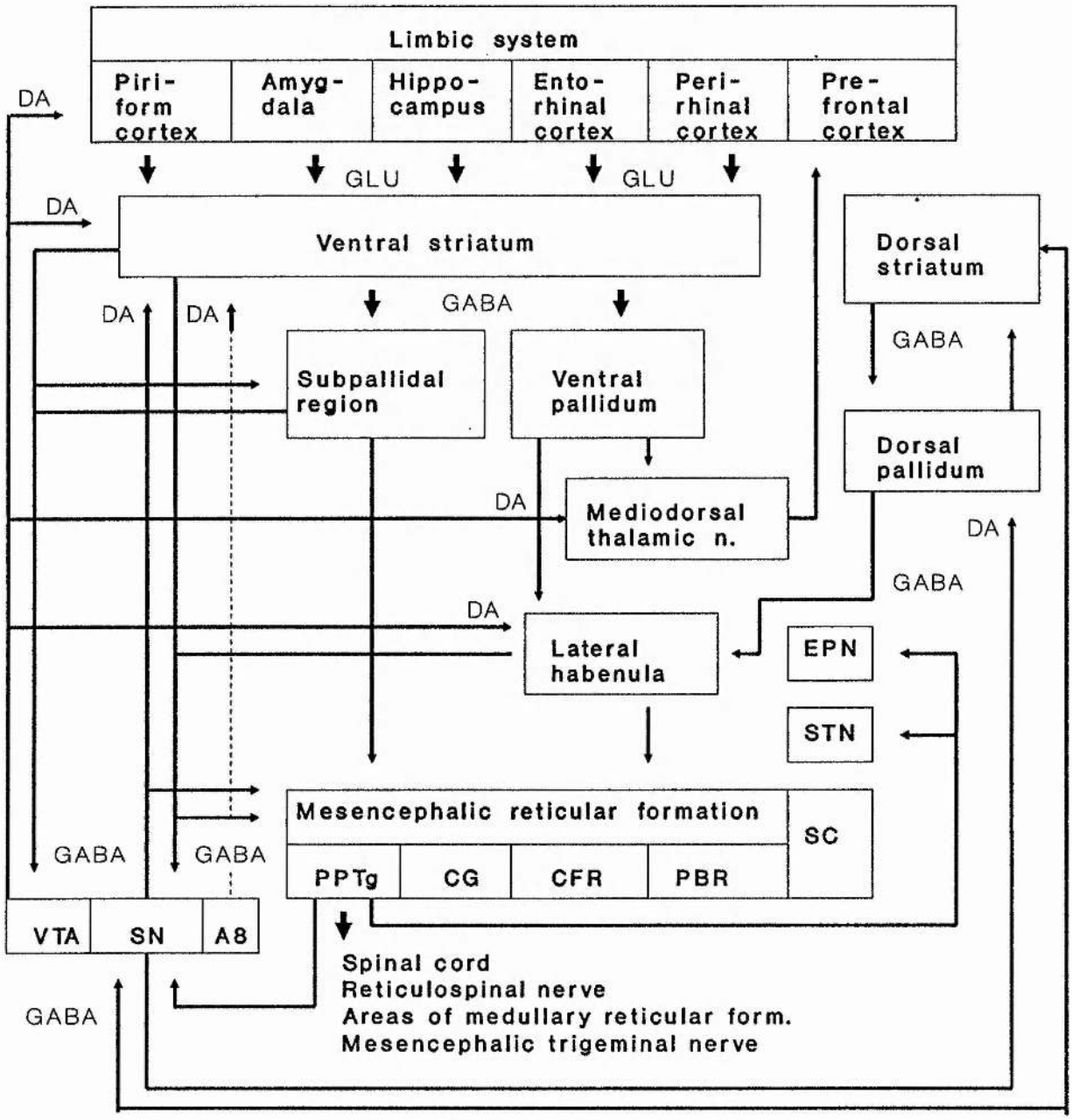
nucleus) and arranged on a medial-to-lateral axis in relation to terminal areas, with single neurones innervating more than one terminal area (Bjorklund and Lindvall, 1986). Anterograde and retrograde labelling has further shown that the ventral striatum also receives extensive limbic input from the amygdala (Kelley et al, 1982; Yim and Mogenson, 1982), hippocampus (Kelley and Domesick, 1982; Yang and Mogenson, 1984, 1987), cortical areas associated with the limbic system (entorhinal, perirhinal and piriform cortices) and the prefrontal cortex (Bjorklund and Lindvall, 1986).

All ventral striatal areas project to the ventral pallidum defined as a rostroventral extension of the globus pallidus underneath the temporal extension of the anterior commissure, including parts of the substantia innominata (Groenewegen and Russchen, 1984; Heimer et al, 1982; Nauta et al, 1978). Additional projections from the N.Acc. extend into the subpallidal region including areas associated with limbic circuitries, such as the sublenticular part of the substantia innominata, lateral preoptic area, lateral septum and lateral hypothalamus, as well as lateral aspects of the amygdala (Groenewegen and Russchen, 1984; Mogenson et al, 1983; Nauta et al, 1978; Swanson et al, 1984). However, these projections are diffuse and sparse in comparison with striatopallidal afferents. The ventral pallidum in turn innervates the mediodorsal thalamic nucleus projecting to the prefrontal cortex (Heimer et al, 1982). Other targets of the ventral pallidum are the lateral habenula (Herkenham and Nauta, 1977) and the pedunculopontine tegmental nucleus (PPTg) of the mesencephalic reticular formation (Swanson et al, 1984). Neurones in the subpallidal region project to a number of areas along the medial forebrain bundle, including the PPTg, central grey, and superior colliculus (Swanson et al, 1984).

Ventral striatal circuitry

In addition to its pallidal projections, ventral striatal output feeds back to mesencephalic DA cell groups. The bed nucleus of stria terminalis projects primarily to the VTA, while N.Acc. fibres terminate mainly in the medial part of the substantia nigra pars compacta, and the pars reticulata (Nauta et al, 1978). Somogyi and colleagues (1981) were able to show that some of the projections from the N.Acc. to substantia nigra synapse on neurones innervating dorsal striatal regions. Thus, the substantia nigra seems to be a point of convergence for efferents from the ventral and dorsal striatum. Other areas where inputs from both regulatory circuits overlap are the lateral habenula and the PPTg. As well as feeding back into dopaminergic cell groups, descending ventral striatal and ventral pallidal efferents also project to the mesencephalic reticular formation (cuneiform and parabrachial regions, central grey, and raphe nuclei) which includes the PPTg (Groenewegen and Russchen, 1984; Nauta et al, 1978; Swanson et al, 1984). Since the same is true for descending projections transmitted via the lateral habenula and substantia nigra, *all* descending output from the ventral striatum feeds into dorsal and medial parts of the mesencephalic reticular formation. In turn, parts of this formation, the PPTg in particular, feed back to structures of the dorsal striatal circuitry (substantia nigra pars compacta, subthalamic nucleus, entopeduncular nucleus and dorsal striatum), as well as to ponto-medullary regions (Blaha and Winn, 1992; Garcia-Rill, 1986; Moon Edley and Graybiel, 1983). The mesencephalic reticular formation, including the PPTg, has been shown to play an important role in the integration of sensory inputs and motor responses, and may play a role in the motor deficits associated with basal ganglia disorders, such as Parkinson's disease (Garcia-Rill, 1986). Fig. 1.2 provides an overview over the main structures and projections

Fig. 1.2 Ventral striatal circuitry and its interaction with the dorsal striatal complex (adapted from Bjorklund and Lindvall, 1986). CFR, cuneiform region; CG, central gray; DA, dopamine; EPN, entopeduncular nucleus; GLU, glutamate; PBR, parabrachial nucleus; PPTg, pedunculopontine tegmental nucleus; SC, superior colliculus; SN, substantia nigra; STN, subthalamic nucleus; VTA, ventral tegmental area.



involved in the ventral striatal circuitry, as well as their interactions with the dorsal striatum.

Neurotransmitter systems in the ventral striatum

DA-containing neurones have been found to interact primarily with GABAergic, glutamatergic and cholinergic neurones in the ventral striatal circuitry. Descending N.Acc. output to the ventral pallidum, substantia nigra and VTA is relayed via GABAergic and cholinergic projections (Churchill et al, 1990; Mogenson et al, 1983). There is, for example, electrophysiological evidence for amygdala stimulation to activate inhibitory GABAergic neurones in the N.Acc. which in turn inhibit ventral pallidal neurones (Yim and Mogenson, 1983) and for glutamatergic projections from limbic areas and the prefrontal cortex to provide excitatory input to the ventral striatum (Walaas, 1981). Yim and Mogenson (1989) have shown that glutamatergic projections from the basolateral nucleus of the amygdala to the N.Acc. inhibit spontaneous locomotion, although it is not clear whether this effect is mediated through projections from the N.Acc. to the ventral pallidum, or whether descending pathways to substantia nigra or VTA are involved. Finally, choline-acetyltransferase-positive staining has identified cholinergic and cholinoceptive neurones in the ventral striatal complex, especially the ventral pallidum, nucleus accumbens and olfactory tubercle (Kimura et al, 1981) (see Fig. 1.2).

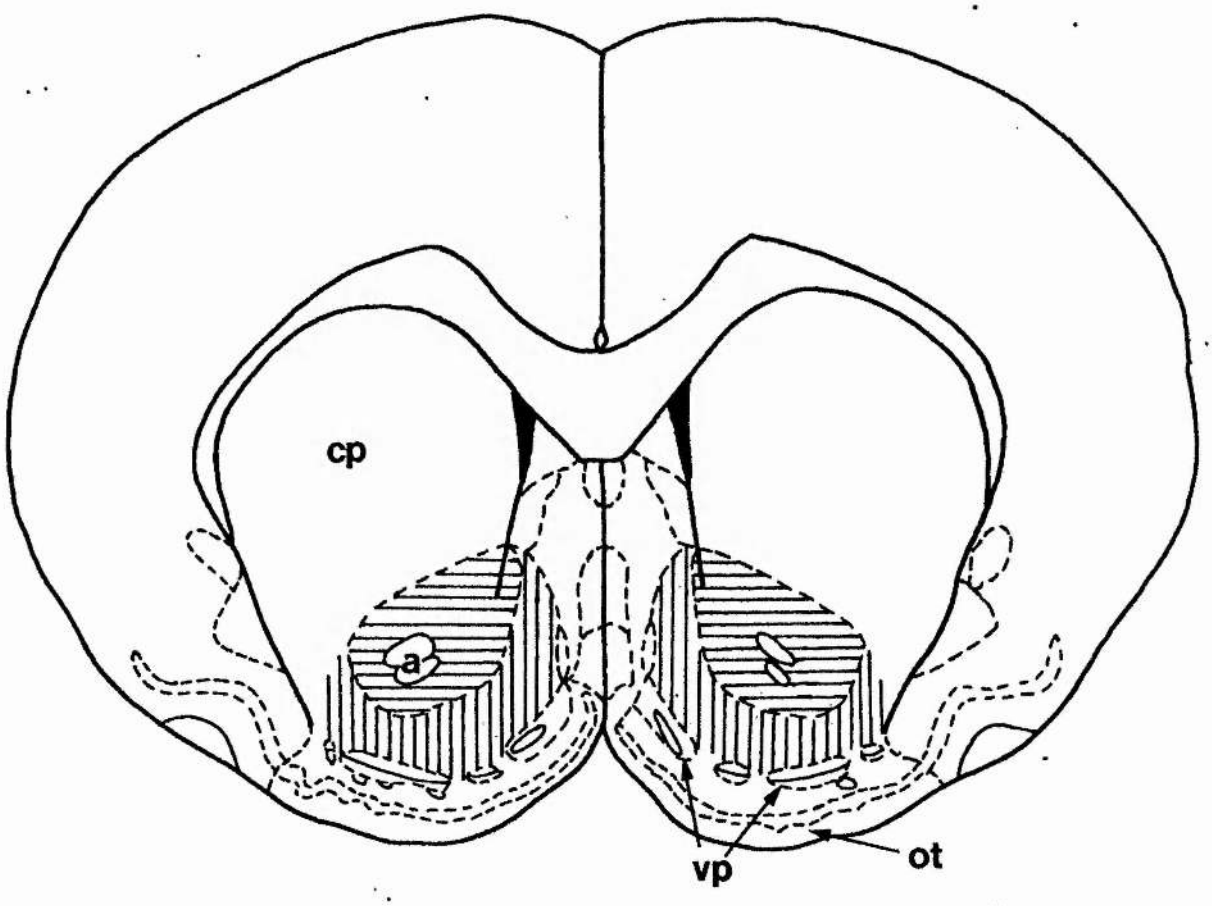
Recent work by Halpain, Girault and Greengard (1990) has identified some of the biochemical processes involved in dopaminergic and glutamatergic control of striatal output. According to these authors, glutamatergic input from the neocortex and dopaminergic input from the substantia nigra have opposing effects on striatal activity. It is known that

DA exerts its effects by stimulating cAMP-dependent phosphorylation of DARPP-32 (a DA- and cAMP-regulated phosphoprotein) which then inhibits protein phosphatase in striatal tissue. This process is reversed by activation of a subclass of glutamate receptor, leading to dephosphorylation of DARPP-32 and activation of protein phosphatase. Though the exact effects of protein phosphatase are not yet established, a number of substrates of the enzyme have been identified, e.g., ion channels, synaptic vesicle proteins as well as other proteins. Thus, DARPP-32 activity and resulting striatal output are regulated by two different neurotransmitters with distinct but interacting second messenger systems.

Subdivisions of the nucleus accumbens

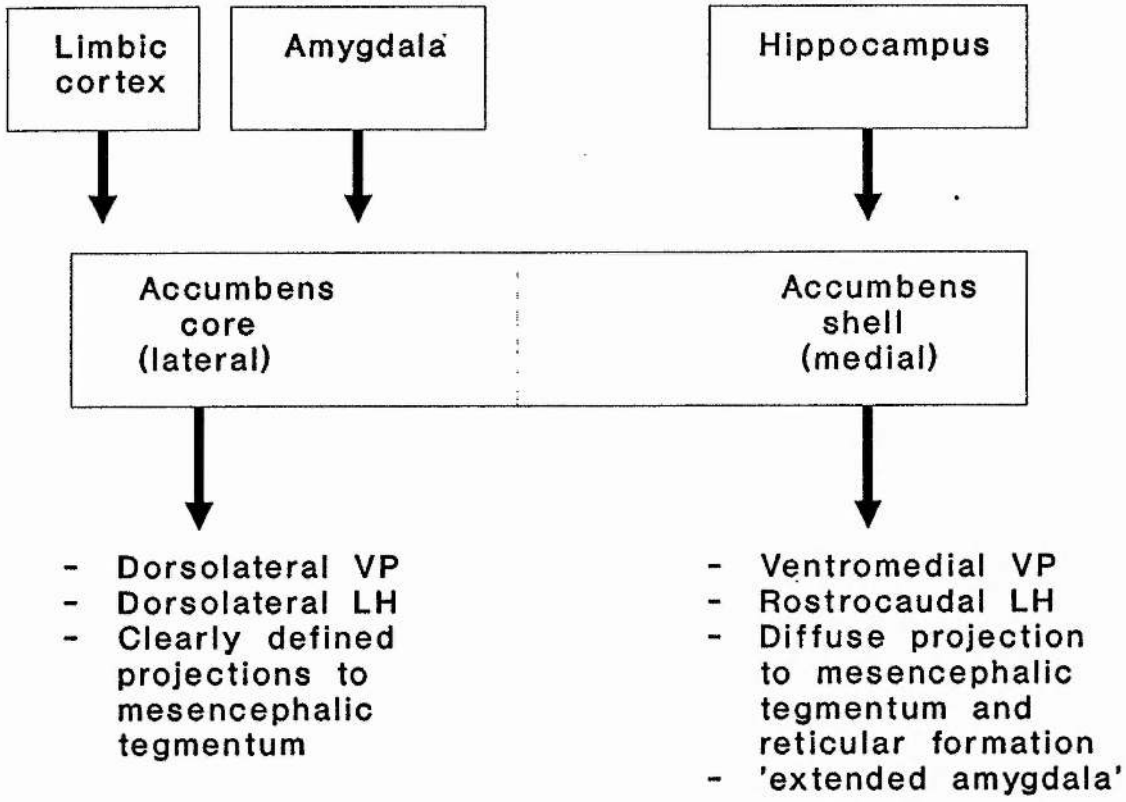
It is important to note that the ventral striatum, as well as its principal structure, the N.Acc., cannot be considered as homogeneous areas. Ventral striatal afferents from the hippocampus, for example, synapse on a distinct region of the medial N.Acc., whereas input from the basolateral amygdala terminates within the entire ventral striatum; afferents from the piriform cortex innervate the olfactory tubercle and ventral part of the N.Acc.; afferents from entorhinal and perirhinal cortices terminate in the olfactory tubercle and a wider area of the N.Acc.; the bed nucleus of stria terminalis receives limbic and allocortical input mainly from the amygdala and hippocampus (Groenewegen and Russchen, 1984; Kelley and Domesick, 1982; Kelley et al, 1982). Based on the distribution of a number of neuropeptides (including cholecystokinin, angiotensin II and neurotensin) and other substrates, the N.Acc. itself can be subdivided into a *core* and a *shell* region, with the shell covering medial, lateral and ventral sides of the core (Heimer et al, 1991;

Fig. 1.3 Core and shell areas of the nucleus accumbens. Horizontal stripes: accumbens core; vertical stripes: accumbens shell; a, anterior commissure; cp, caudate putamen,; ot, olfactory tubercle; vp, ventral pallidum. Section (1.20 mm anterior to bregma) drawn from the atlas of Paxinos and Watson (1986).



Zaborszky et al, 1985) (see Fig. 1.3). Despite their neurochemical differences, these two regions are strongly interrelated via an *intrastriatal association system*, consisting of an extensive network of fine fibres distributed throughout the accumbens and ventral caudate-putamen (Heimer et al, 1991). Projection patterns have further led to a distinction between *medial* and *lateral* aspects of the N.Acc., and since the medial N.Acc. corresponds almost entirely to the area defined as accumbens shell, it is argued that the distinction between lateral and medial N.Acc. may reflect differences between accumbens core and shell (Groenewegen and Russchen, 1984; Heimer et al, 1991). Fig. 1.4 indicates differential afferent and efferent connections of accumbens core and shell. The accumbens shell receives strong input from the subiculum and projects predominantly to the ventromedial part of the ventral pallidum, the ventral tegmental area and also sends some diffuse projections to the mesencephalic tegmentum and reticular formation. In contrast, the accumbens core is innervated mainly by limbic cortices and the amygdala, with projections primarily to the dorsolateral ventral pallidum, substantia nigra and mesencephalic tegmentum. For example, specific lesions of neurones intrinsic to accumbens core or accumbens shell areas have been shown to result in differential up-regulation of $GABA_A$ receptors in the ventral pallidum. While neuronal degeneration in the dorsomedial core enhanced muscimol binding (indicating increased number or affinity of $GABA_A$ receptors) in the dorsal ventral pallidum, lesions in the accumbens shell or lateral core did not change $GABA_A$ receptor binding in the ventral pallidum (Churchill et al, 1990). The differences between shell and core are most pronounced with regard to their efferents to the lateral hypothalamus and the 'extended amygdala'. While core efferents terminate primarily in a region where the entopeduncular nucleus extends

Fig. 1.4 Differential afferent and efferent connections of nucleus accumbens core and shell. LH, lateral hypothalamus; VP, ventral pallidum.



into the lateral hypothalamus, the shell projects diffusely to the rostrocaudal extent of the lateral hypothalamus. The concept of an *'extended amygdala'* was introduced by Alheid and Heimer (1988) defining it as a major basal forebrain structure formed by the bed nucleus of the stria terminalis, the sublenticular substantia innominata and the ventromedial amygdala. Since this 'extended amygdala' has histochemical and connectional features resembling the accumbens shell it is suggested that some parts of the shell represent a rostral extension of the extended amygdala, or that parts of the ventral striatum and extended amygdala overlap within the area of the accumbens shell (Alheid and Heimer, 1988). Thus, only shell projections terminate diffusely in the sublenticular part of the extended amygdala (Heimer et al, 1991).

Although the subdivision of the N.Acc. is based on elaborate tracing techniques that allow accurate identification of terminal areas it is of course subject to some simplification. Nevertheless, the data suggest that it is necessary to differentiate functionally and anatomically not only between the caudate-putamen and N.Acc., but, more importantly, between the core and shell of the N.Acc. The precise functional implications of such subsystems in the N.Acc. are at present unclear and remain to be investigated (Groenewegen and Russchen, 1984; Heimer et al, 1991).

Compartmental organization of the striatum

The complexity of striatal structure is further emphasized by recent work by Gerfen and colleagues who have identified a patch-matrix pattern of striatal compartmentalization that is superimposed on the classically accepted dorsal-ventral division. Analyses of neuroanatomical markers as well as of afferent and efferent connections have shown that striatal neurones are subdivided into patch and matrix compartments with

differential dopaminergic systems, terminal fields and projections to dopaminergic and GABAergic neurones. Patch-matrix organization was first reported following labelling of high densities of *u* opiate receptors in patches. Subsequently, other neurochemical markers (especially the neuropeptides enkephalin and substance P, as well as DA) were identified confirming a patch-matrix pattern throughout the striatum (Gerfen, 1992), interestingly with the exception of the shell region of the nucleus accumbens, which is thought to consist of several compartments with different afferent and efferent connections and neurochemical characteristics, rather than just a patch and a matrix compartment (Voorn et al, 1989). The dendrites of neurones in both patches and matrix are contained within the respective compartments, thereby maintaining the separation of compartmentally organized inputs (Gerfen, 1985). Striatal matrix and patches have been shown to be innervated by distinct dorsal and ventral sets of mesencephalic DA neurones, respectively, with the matrix receiving additional input from non-dopaminergic neurones located in the substantia nigra (Gerfen et al, 1987). Similarly, distinct patch and matrix striatonigral projections have been identified (Gerfen, 1984, 1985), although interactions between striatonigral and mesostriatal projection systems are reciprocal as well as non-reciprocal. Patches and matrix can further be differentiated on the basis of cortical afferents. Cortical inputs to the patch compartment arise from the deep layer V and layer VI of the cortex, whereas the matrix is innervated by superficial layer V and the supragranular layers (Gerfen, 1989). Initially, it was therefore argued that patches and matrix correspond to limbic and non-limbic cortical inputs, respectively (Gerfen, 1984). However, considering evidence of periallocortical projections to striatal matrix (Gerfen, 1989), it seems more appropriate to distinguish two neuroanatomical circuits formed by patch-

matrix compartmentalization. One consists of widespread and nonspecific connections typical of limbic cortex, the other of indirect specific connections typical of neocortex. Although both circuits can be identified throughout most of the striatum, their combination varies such that patches are found primarily in ventral striatum innervated by allocortical structures, whereas the caudate-putamen receiving input from the neocortex has a predominantly matrix organization and relatively few patches (Gerfen, 1992). Thus, patch-matrix organization should be viewed as a mechanism retaining information typical of limbic cortex and neocortex, respectively. Since patches and matrix project to different dopaminergic and GABAergic neurones, an interaction between the two systems may provide an additional mechanism through which DA can modulate striatonigral and striatopallidal output pathways (Gerfen, 1992).

Patch-matrix organization therefore reflects the heterogeneity of the striatum and further emphasizes the complexity of interactions in the mesotelencephalic DA system. It is important to bear in mind the implications of striatal heterogeneity and compartmentalization, especially during the following sections reviewing ventral striatal functions in more detail.

FUNCTIONAL ASPECTS OF THE VENTRAL STRIATAL CIRCUITRY

Both dorsal and ventral striatal neurones are thought to be involved primarily in integrating cognition, perception and action (Chevalier and Deniau, 1990). The neurological disorders most frequently associated with impairments in striatal function and dopaminergic transmission are

Parkinson's disease and schizophrenia. Parkinson's disease is caused by degeneration of mesencephalic DA-containing cell groups resulting in abnormal neuronal activity (both *increased* and *decreased*) in the basal ganglia circuitry and projections from the dorsal striatum to the globus pallidus, subthalamic nucleus, thalamus and PPTg in particular (Mitchell et al, 1989). In the parkinsonian patient, this change in neuronal activity is typically characterized by a deficit in the initiation of voluntary movements. Recently, animal models of the Parkinson's disease have been developed and the symptoms in affected rats include akinesia, catalepsy, postural abnormalities, sensory neglect, aphagia and adipsia.

By contrast, hyperactivity and stereotyped behaviours are observed in rats with *increased* dopaminergic transmission and overactivity of the striatal system. Mesolimbic DA hyperactivity is assumed to occur in schizophrenic patients and this overactivity can lead to involuntary movements, dystonia, stereotypy, excitation as well as psychosis. It may further result in deficiencies of sensorimotor gating, i.e., the appropriate responding to sensory stimuli (Swerdlow et al, 1990b).

Striatal control of motor systems

Striatal control of motor output in response to cognitive or sensory inputs is thought to be promoted via GABAergic *disinhibition* (Chevalier and Deniau, 1990). Regarding the dorsal striatal circuitry, it has been shown that striatal efferents are GABAergic and have an inhibitory action on the entopeduncular nucleus and substantia nigra, which are in turn GABAergic and disinhibit neurones in the thalamus and mesencephalic reticular formation. The majority of striatal neurones are inactive when the organism is immobile, while pallidal and nigral neurones are tonically active. Experiments combining single cell recordings in the substantia

nigra with pharmacological stimulation of the striatum have shown that increased striatal activity does indeed inhibit the entopeduncular nucleus and substantia nigra, resulting in disinhibition of neurones in the thalamus and mesencephalic reticular formation. The advantage of a disinhibitory mechanism - rather than direct excitation - translating cognitive and sensory information into action is that the striatum can exert control over motor output pathways in two ways: on one hand, when striatal cells are inactive, target neurones are under inhibitory control; activity in the striatum, on the other hand, allows the activation of brain stem motor systems (Chevalier and Deniau, 1990). Similar disinhibitory mechanisms are likely to process information in the ventral striatal circuitry also based upon GABAergic transmission.

Disinhibition has a central role in the model of striatal motor systems proposed by Swerdlow and Koob (1987a), according to whom both the dorsal and the ventral striatal motor systems consist of three interacting feedback loops: a cortico-thalamo-cortical positive feedback loop; a cortico-striato-pallido-thalamo-cortical positive feedback loop; and a striato-pallido-tegmento-striatal negative feedback loop. The circuitry constituting the N.Acc. motor system will be described in more detail here. Loop I is formed by excitatory input from the limbic cortex to the dorsomedial thalamic nucleus (DMT) and back to the limbic cortex. This loop may serve to maintain an ongoing stream of impulses necessary in a continuous motor program. Loop II consists of excitatory, glutamatergic projections from the limbic cortex to inhibitory GABAergic efferents from the ventral striatum to the ventral pallidum, which in turn has inhibitory effects on activity in the DMT, thereby further strengthening the excitatory Loop I through disinhibition. This pattern of activity is maintained by *lateral inhibition*, where firing in one subset of neurones

will inhibit activity in adjacent sets of neurones (Groves, 1983). Loop III is involved in the termination of this excitatory feedback: excitatory input from the limbic cortex leads to activation of inhibitory striatal efferents projecting to the ventral pallidum, thereby reducing the inhibitory GABAergic output of the ventral pallidum to ascending dopaminergic projections to the striatum. Since this dopaminergic input is in turn inhibitory, ventral striatal firing is reduced and can switch to a new pattern of activity. According to this model, dopaminergic input to the striatum has a permissive, or '*gating*' function, rather than a directional one (Gray et al, 1991). Further striatal inputs are necessary to provide sensory or cognitive information about direction of change. A diagram of the limbic cortico-striato-pallido-thalamic-midbrain circuitry is shown in Fig. 1.5.

Substantial support for this 'gating' hypothesis of dopaminergic control of limbic afferents has been provided by studies by Mogenson and colleagues. Owing to its converging limbic afferents and projections to the mesencephalic reticular formation and substantia nigra, the N.Acc. has been ascribed the role of a '*limbic-motor interface*' translating 'motivation' into 'action' by acting as a gating mechanism for signals from limbic to motor systems (Mogenson et al, 1980). Dopaminergic projections from the VTA were found to attenuate excitatory responses of N.Acc. neurones to hippocampal stimulation (Yang and Mogenson, 1984). Similarly, DA applied iontophoretically to the N.Acc. and VTA stimulation both attenuated the response of N.Acc. neurones to amygdala stimulation (Yim and Mogenson, 1982). The *neuromodulatory* role of ventral striatal DA was further underlined by the observation that intra-accumbens administration of low doses of DA did not induce hyperactivity observed following administration of larger doses, but significantly attenuated locomotor activity by amygdala stimulation (Yim and Mogenson, 1989).

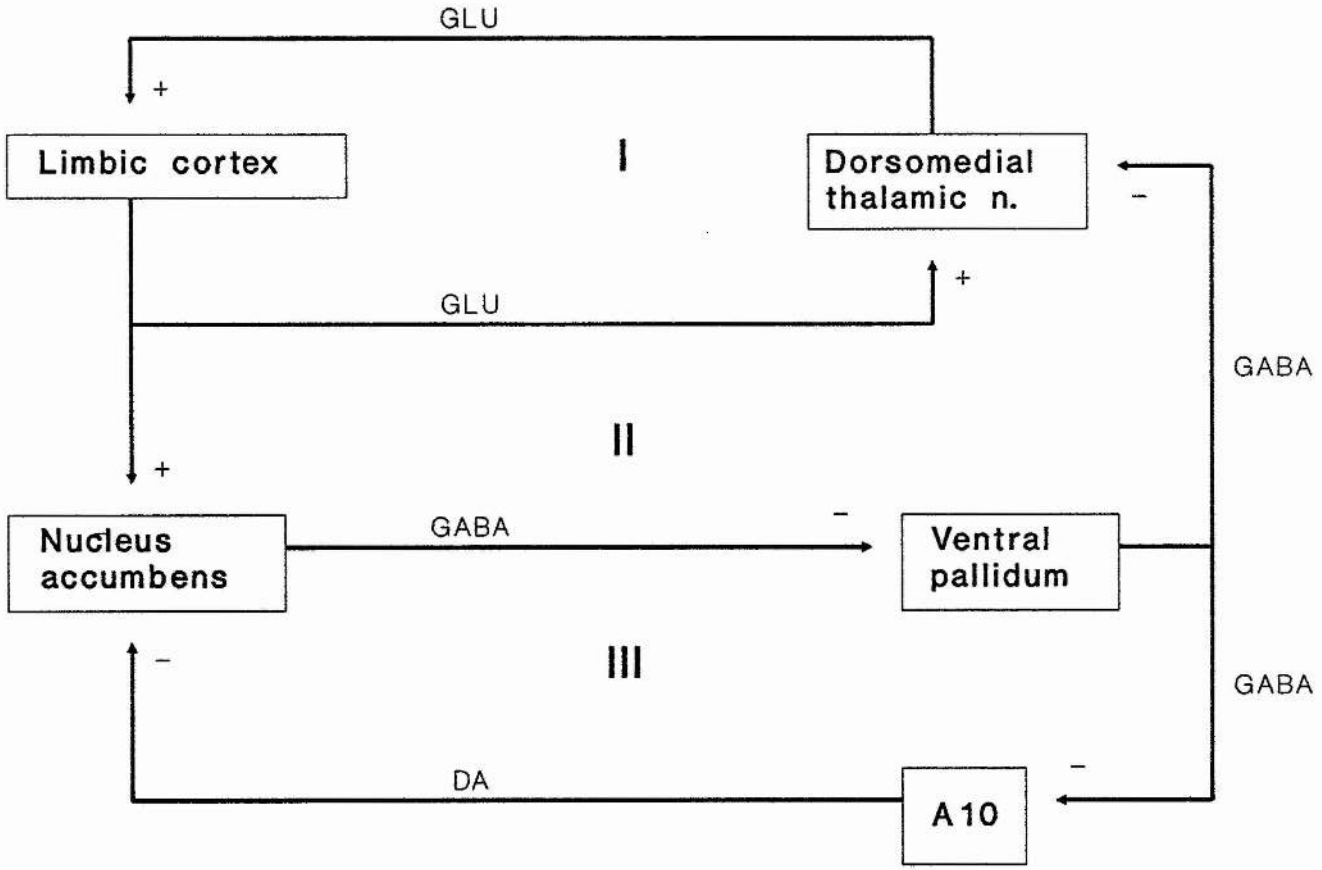


Fig. 1.5 Nucleus accumbens motor system. Limbic cortico-striato-pallido-thalamic-midbrain circuitry (Swerdlow and Koob, 1987).

According to the model of the N.Acc. as a 'limbic-motor interface', degeneration of mesencephalic cell groups projecting to the ventral (and dorsal) striatum, as in Parkinson's disease, causes breakdown of the gating mechanism and appropriate information no longer reaches the ventral pallidum (and globus pallidus), resulting in severe motor deficits and a failure to initiate motivated behaviour (Mogenson et al, 1980).

Recent work summarized in a review by Carlsson and Carlsson (1990) implies not only strong interactions between glutamatergic and dopaminergic transmission in the striatum, but also glutamatergic activity unrelated to dopaminergic systems in the generation and initiation of motor output. Although most of the evidence comes from studies concerned with dorsal aspects of the striatum, it is reasonable to assume that similar processes occur in the ventral striatal complex. In accordance with Swerdlow and Koob (1987a, see above), Carlsson and Carlsson (1990) put forward the notion of cortico-striatal thalamo-cortical negative feedback controlling cortical activity. Activation of cortical projections to the thalamus (relayed via the striatum, pallidum and subthalamic nucleus) would result in reduced thalamic responding to sensory inputs and therefore attenuate the amount of input that reaches cortical areas. Dopaminergic projections, on the other hand, would enhance thalamic activity and enhance the information flow to the cortex. A similar mechanism underlies the control of neuronal firing in the mesencephalic reticular formation. Thus, glutamatergic and dopaminergic projection systems act independently of each other but ultimately synapse on the same target neurones that they influence in opposite directions. Administration of a selective glutamate antagonist acting at NMDA receptor subtypes was found to significantly enhance locomotor activity in rats and mice depleted of monoamines following pretreatment with

reserpine (blocking catecholamine uptake and storage) and alpha-methyl-*p*-tyrosine (inhibiting tyrosine hydroxylase). Enhanced effects obtained by combining the NMDA antagonist with adrenergic or dopaminergic agonists or muscarinic antagonists suggest that several transmitter systems may interact to initiate locomotion. Glutamatergic NMDA receptors in the striatum therefore seem to be strongly involved in the control of locomotion, whereas dopaminergic mechanisms may be less critically implicated than previously thought. Together with reports that phencyclidine (PCP), a drug which induces behavioural patterns closely resembling schizophrenic symptoms, acts as an NMDA antagonist, these findings support suggestions that schizophrenia may be caused by a deficiency in corticostriatal glutamatergic transmission, rather than by a hyperactive DA system as previously suggested (Carlsson and Carlsson, 1990). Conversely, parkinsonian patients might benefit from administration of NMDA antagonists as part of the pharmacological intervention. In the light of an interaction between NMDA antagonists and catecholaminergic agonists or muscarinic antagonists both schizophrenic and parkinsonian symptoms may be alleviated through manipulation of adrenergic and cholinergic systems.

In summary, striatal neurones appear to control motor outflow through GABAergic disinhibition of the thalamus and premotor neurones in the mesencephalic reticular formation. According to work by Mogenson and colleagues, and Swerdlow and Koob, the N.Acc. acts as a gating mechanism, allowing the organism to switch between patterns of activity in response to directional sensory or cognitive information. Corticostriatal glutamatergic and mesostriatal dopaminergic projections act in opposing directions to influence firing in thalamic efferents to cortical areas and the mesencephalic locomotor region. However, evidence has been presented

suggesting that glutamatergic transmission alone can regulate motor outflow, while dopaminergic mechanisms may be less strongly involved than previously assumed.

Behavioural correlates of dopamine activity in the ventral striatum

The ventral and dorsal striatum mediate different and interacting behavioural responses. Unilateral DA depletion in the dorsal striatum (caudate-putamen) produce postural asymmetry, rotation in response to *d*-amphetamine, and impairments in response initiation to contralateral space (Carli et al, 1985; Ungerstedt, 1971b). Robbins and Brown (1990) argue that this so-called 'striatal neglect' is not the result of a sensory deficit, but rather reflects impaired control over response output. Thus, the striatum is believed to control response selection to influence spatial and temporal preference. By contrast, DA release in the N.Acc. (ventral striatum) is implicated in mediating locomotor activity, exploration of novel environments, feeding, the control of conditioned reinforcement and reward-related processes. The activity-enhancing effect of intra-accumbens injections of DA or DA receptor agonists has been widely reported (Costall and Naylor, 1975; Dreher and Jackson, 1989; Hamilton et al, 1986; Pijnenburg et al, 1973, 1976), while dopaminergic depletion of the N.Acc. has been shown to attenuate spontaneous locomotion as well as the locomotor response to DA receptor agonists (Evenden and Carli, 1985; Fink and Smith, 1980a/b; Kelly and Roberts, 1983; Kelly et al, 1975; Koob et al, 1978, 1981; Oades et al, 1986; Winn and Robbins, 1985). Contradictory evidence exists with regard to the exploration of novel environments which was reported to be abolished by dopaminergic depletion of mesolimbocortical terminal areas (Fink and Smith, 1980a), while such deficits were not observed by Winn and Robbins (1985).

Lesions of DA terminal fields in the N.Acc. have also been shown to increase food intake during restricted feeding (Koob et al, 1978), as well as the length of feeding and feeding bouts (Evenden and Carli, 1985). Extensive research has also been directed at the control of reinforcement and reward-related processes, such as drug self-administration, electrical self stimulation, and conditioned preference paradigms which appear to be mediated through dopaminergic mechanisms in the ventral striatum (Aulisi and Hoebel, 1983; Carr and White, 1983, 1986; Everitt et al, 1991; Gold et al, 1988; Kelley and Delfs, 1991a/b; Koob, 1992; Spyraki et al, 1982a/c; Spyraki et al, 1983; Taylor and Robbins, 1984, 1986). The ventral striatum has further been suggested to play a role in switching between motivated behaviours (Evenden and Carli, 1985; Evenden and Robbins, 1983; Robbins and Koob, 1980) and the acquisition of excessive drinking in response to intermittent food-reinforcement (Mittleman et al, 1990; Robbins and Koob, 1980; Wallace et al, 1983) seen as an indicator of behavioural 'inflexibility' and 'stimulus-bound' behaviour (Dantzer at al, 1988). Recently, a number of studies have focused on limbic-striatal interactions underlying locomotor activity and reward-related processes (Cador et al, 1989; Everitt et al, 1991; Mogenson et al, 1980; Yang and Mogenson, 1984; Yim and Mogenson, 1989). Simon and colleagues (1988) showed that depletion of DA terminals in the amygdala led to an increase in DA activity in the N.Acc., an enhanced locomotor response to *d*-amphetamine, as well as an increase in the acquisition of *d*-amphetamine self-administration (Deminiere et al, 1988). Cador, Robbins and Everitt (1989) replicated earlier findings of enhanced conditioned reinforcement in response to intra-accumbens *d*-amphetamine; these data were extended by demonstrating that lesions of neurones intrinsic to the amygdala attenuated responding for the conditioned reinforcer selectively, without affecting the

non-reinforced response. Thus, it was suggested that while ventral striatal DA activity enhances basal levels of instrumental responding in operant conditioning, the amygdala is involved in mediating choice mechanisms between reward and non-reward, underlining interactions between the limbic system and ventral striatal DA in the mediation of operant behaviour (Cador et al, 1989; Robbins et al, 1989). In addition, Everitt and colleagues (1991) suggested that responding to previously neutral stimuli which have been associated with reward ('conditioned place preference') is dependent on interactions of the basolateral amygdala with ventral striatal mechanisms.

Finally, one of the dysfunctions underlying acute schizophrenic symptoms is a disruption in the interaction between the striatal motor programming system and the septohippocampal monitoring system, caused by damage to projections from the subiculum to the N.Acc., according to the model of the neuropsychology of schizophrenia proposed by Gray and colleagues (1991). This hypothesis is supported by abnormalities in several areas of schizophrenic brain, including the hippocampus and amygdala, as well as by the finding that hippocampal lesions increased dopaminergic activity in the N.Acc. (Gray et al, 1991). One of the experimental models used to assess cognitive deficits in schizophrenic patients is the phenomenon of 'prepulse inhibition', where presentation of a weak lead stimulus attenuates the startle response to a strong sensory stimulus, such as a loud tone. Performance on this test of sensorimotor gating is significantly impaired in schizophrenic patients, and several studies have implicated DA overactivity and its effects on GABAergic activity in the ventral pallidum in causing this deficit (Swerdlow et al, 1990a/b).

SUMMARY

To conclude, the N.Acc. forms an important part of the ventral striatal circuitry, where inputs from midbrain dopaminergic neurones, limbic structures and cortical areas influence behavioural responding by controlling N.Acc. output and feedback mechanisms to brainstem locomotor systems, substantia nigra and the VTA. Dopaminergic input to the ventral striatum removes inhibitory control of striatal function, thereby allowing the initiation of motivated behaviour. The N.Acc. in particular appears to constitute a 'limbic-motor interface' responsible for the translation of motivation into action by filtering limbic input subsequently relayed to mesencephalic locomotor areas. Locomotor activity, exploration, feeding, reinforcement and reward, as well as behavioural flexibility have all been associated with ventral striatal activity.

CHAPTER II

BEHAVIOURAL MODELS OF VENTRAL STRIATAL DOPAMINE SYSTEMS

The following section reviews previous research concerned with the role of the ventral striatum in mediating motivated responses in the rat. The review is intended as a background to the experiments described in subsequent chapters here and therefore concentrates on three types of behavioural responding: i) locomotor activity and exploration; ii) schedule-induced polydipsia; iii) conditioned place preference.

LOCOMOTOR ACTIVITY AND EXPLORATION

A substantial number of studies have demonstrated the significant role of the ventral striatum in mediating locomotor activity. Research has focused primarily on the locomotor response to stimulating drugs following dopaminergic depletion of terminal fields of mesencephalic projection systems. Since locomotor activity represents an easily assessed unconditioned response it has also widely been used by authors investigating the neuroanatomical connections of the ventral striatal complex, as outlined above.

Intra-accumbens injections of DA, dopaminergic receptor agonists (e.g., apomorphine), or amphetamine, a close structural analogue of the catecholamines, induce increased levels of locomotion in rats (Costall and Naylor, 1975; Pijnenburg et al, 1973, 1976). Research by Dreher and Jackson (1989) proposes that although administration of selective D_1 and D_2 DA receptor agonists into the N.Acc. stimulates locomotion in a dose-

related manner, simultaneous injection of both types of drugs has at least an additive effect on locomotor activity. Therefore, concurrent activation of both D_1 and D_2 DA receptors, which interact positively, appears to be necessary for the expression of locomotor behaviour. 6-hydroxydopamine (6-OHDA) lesions destroying DA-containing cells in the VTA or depleting DA terminal fields in the N.Acc. reduce spontaneous locomotion as measured in photocell activity cages (Evenden and Carli, 1985; Koob et al, 1978) or in home cage running wheels (Kubos et al, 1987), although this change was not observed by others (Koob et al, 1981). 6-OHDA lesion of the N.Acc. attenuate the locomotor response to low doses of *d*-amphetamine (Fink and Smith, 1980a/b; Kelly et al, 1975; Koob et al, 1981; Winn and Robbins, 1985), while potentiating apomorphine-induced hyperactivity (Kelly et al, 1975; Koob et al, 1981; Winn and Robbins, 1985). Hyperactivity following administration of *d*-amphetamine is further blocked by post-synaptic DA receptor blockade within the N.Acc. with neuroleptic drugs (Shaefer and Michael, 1984). Recovery from lesion effects has been noted by Kelly and colleagues (1975) who observed maximal locomotor responses to *d*-amphetamine and apomorphine 2 weeks after surgery and a subsequent attenuation over a 90-day period, reflecting an increase of DA levels in the N.Acc.. The stereotyped responding induced by higher doses of *d*-amphetamine is unaffected by N.Acc. lesions, supporting the differentiation drawn between dorsal and ventral DA systems (Jones et al, 1989; Kelly et al, 1975). Moreover, 6-OHDA lesions of dopaminergic terminal fields in the dorsal striatum enhance the locomotor response to *d*-amphetamine, as well as attenuate stereotyped behaviour (Joyce and Iversen, 1984). In conjunction with previous observations by Lyon and Robbins (1975) of competition between amphetamine-induced locomotor activity and stereotypy, Joyce and

Iversen (1984) propose that nigrostriatal neurones prevent the expression of tegmentostriatal neurones following *d*-amphetamine administration. Removing this inhibition by lesioning nigrostriatal terminal fields leads to an enhanced locomotor response to *d*-amphetamine. The mechanisms underlying this competition, however, are not explained, but may involve striatal feedback loops.

Some authors have suggested that the locomotion induced by stimulation of the N.Acc. has in particular an exploratory nature. Exploration is typically determined in terms of the duration of investigation of novel objects or environments, or in terms of preference for a novel environment. Methods measuring exploratory behaviours include open fields, mazes, exploration choice boxes, hole boards, or boxes into which novel objects can be introduced (Robbins, 1977). Fink and Smith (1980a) observed reduced exploratory locomotion (measured in a novel open field) and reduced investigatory behaviour (measured as exploration of a novel object) that could be restored with low doses of apomorphine after 6-OHDA lesions of mesolimbocortical DA neurones. However, other authors did not report deficits in exploration (measured in an exploration choice box) four weeks or one week after surgery (Robbins and Everitt, 1982; Winn and Robbins, 1985). It is argued that these contrasting results may be due to methodological differences; in particular, exploration of novel objects and open fields may measure deficits in locomotor activity, rather than exploration, and it is suggested that preference tests for novel and familiar environments would assess exploratory behaviour more adequately (Winn and Robbins, 1985).

On the basis of the above studies, the locomotor-stimulating effects of *d*-amphetamine can be assumed to be mediated via DA release from pre-synaptic terminals within the N.Acc. and the subsequent activation of

post-synaptic DA receptors (Swerdlow et al, 1987a). Since the effects of psychomotor stimulants are blocked following administration of glutamatergic receptor antagonists (Pulvirenti et al, 1991), it has been suggested that endogenous glutamate may have a facilitatory effect on both pre-synaptic dopaminergic terminals (Hamilton et al, 1986) and post-synaptic GABAergic neurones, thereby modulating integrated N.Acc. output. Yim and Mogenson (1989) have demonstrated that N.Acc. DA itself has a modulatory function significantly attenuating locomotor activity in response to amygdala stimulation at low doses which would not by themselves elicit increased activity. Some of the possible output pathways which may contribute to the modulation of locomotor activity include ventral striatal and ventral pallidal projections to the PPTg and DMT (Swerdlow and Koob, 1987b). However, the involvement of the PPTg in mediating drug-induced locomotion is at present unclear. Reports that the PPTg contributes to N.Acc. amphetamine-induced locomotion (Brudzynski and Mogenson, 1985) were not confirmed by Swerdlow and Koob (1987b) who observed attenuation of apomorphine 'supersensitivity' (in response to DA terminal depletion in the N.Acc.) only after lesions of the DMT, but not the PPTg. Since DMT lesions only partially blocked locomotor activity elicited in the N.Acc. or ventral pallidum, additional efferents to thalamic or mesencephalic structures need to be identified that mediate the motor output of ventral striatal activity (Swerdlow and Koob, 1987b). These data are supported by recent studies which did not report impairments in spontaneous locomotion or the locomotor response to systemic or intra-accumbens administration of *d*-amphetamine in rats with PPTg lesions (J. Dunbar, personal communications; W. Inglis, personal communications). It is possible that the PPTg may indeed be a critical area of drug reward rather than locomotor activity since the nucleus has been

associated with the reinforcing effects of morphine, *d*-amphetamine and nicotine (Bechara and Van der Kooy, 1989; Iwamoto, 1990). Finally dopaminergic terminals in the olfactory tubercle have also been implicated in locomotor control since administration of both DA and apomorphine into this area has been reported to lead to dose-dependent increases in activity levels in familiar environments (Cools, 1986).

SCHEDULE-INDUCED POLYDIPSIA

Determinants and possible functions

Schedule-induced polydipsia (SIP) is a type of adjunctive behaviour that occurs when food-deprived rats are allowed to lever-press for small food rewards on a fixed-interval schedule with water freely available. Most animals will consume excessive amounts of water, although they are *not* water-deprived at any time. This form of adjunctive behaviour was first reported by Falk in 1961 and has been described as a laboratory analogue of displacement behaviours observed in the wild.

The possible functions of SIP cannot easily be explained in terms of adjustment and adaptation. SIP is displayed by animals that have no physiological water deficit and therefore represents a form of non-homeostatic drinking demanding the use of substantial energy reserves in an already food-deprived rat. Neither can SIP be explained as a response to a particular reinforcement schedule, since excessive drinking develops over time and is not usually observed during the first test sessions, even if the animal already responds appropriately to the scheduled delivery of food pellets. Finally, it is necessary to account for the specific drinking pattern induced by intermittent food reinforcement, with excessive drinking occurring immediately after the ingestion of the food pellet and

reaching its peak early in the inter-reinforcement interval (Falk, 1971).

Therefore, although SIP is a well-defined phenomenon that has been investigated in some detail, the exact reasons for its occurrence are still unclear (Falk 1971, 1977; Keehn and Stoyanov, 1986). According to *'activation models'* of SIP the response develops as the consequence of an unstable situation, where the emission of an on-going, highly motivated behaviour (e.g., deprivation-induced feeding) is interrupted (in this case by the FI schedule). It is argued that this 'thwarting' of motivation leads to increased levels of general arousal which in turn facilitate the emission of other activities such as drinking, cued by environmental stimuli (Levine and Levine, 1989).

Since concepts such as arousal, stress and activation are used extensively but elusively in the context of SIP research, a brief definition is appropriate here. Hebb (1955), for example, stated that '... we can now distinguish two quite different effects of a sensory event. One is the 'cue function', guiding behaviour; the other, less obvious but no less important is the 'arousal function'. Without a foundation of arousal, the cue function cannot exist' (Hebb, 1955, p. 249). Thus, arousal will be defined here as a process that increases an organism's ability to respond to sensory input and is associated with specific neurochemical, physiological and behavioural alterations, while stress is regarded as a consequence of high levels of arousal.

Activation models

Support for 'activation models' is based on studies which have shown that the ability to engage in SIP reduces high levels of arousal, measured as reductions in plasma corticosterone (CCS) levels in rats (Dantzer et al, 1988; Levine and Levine, 1989; Mittleman et al, 1986;

Tazi et al, 1986), attenuates the locomotor response to *d*-amphetamine (Tazi et al, 1988) and blocks activation of endogenous pain inhibitors (Tazi et al, 1987). However, contradicting evidence has shown that removal of water during SIP does not lead to increases in plasma CCS levels, and that levels increase rather than decrease during SIP with water available (Mittleman et al, 1988). Mittleman and colleagues (1992) recently reported both increases and decreases in plasma CCS levels associated with reductions in the acquisition of SIP. Other authors did not find any correlation between levels of plasma CCS and water intake during SIP in isolation-reared rats (Jones et al, 1989). These contradictory findings may in part have arisen from methodological difficulties in assessing stress and coping responses in rats. Plasma CCS levels, for example, may not always be an appropriate indicator of coping with stress, since hormonal output may be altered by other variables, such as increased locomotor activity (Tazi et al, 1986). Mittleman and colleagues (1992) argue that plasma CCS levels do not control the acquisition of SIP but may influence the behaviour once it has been established.

Neuronal basis of schedule-induced polydipsia

The neurophysiological substrates at present most strongly associated with the acquisition of SIP are mesencephalic DA projections to the N.Acc.. It has been repeatedly shown that depletion of DA from the N.Acc., but not the caudate-putamen (dorsal striatum), induced by 6-OHDA blocks the acquisition of SIP, while leaving intact normal regulatory behaviour and responses to water deprivation (Koob et al, 1978; Mittleman et al, 1990; Robbins and Koob, 1980; Wallace et al, 1983). It is important to note here that 6-OHDA N.Acc. lesions do not significantly affect normal regulatory activities such as feeding and

drinking (Kelly et al, 1975; Winn and Robbins, 1985), although mild hyperphagia during restricted feeding has been reported (Koob et al, 1978). Robbins and colleagues (1983) further demonstrated that 6-OHDA lesions of N.Acc. terminal fields could block the interference of high doses of *d*-amphetamine with SIP. However, the same authors point out that the disruptive effects of 6-OHDA lesions of the N.Acc. appear to be specific to the acquisition of SIP. By contrast, the already established behaviour was less affected and only the pattern of lever-pressing and licking within the inter-reinforcement interval was changed (Robbins et al, 1983). Assuming that plasma CCS has significant behavioural effects on SIP, Mittleman and colleagues (1992) subsequently showed that administration of CCS dose-dependently increased DA efflux in the N.Acc., as measured with *in vivo* electrochemistry, while metyrapone, a drug blocking CCS synthesis, reduced extracellular levels of N.Acc. DA. Thus, it was concluded that changes in DA efflux in the N.Acc. may disrupt SIP acquisition, although the exact mechanisms still remain to be explained.

Other structures associated with acquisition of SIP are the lateral septum, hippocampus and lateral hypothalamus, all of which are anatomically related to the N.Acc.. 6-OHDA lesions of dopaminergic terminals in the lateral septum, which is thought to play a role in the control of stressful or aversive situations and is innervated by both dopaminergic VTA and GABAergic N.Acc. efferents, significantly enhanced the expression of SIP (Taghzouti et al, 1985). Similarly, Devenport (1978) reported enhanced SIP acquisition following electrolytic lesions of the hippocampus, although this finding was not replicated by Mittleman and colleagues (1990) who observed reduced water intake behaviourally specific to SIP as a consequence of aspiration lesions of the

hippocampus. In addition, excitotoxic lesions of neurones intrinsic to the lateral hypothalamus significantly enhance SIP (Winn et al, 1992). Further research needs to clarify whether this may be an effect of mild hypophagia observed after this type of lesion, or whether the lateral hypothalamus does have a genuine role in modulating the expression of adjunctive behaviour.

Finally, it has frequently been observed that not all rats drink excessively in response to intermittent food reinforcement. These individual differences in the propensity to develop SIP may reflect differences in dopaminergic activity. Tazi and colleagues (1988) found that polydipsic rats engaged in less locomotor activity in response to *d*-amphetamine than did non-polydipsic rats. Since the locomotor effects of *d*-amphetamine are mediated through dopaminergic projections in the N.Acc. (Koob et al, 1981; Oades et al, 1986; Pijnenburg et al, 1973, 1976), it was proposed that the ability to engage in SIP was related to reduced dopaminergic activity in the N.Acc.. Dantzer and colleagues (1988) showed patterns of differential reactivity to aversive situations (such as potential threat, aversive stimuli or the administration of *d*-amphetamine) in polydipsic and non-polydipsic rats. While animals that developed SIP were 'stimulus-bound' and became increasingly stereotyped in their behavioural patterns, non-polydipsic rats were able to chose between a number of responses available and remained behaviourally flexible. This claim was supported by Evenden and Robbins (1983) who showed that response switching in rats was significantly enhanced following systemic injections of *d*-amphetamine.

Summary

On the basis of the data outlined above it appears that DA activity

in the ventral striatum is involved in the acquisition of SIP. While dopaminergic depletion of terminal fields in the N.Acc. prevents responding, it could be hypothesized that *reduced* activity of mesencephalic afferents to the ventral striatum facilitates adjunctive behaviours and *increased* activity of these projections allows selection between different behaviours. The role of anatomically related areas, such as the lateral septum, hippocampus and lateral hypothalamus for SIP acquisition and expression should also be considered.

CONDITIONED PLACE PREFERENCE

Conditioned place preference paradigm

An event or drug is defined as a reinforcer if the presence of the event or drug increases the probability of a response, usually with an hedonic value. Since quantification of reinforcement and reward is difficult due to the pleasurable properties of the particular event, measurement of reward typically focuses upon events or drugs for which an animal will perform an operant response (Koob, 1992). Three main animal models are used to investigate the neuronal basis of reward: conditioned place preference, or CPP (Carr et al, 1989; Phillips and Fibiger, 1987; White and Carr, 1985), drug self-administration (Koob and Goeders, 1989) and intracranial electrical self-stimulation (Phillips and Fibiger, 1989). The CPP paradigm assumes that some drugs act as primary reinforcers and that the pairing of drug-induced reinforcement with neutral environmental stimuli will cause these previously neutral stimuli to become conditioned, or secondary, reinforcers. On subsequent occasions, the animal will spend more time in the paired environment,

indicating that the previously neutral stimuli have gained incentive properties that support approach behaviour in the absence of the primary reinforcer (White and Carr, 1985).

Several criticisms have been raised regarding the validity of the CPP paradigm in measuring the rewarding properties of drugs. First, it is argued that since rats show preferences for relatively novel compartments and many drugs produce state dependent learning (SDL), this could interfere with the CPP paradigm. If the drug induced some kind of memory loss, then the rat may prefer a drug-paired environment due to its perceived novelty (Carr et al, 1989). However, a number of studies have shown that SDL does not appear to be a problem since CPP was observed whether testing took place in a drugged or drug-free state (Mucha and Iversen, 1984; Spyraki et al, 1985). Second, the data of White and Carr (1985) suggest that CPP may depend on the rewarding as well as the memory-improving properties of a drug. In their experiment, rats consumed equal amounts of saccharin and sucrose, but showed place preference only for sucrose. It was argued that both solutions had rewarding properties, but only sucrose improved memory. In addition, saccharin CPP was observed when glucose or *d*-amphetamine were given post-training to improve memory for the saccharin pairings (Messier and White, 1984; White and Carr, 1985). Finally, it has been pointed out that since rewarding drugs tend to enhance spontaneous locomotor activity, this unconditioned locomotor response may contribute to place preference (Swerdlow and Koob, 1984; Swerdlow et al, 1987). Swerdlow and Koob (1984) demonstrated that if rats were restrained in plexiglas restrainers during conditioning amphetamine CPP was abolished but animals still engaged in conditioned locomotion in the amphetamine-paired compartment, indicating that only a drug-place association but no place

preference had been learned. This study was later replicated by Carr and colleagues (1988) who argued that the restraints had maintained the novelty of both compartments and CPP was abolished. However, when rats were pre-exposed to the compartments prior to conditioning, restricted movement no longer blocked amphetamine CPP. In addition, it has been shown that drugs attenuating locomotor activity (e.g., some neuroleptic drugs) do not significantly affect CPP (Spyraki et al, 1982a/c).

Neuronal basis of reward

Rats will readily self-administer drugs (both intravenously and intracranially) and will work for intracranial self-administration. Dopaminergic mechanisms in the ventral striatum and its interactions with other brain sites are considered to be the important substrates for drug self-administration and electrical self-stimulation, and the same processes are suggested to underlie the approach behaviour during CPP (Koob, 1992; Koob and Goeders, 1989; Phillips and Fibiger, 1989).

Intra-accumbens injections of psychostimulant drugs such as *d*-amphetamine and cocaine reliably produce CPP (Aulisi and Hoebel, 1983; Carr and White, 1983, 1986); these effects are blocked by addition of DA receptor antagonists to the injection fluid (Aulisi and Hoebel, 1983). Spyraki and colleagues (1982a) further showed that CPP induced by systemic administration of *d*-amphetamine was attenuated by 6-OHDA lesions of the N.Acc.. These effects were specific to ventral striatal activity since *d*-amphetamine injections into other dopaminergic sites (medial prefrontal cortex, caudate-putamen, amygdala, area postrema region) did not affect place preference (Carr and White, 1983, 1986). However, contradictory data have shown that cocaine CPP was not blocked by DA receptor antagonists, or by dopaminergic depletion of

N.Acc. terminal fields (Spyraki et al, 1982b). Thus, it seems that dopaminergic innervation of the N.Acc. contributes to the rewarding effects of cocaine in conjunction with other substrates. One of the possible explanations are rewarding peripheral anesthetic properties of cocaine (Carr et al, 1989). In addition, Brown and Fibiger (1992) have recently shown a dissociation between the behavioural and neurochemical effects of cocaine-induced conditioned locomotion. While administration of cocaine significantly increased DA concentrations in the N.Acc., DA levels did not differ in conditioned and unconditioned rats when exposed to the test environment. It is therefore concluded that only the development of conditioned locomotion induced by cocaine, but not the conditioned response itself, is mediated via dopaminergic mechanisms (Brown and Fibiger, 1992).

CPP is further elicited by central injection of morphine or heroin into the VTA or N.Acc. (Bozarth and Wise, 1981; Phillips and LePiane, 1980; Spyraki et al, 1983; Van der Kooy et al, 1982). Spyraki and colleagues (1983) found that heroin CPP was blocked by DA receptor antagonists, as well as by 6-OHDA lesions of N.Acc.. CPP was not observed following opiate injections into the amygdala, caudate-putamen, or nucleus ambiguus (Van der Kooy et al, 1982). However, self-administration studies have indicated that other, both DA-dependent and -independent substrates are involved in opiate reward (Pettit et al, 1984): the first mediated through DA-containing neurones in the VTA, the second through activation of opiate receptors within the N.Acc. (Koob, 1992). Therefore, integrity of DA terminal fields in the N.Acc. originating from neurones within the VTA is critical for the rewarding effects of psychostimulant drugs such as cocaine and *d*-amphetamine, while opiate reward appears to depend on neurones within the VTA, as well as on

opiate-receptors of neurones intrinsic to the N.Acc..

Although tegmentostriatal DA activity has been attributed a critical role in the mediation of reinforcement and reward, other brain sites also appear to be involved. Everitt and colleagues (1991) demonstrated that established sucrose CPP was abolished by excitotoxic lesions of the basolateral amygdala projecting to the ventral striatum, as well as by asymmetric lesions of the basolateral amygdala and ventral striatum and by ventral striatal lesions. In line with previous work investigating reward-related processes, this suggests that the two structures interact in the mediation of CPP; the amygdala being involved in associating environmental stimuli and reward, and the ventral striatum controlling motor responses directed towards those stimuli. Excitotoxic lesion data from Hubner and Koob (1990), although based on drug self-administration experiments rather than CPP, suggest the ventral pallidum as an important site mediating the reinforcing effects of cocaine and heroin. Finally, Bechara and Van der Kooy (1989) have identified the PPTg as a critical area of drug reward. Excitotoxic lesions of neurones in the ventromedial part of this nucleus (containing mostly non-cholinergic cells bodies) abolished acquisition of morphine and amphetamine CPP, but did not interrupt the response once it had been established. These data are extended by Iwamoto (1990) who reported place preference induced by administration of nicotine into the PPTg.

Summary

The research outlined above suggests that neuronal information about reinforcement and reward originating from DA-containing neurones in the VTA and VTA - N.Acc. projections may be modulated by limbic structures and finally influence appetitive and approach responses via

striatal output mechanisms, i.e., through the ventral pallidum and the PPTg region of the midbrain, which in turn sends descending output to the medulla and spinal cord. This, however, is a very hypothetical model of the neuronal mechanisms of CPP and drug reward which does not account for differences in neuropharmacological properties of psychostimulant drugs, opiates, ethanol and sedative substances. Similarly, different mechanisms may be activated during the processing of information about rewarding events and rewarding drugs, and during the acquisition and expression of conditioned responses. Other brain sites which should receive further attention are limbic structures, such as the amygdala, frontal cortex and hippocampus, feedback mechanisms within the ventral striatal circuitry, as well as descending striatal projections.

CHAPTER III

METHODS TO INVESTIGATE DOPAMINERGIC MECHANISMS IN THE VENTRAL STRIATUM

NUCLEUS ACCUMBENS LESIONS

Excitotoxic lesions

In order to examine the functional role of the N.Acc. *per se*, it is essential to look at the consequences of selective damage to neurones intrinsic to the nucleus. The behavioural effects of such lesions may differ from those observed following dopaminergic depletion of terminal fields in the N.Acc.. Endogenous excitatory amino acid receptors are normally responsible for mediating fast excitatory neurotransmission in brain and a variety of receptors selective for different exogenous excitatory amino acid receptor agonists have been identified. Exogenous agonists include ibotenate, *N*-methyl-D-aspartate (NMDA), quisqualate, kainate and AMPA; the main endogenous agonists are glutamate, aspartate, quinolinate and glycine (Collingridge and Lester, 1989). However, activation of excitatory amino acid receptors may also have '*excitotoxic*' consequences causing fibre-sparing neuronal degeneration (Olney, 1974). Excitotoxins exert their effects by binding to glutamate receptors on neuronal dendrites and promoting the uncontrolled entry of Ca^{++} into neurones, which has toxic consequences. While studies examining the neurotoxic effects of the glutamate receptor agonist AMPA have identified a 'delayed' degenerative mechanism depending on intracellular Ca^{++} (Garthwaite and Garthwaite, 1991), Ca^{++} overload which cannot be sufficiently cleared (thereby leading to overstimulation of phospholipase and protease) appears to be responsible for rapid neuronal death following

administration of NMDA to specific brain sites (Garthwaite and Garthwaite, 1986). Neuronal damage can be assessed using standard cresyl violet to stain Nissl substance which indicates cell loss and gliosis. Immunohistochemical and high performance liquid chromatography (HPLC) analyses can be used to demonstrate damage to particular cell groups with high concentrations of a specific neurotransmitter. Different excitotoxic compounds not only bind to different receptors but also interact with the lesion site and subgroups of neurones. NMDA, kainate, AMPA and ibotenate, for example, bind to different types of glutamate receptors, while quisqualate acts at AMPA and quinolinate at NMDA receptors. Quisqualate has been shown to induce effective lesions in the PPTg (Rugg et al, 1992), while being less potent than NMDA in the lateral hypothalamus (Hastings et al, 1985). Ibotenate has been reported to destroy parvocellular but not magnocellular neurones in the paraventricular nucleus of the hypothalamus in the hamster (Hastings and Herbert, 1986).

Several problems have emerged recently regarding the specificity of damage induced by injections of excitotoxic substances. Following ibotenate injections into the rat septum, Coffey and colleagues (1990) observed significant damage to axons passing through the lesion area in the form of demyelination caused by an increase in microglia and macrophages associated with neuronal loss. In addition, ibotenate caused breakdown of the blood-brain barrier, leading to an invasion of macrophages and further demyelination. However, recent experiments in this laboratory using stains for myelin and a blood-brain barrier protein have indicated that while neuronal degeneration was complete 24 hours after infusion of NMDA into the rat lateral hypothalamus, the blood-brain barrier was reinstated after 12 days and myelin was found to begin to

recover 3 weeks after infusion of the toxin (H. Brace, personal communications). Thus, it is important to take into consideration that excitotoxic substances may induce damage in addition to neuronal loss. However, it is suggested that demyelination is most likely to occur in areas where neurones and fibres are in close proximity, which is the case in septal areas, for example. By comparison, axons are likely to remain intact in striatal regions where fibres occur in compact bundles (Coffey et al, 1990).

Relatively few studies investigating the behavioural effects of excitotoxic lesions in the N.Acc. have been published. Mazzari and colleagues (1986) reported survival of dopaminergic afferents following quinolinate lesions of the striatum, thereby demonstrating its selectivity and fibre-sparing properties. The authors also observed an increase in firing of dopaminergic neurones shortly after administration of the excitotoxin (as a reaction to loss of post-synaptic target neurones), as well as increased striatal DA turnover and metabolite levels 4 and 11 days post-lesion. At these points in time, no biochemical changes were detected in the substantia nigra. It is proposed that increased striatal DA turnover reflects a response of DA terminal fields to the interruption of neuronal feedback circuitries (Mazzari et al, 1986). The implications of this observation for behaviour remain to be tested. Churchill and colleagues (1990) found that quinolinate lesions of the accumbens core or shell resulted in topographically organized upregulation of $GABA_A$ receptors in the ventral pallidum, without affecting dopaminergic afferents to the N.Acc. (Churchill et al, 1990). Behavioural studies using ibotenate (Annett et al, 1989) and quisqualate (Everitt et al, 1991) to induce N.Acc. lesions report impairments in spatial learning and the abolition of place preference conditioning. However, both studies failed to state the quality

of damage and individual lesion volumes. Given that excitotoxic ventral striatal lesions have at present not been widely examined, and considering the heterogeneity of the N.Acc., it is important to make detailed assessments of the nature of the damage induced. Such methods also allow correlations of lesion volumes with behavioural responding.

Development of an excitotoxic lesion in the nucleus accumbens

As yet, there is no standard procedure for producing an effective excitotoxic lesion in the N.Acc.. It was therefore necessary to carry out a pilot experiment to establish which excitotoxin at which dose and concentration would produce the most efficient lesion of intrinsic N.Acc. neurones without damaging adjacent areas. In this preliminary experiment, unilateral lesions were made by injecting 1 *u*l of either NMDA at 120 nmol (17.652 *u*g; $n = 4$) or 60 nmol (8.826 *u*g; $n = 4$) or quinolinate at 60 nmol (10.026 *u*g; $n = 4$) or 30 nmol (5.013 *u*g; $n = 4$) into the right or left accumbens core. Previous work in this laboratory has shown that NMDA produced significant lesions of the lateral hypothalamus and PPTg at 60 and 120 nmol. In contrast with ibotenate, which has been reported to diffuse into the septal nuclei, NMDA has further been shown to be regionally specific following injection into the N.Acc. (A.J.M. Clark, personal communications). Since NMDA receptors are located primarily on cell bodies rather than on nerve terminals, NMDA would induce selective degeneration of GABAergic and cholinergic cell bodies located within the ventral striatum, leaving intact passing fibre tracts (Carlsson and Carlsson, 1990). Quinolinate has previously been shown to induce neuronal degeneration in the striatum (Churchill et al, 1990; Mazzari et al, 1986) and PPTg (Rugg et al, 1992). Rats were sacrificed 10 days and 4 weeks after surgery and brain sections were stained for Nissl substance.

Lesion boundaries were identified on the basis of detectable cell loss or reactive gliosis present. No cell loss had occurred following the low dose of quinolinate and little or no damage was seen following the high dose of the excitotoxin. The low dose of NMDA induced some cell loss in the accumbens core in all animals, whereas the high dose of NMDA caused extensive damage to accumbens core and shell, olfactory tubercle and ventromedial caudate-putamen, as well as to adjacent areas (dorsolateral caudate-putamen and lateral septum). It was therefore decided to use NMDA at the 60 nmol and 90 nmol doses in subsequent experiments. Since the aim was to induce as much cell loss as possible in the N.Acc., without destroying neighbouring areas the dose of NMDA was slightly adjusted as more information about the lesion properties was obtained in the course of later experiments.

Quality and volume of excitotoxic lesions

Here, an overall evaluation of NMDA accumbens lesions is given; detailed histological analyses of individual experiments are presented in the relevant chapters.

Using a digitized tablet ('Grafpad') connected to a BBC microprocessor, the areas of the intact accumbens core, accumbens core and shell, and ventral striatum (N.Acc., olfactory tubercle, ventromedial caudate-putamen, bed nucleus of the stria terminalis) as represented on a set of 8 appropriate standardized sections were calculated and repeated estimates (n = 10) of volumes made on these standardized sections. Volumes were found to be appropriately 3.63 mm^3 (SD = $\pm$ 0.2), 6.47 mm^3 (SD = $\pm$ 0.22) and 10.22 mm^3 (SD = $\pm$ 0.59) respectively. Lesion volumes resulting from bilateral infusions of NMDA into the N.Acc. were expressed as percentages of total area damaged in the

accumbens core or core and shell combined. The data of all animals with 100% or less cell loss in the ventral striatum was included in the statistical analysis (n = 30). Lesion boundaries were again identified on the basis of detectable neuronal loss or reactive gliosis present. The average lesion volume obtained by infusion of 60 nmol NMDA (n = 24) was 96.37% (SE = $\pm$ 9.65) of total accumbens core volume and 59.79% (SE = $\pm$ 5.25) of total accumbens core and shell area as calculated on standardized sections. Infusion of 90 nmol NMDA induced core and shell lesions of 95.54% (SE = $\pm$ 21.19) on average. With the smaller dose of NMDA, rats sustained cell loss in the accumbens core, whereas the larger dose produced cell loss in the accumbens core and shell. Other ventral striatal structures were also damaged in some cases. At the 60 nmol dose, the olfactory tubercle was damaged in 2 rats, while following infusion of 90 nmol NMDA, 1 rat had sustained damage to the olfactory tubercle, 1 rat had sustained damage to the ventromedial caudate-putamen, and in 1 rat both structures were affected. Fig. 3.1 and 3.2 represent the smallest (A) and largest (B) ventral striatal lesions induced by 60 nmol and 90 nmol NMDA respectively.

It can therefore be concluded that NMDA at the doses mentioned above has been successfully used to induce selective lesions in the N.Acc.. The toxin acts regionally specifically and does not diffuse away from the original lesion site into the septal nuclei, as has been reported following injection of ibotenate into the N.Acc.. However, it is necessary to take into account considerable between-subject variability in the volume of neuronal loss. A possible explanation for this consistent observation may be subtle regional differences in the distribution of NMDA receptors, so that slight variations in the infusion site result in different lesion volumes.

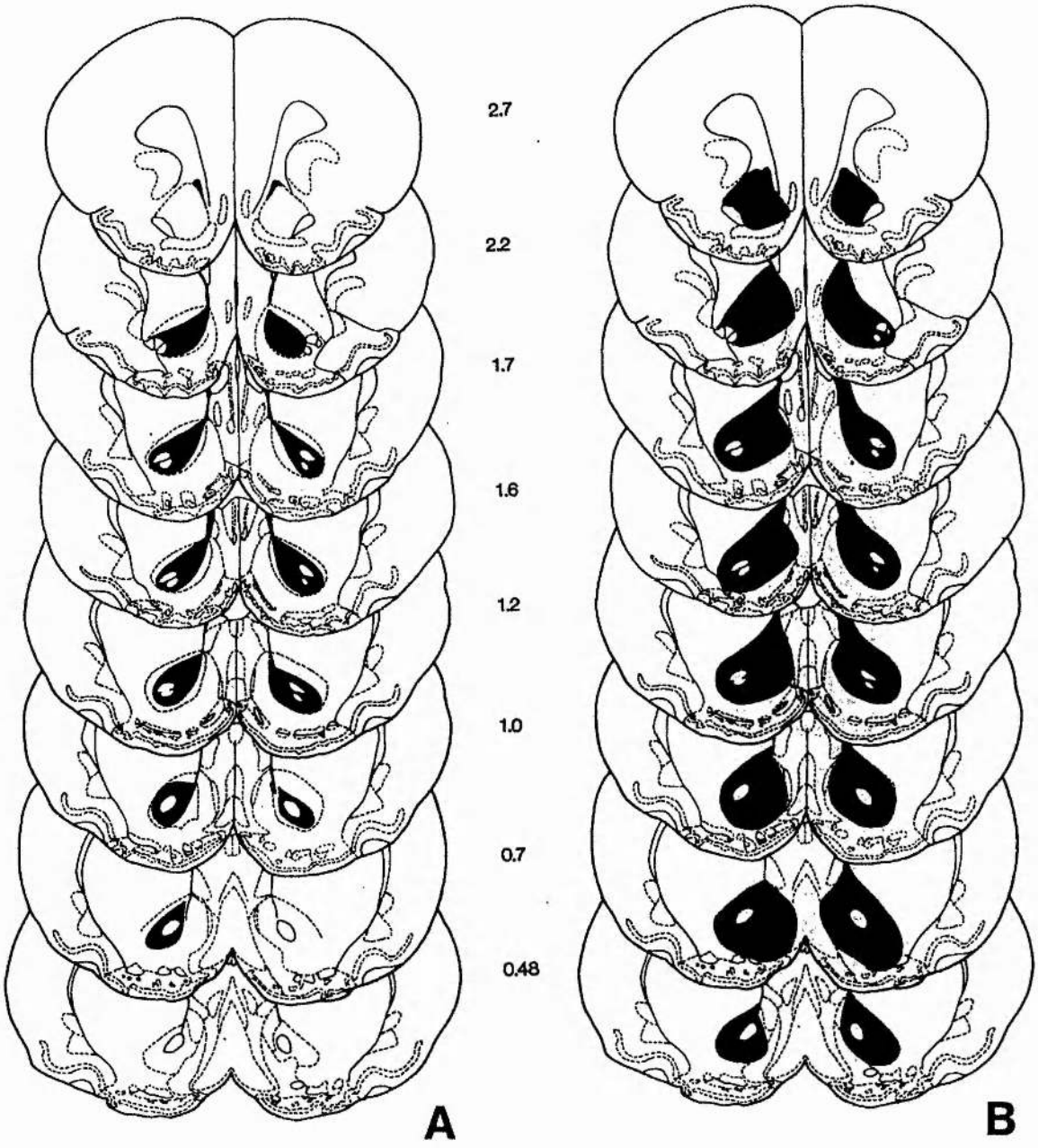


Fig. 3.1 Smallest (A) and largest (B) ventral striatal lesions induced by 60 nmol NMDA. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm.

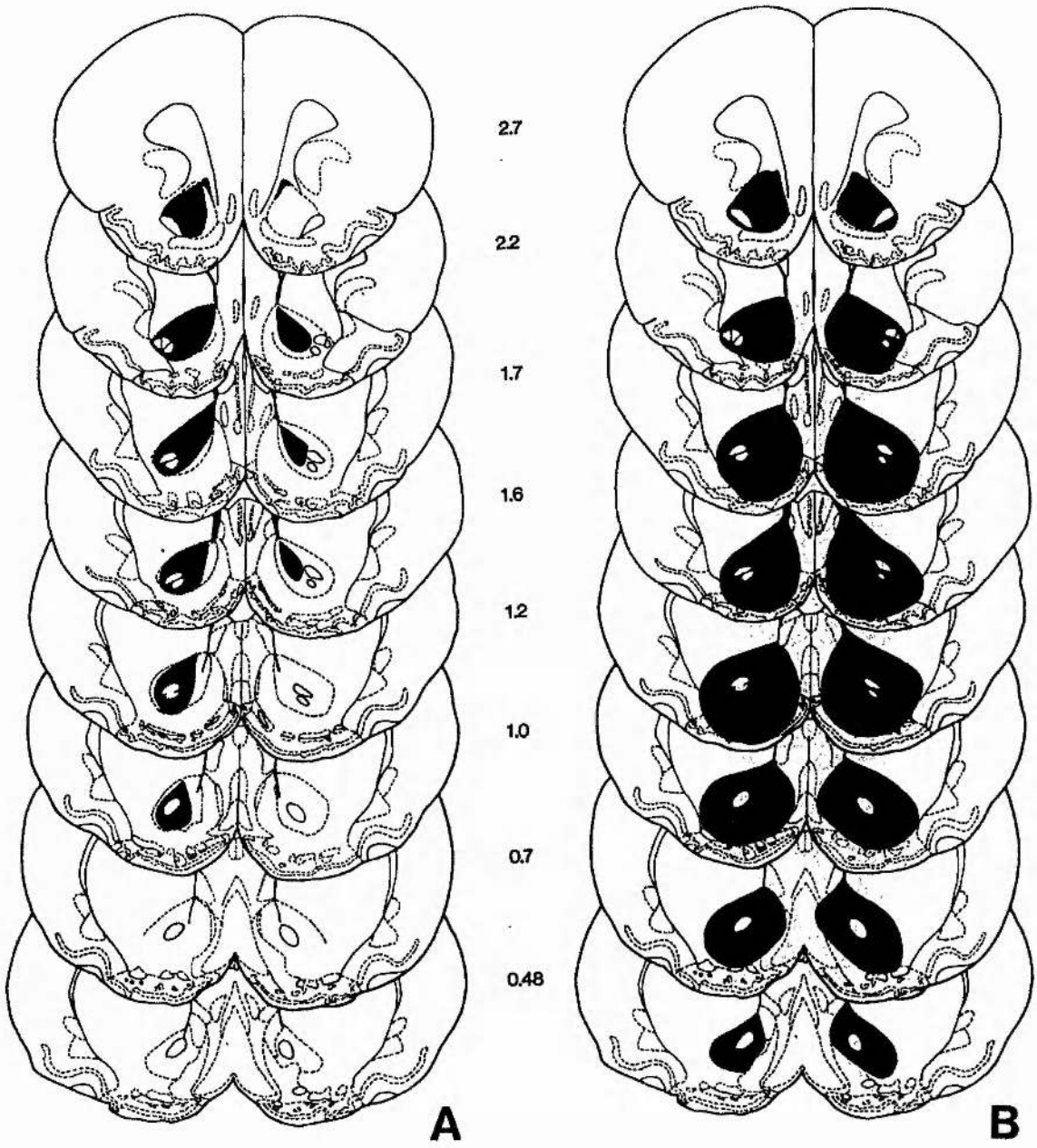


Fig. 3.2 Smallest (A) and largest (B) ventral striatal lesions induced by 90 nmol NMDA. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm.

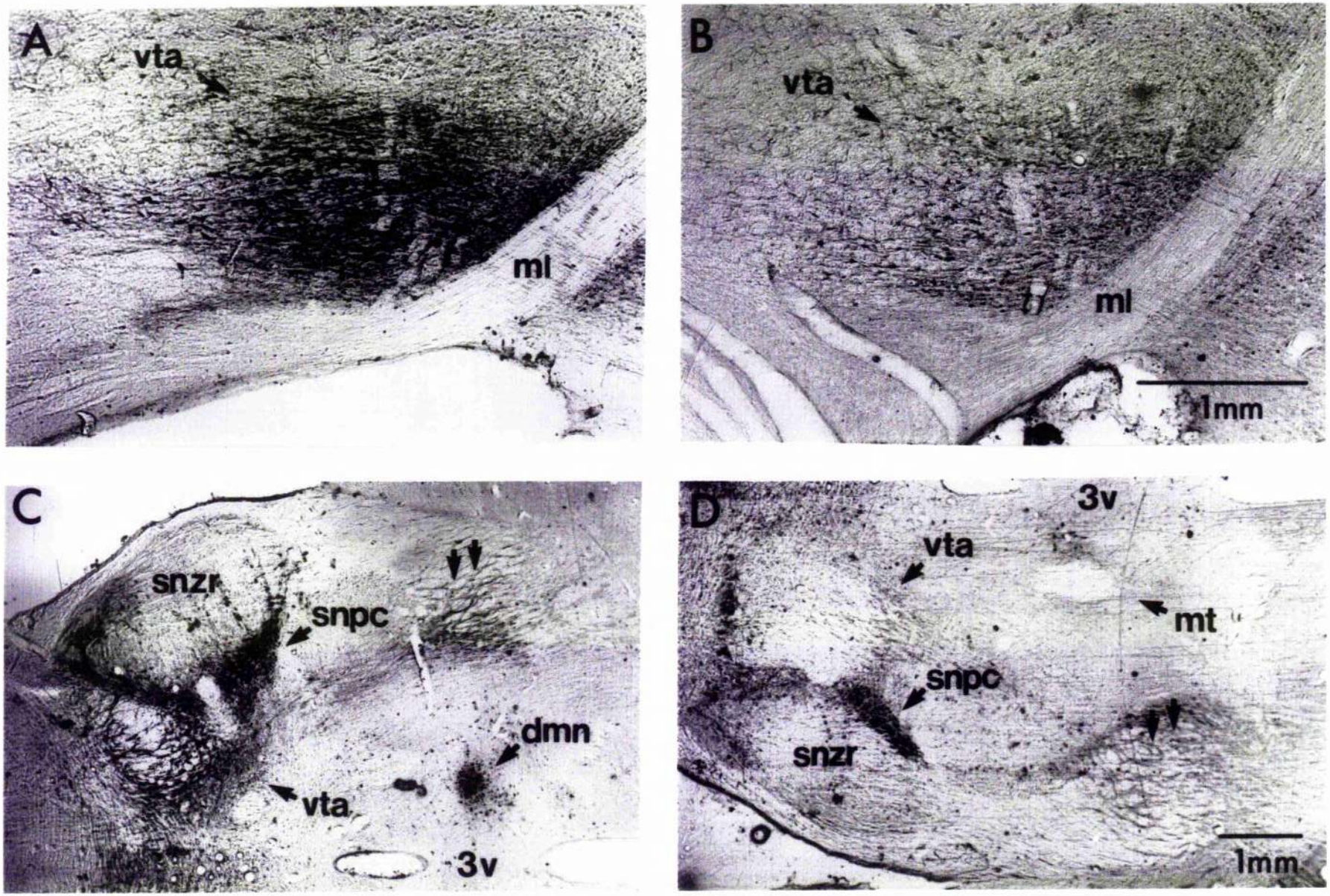
Depletion of dopamine terminal fields in the nucleus accumbens

The majority of studies outlined in previous chapters have used the neurotoxin 6-hydroxydopamine (6-OHDA) to disrupt dopaminergic projections to the ventral striatum originating in the VTA. 6-OHDA is a chemical analogue of catecholamine neurotransmitters and exerts its toxic effects by being taken up selectively into the terminal fields, axons or cell bodies of catecholamine-containing neurones where it is degraded. Its metabolites - principally hydrogen peroxide - cause neuronal degeneration. Infusion of 6-OHDA results in a long-lasting loss of uptake sites, as well as in a reduction of the activity of enzymes necessary for catecholamine synthesis (Uretsky and Iversen, 1970). Noradrenergic neurones can be protected by administration of a noradrenaline reuptake blocker prior to surgery, so that when injected into the N.Acc., 6-OHDA can induce lesions specifically depleting DA from terminal fields in this area. Immunohistochemical analysis of such lesions shows significant retrograde degeneration of neurones in the VTA, while DA-containing nigral neurones are less affected. Fig. 3.3 shows sections through the midbrain and medial forebrain stained for the DA precursor tyrosine hydroxylase (TOH) after sham and 6-OHDA lesions of the N.Acc.. TOH-positive staining confirmed the biochemical findings reported previously (Winn and Robbins, 1985) - based on HPLC analyses - where terminal lesions using 8 *u*g/2 *u*l 6-OHDA in the N.Acc. produced large depletion of DA in this area but had no effect on DA in the caudate-putamen. Immunohistochemical data obtained following 6-OHDA accumbens lesions will be discussed in more detail in the relevant experimental chapter.

Summary

In conclusion, the excitotoxin NMDA can reliably induce selective

Fig. 3.3 Photographs of sections through the midbrain and medial forebrain stained for tyrosine hydroxylase (TOH). (A) and (B) show the distribution of TOH-positive cells in the ventral tegmental area (VTA) in sagittal sections after sham lesions (A) and after 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens (B); (C) and (D) show the distribution of TOH-positive neurones in the VTA, substantia nigra and mesostriatal projection sites after sham lesions (C) and after 6-OHDA lesions of the nucleus accumbens (D); dmn, dorsomedial hypothalamic nucleus, ml, medial lamniscus; mt, mammillothalamic tract; snpc, substantia nigra pars compacta; snzr, substantia nigra zona reticulata; vta, ventral tegmental area; 3v, 3rd ventricle; vertical arrows indicate mesostriatal fibre systems. Scale bars = 1 mm.


A
vta
ml
B
vta
ml
1mm
C
snzr
snpc
dmn
vta
3v
D
3v
vta
mt
snpc
snzr
1mm

lesions in the N.Acc.. Cell loss following infusion of 60 nmol and 90 nmol NMDA occurs primarily in the accumbens core, with some damage extending to the accumbens shell, as well as the olfactory tubercle and the ventromedial caudate-putamen. Using a 60 nmol dose of NMDA, 96.37% of cells were lost from the accumbens core. Depletion of DA terminal fields in the N.Acc. using 6-OHDA induces specific, retrograde degeneration of dopaminergic neurones in the VTA. It is therefore reasonable to assume that the two techniques allow one to compare the behavioural effects of loss of neurones intrinsic to the N.Acc. and depletion of dopaminergic terminals in this area.

IN VIVO VOLTAMMETRY

Measurement of neurotransmitter release *in vivo* is essential for an understanding of the neuronal basis of behaviour. One relatively novel method of recording neurotransmitter release in the behaving animal is *in vivo* voltammetry which was first reported in the 1970's and has since developed into a reliable and widely applicable tool in neuroscience (Adams, 1978; Lane et al, 1979; Stamford, 1989). Briefly, *in vivo* voltammetric recordings are based on the application of voltages to graphite paste or carbon fibre working electrodes implanted into brain tissue. Any electroactive compound at the electrode surface is oxidized, giving off electrons and altering the flow of current (Stamford, 1989). Only the catecholamine neurotransmitters (DA and noradrenaline), serotonin and their metabolites are electroactive, while most other neurotransmitters cannot be oxidized (e.g., GABA, acetylcholine, glycine, glutamate, aspartate and taurine) or are present in such low concentrations

that the resulting current cannot be measured (e.g., endogenous opiates and substance P). In practice, the animal is implanted with a working electrode placed in the area of brain from which measurements are to be taken, as well as with an Ag/AgCl reference and auxiliary electrode combination. The implant is covered with dental cement and can be connected to a potentiostat when necessary.

To record from the working electrode, a computer-generated digital waveform is converted into an analog waveform by an interface and the appropriate voltage is applied through an amplifier circuit in the potentiostat. The resulting current is measured at the working electrode through a current-to-voltage converter, the analog signal is converted into a digital signal and stored in the computer for analysis. What kind of potential waveform is applied to the electrode depends on the type of measurement required. Waveforms can be classified into potential step, or pulse methods, and potential sweep methods. Pulse methods are usually used to obtain quantitative information on concentrations of electroactive components, while sweep methods can give indications about qualitative properties of a compound (Justice, 1987). Slow application of the potential waveform results in high resolution between compounds, whereas rapid voltage scans can measure changes in transmitter efflux over short periods of time (Stamford, 1989). One of the most frequently used pulse methods is chronoamperometry, where the current potential is changed instantly from a value at which no reaction occurs to one at which an electrochemical reaction takes place. This method has the advantage of allowing recordings to be taken at frequent intervals, without causing much tissue reaction because the potential is only applied for a short period of time. On the other hand, chronoamperometry only measures a single oxidation peak, compared to linear sweep methods that can detect

two or more peaks (Justice, 1987).

Methodological considerations: electrode selectivity

Electroactive compounds such as ascorbic acid (AA) or the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), as well as DA oxidize within a narrow potential range of about +0.1 V to +0.5 V. Normal graphite paste or carbon fibre electrodes fail to discriminate between the three compounds, a particular problem as extracellular levels of DOPAC and AA are significantly larger than those of DA. For this reason, electrodes are typically modified in different ways. For example, stearate can be added to the graphite paste at the electrode tip, creating an anionic environment attracting cations such as DA, while shifting the oxidation potentials of interfering anionic compounds like DOPAC and AA to more positive values than that at which DA normally oxidizes. Indeed, evaluations of these modified electrodes have shown that they selectively measure extracellular levels of DA *in vitro* (Broderick, 1989), in brain homogenate solutions (Blaha and Jung, 1991) and *in vivo* striatal tissue (Blaha and Lane, 1983; Lane et al, 1987). Blaha and Jung (1991) have additionally demonstrated that stearate-modified graphite paste electrodes selectively detect DA after exposure to brain tissue without interference from DOPAC or AA using both chronoamperometry and rapid scan voltammetry. The selectivity of carbon fibre electrodes can be improved by coating the fibres with a thin film of an ionic polymer, such as Nafion (Brazell et al, 1987).

Methodological considerations: voltammetric measurements and brain tissue

It is important to bear in mind that no matter how carefully

controlled the experiment, voltammetric measurements will always have an effect on brain tissue. First, implantation of the working electrode induces tissue damage, although carbon has been found to cause only minor tissue reactions, even in chronic implants (Stamford, 1986). Reference and auxiliary electrodes should be placed on the skull or into muscle tissue to avoid unnecessary interactions with brain tissue. Secondly, the current flow between auxiliary and working electrodes may stimulate the brain and change the neurochemical environment around the tip of the working electrode. Chronoamperometry at carbon paste electrodes has been shown to alter neuronal activity in the striatum, although it is not clear whether this change had an effect on subsequent chronoamperometric measurements (Stamford, 1986). Gratzl and colleagues (1991) showed significant depletions of monoamine levels at the electrode tip following *in vivo* staircase voltammetry in the cat thalamus. Such depletion can be minimized by increasing the length of sampling intervals to allow for relaxation of the electrode surroundings, although this results in poorer temporal resolution. Scan rates during linear sweep voltammetry are typically less than 200 nA (Lane et al, 1978) and there is at present no evidence suggesting neurochemical changes in brain tissue following these scanning techniques. Third, voltammetric measurements are based on the oxidation of electroactive compounds, and the products of this reaction may develop into potential neurotoxins. Since the formation of such neurotoxins depends on the length of the voltammetric measurement, longer scans make follow-up reactions more likely (Stamford, 1986).

In the same way as voltammetry affects brain tissue, the tissue itself may affect voltammetric measurements in different ways. First, one of the consequences of acutely implanting an electrode into brain tissue is

the formation of a pool of fluid at the electrode tip, making access to the electrode surface difficult and responding to concentration changes in extracellular fluid less accurate. It is thought that the fluid in this pool is similar to extracellular fluid, but concentrations of neurochemicals may differ. In chronic implants, the electrode tip is surrounded by neuroglia which may interact with extracellular neurotransmitters and therefore interfere with voltammetric recordings. Second, following implantation into brain tissue, the properties of an electrode change in terms of sensitivity, resolution between peaks and position of oxidation peak. Carbon paste electrodes are more sensitive after than before surgery, which is thought to be due to priming of the electrode with free amines from extracellular fluid. Third, voltammetric recordings may be distorted by administration of drugs with electroactive properties, such as some of the neuroleptics and apomorphine. Additionally, most drugs are metabolized and the metabolite may be electroactive or an electrode poison, even if the original compound is not. However, drug effects on voltammetric recordings are minimal due to low dosages and differences in oxidation peaks (Stamford, 1986).

In vivo *voltammetry vs microdialysis*

Another well-established method of analyzing neurotransmitter concentrations in the behaving animal is *in vivo* microdialysis. This technique is based on the introduction of a physiological solution into an artificial membrane permeable to water and small solutes, whereas the other side of the membrane is in contact with extracellular fluid. The concentration gradient between the two sides of the membrane will cause substances present in intercellular space to diffuse into the dialysis probe. The fluid in the dialysis probe can then be collected at short intervals for

later analysis (Ungerstedt et al, 1982; Benveniste et al, 1989). The major advantage of microdialysis is that it can extract easily a large variety of transmitters and their metabolites, including those that are not electroactive, and these can be analyzed using a range of appropriate techniques, such as HPLC. However, 'real time' measurements are at best crude and the probe dimensions are significantly larger, making multiple site measurements impossible. Benveniste and colleagues (1989) point out that calibration of dialysis probes in saline solutions with a known concentration of the substance of interest may be misleading since diffusion characteristics for saline and brain tissue differ significantly. Studies using cross-comparisons of *in vivo* microdialysis and voltammetry have reported conflicting results. While Benveniste and co-workers (1989) found similar results for measurements of extracellular levels of K^+ and Ca^{++} in rat cerebral cortex, Blaha and colleagues (1990) reported differential measurements of DA in brain tissue obtained by the two techniques. According to recent data by this group, such discrepancies may reflect depletion of extracellular DA by the dialysis probe (Blaha et al, 1991). Voltammetric measurements taken at the tip of the probe indicated that microdialysis significantly reduced DA levels in the extracellular space surrounding the dialysis probe. Similarly, Benveniste and colleagues (1989) found significant depletions of extracellular Ca^{++} within a radius of 1 mm from the perimeter of the dialysis probe 30 min after the start of perfusion.

To conclude, *in vivo* voltammetry and microdialysis are not mutually exclusive techniques but should rather be used in conjunction for reliable analysis of substances present in extracellular fluid.

Summary

Despite a number of methodological problems which remain to be solved (but meanwhile can be minimized) *in vivo* voltammetry represents an important and reliable technique for selectively measuring extracellular catecholamine levels in brain. Its advantages include high spatial and temporal resolution, little tissue damage, and the possibility of taking 'real time' measurements. Research suggests that rapid scan voltammetry and chronoamperometry at stearate-modified graphite paste electrodes selectively measure DA efflux in the striatum of the behaving rat.

EXPERIMENTAL CHAPTERS: RATIONALE

The experiments outlined in the following chapters aim to use the lesion and *in vivo* voltammetric techniques discussed above in conjunction with several microinjection studies to further investigate the role of the N.Acc. in the mediation of motivated behaviour and reward.

Chapters V, VII and IX evaluate the regulatory and behavioural consequences of excitotoxic N.Acc. lesions. Assessments were made of post-lesion home cage food and water intake and body weight. Chapter V investigates the lesion effects on exploration, spontaneous locomotion and the locomotor and stereotypy responses to systemic administration of *d*-amphetamine and apomorphine, and direct comparisons with depletion of dopaminergic terminals in the N.Acc. induced by 6-OHDA are made. Chapters VII and IX examine the effects of neuronal loss from the N.Acc. following excitotoxic lesions on schedule-induced polydipsia (as well as on drinking in response to deprivation and hypertonic saline) and place preference conditioning respectively. Prior to the lesion experiments, the

effects of intra-accumbens administration of DA on locomotion and schedule-induced drinking were tested. These experiments are reported in Chapter VI. To complement the lesion data, Chapter VIII describes *in vivo* voltammetric experiments investigating the relationship between extracellular DA levels in the N.Acc. and the acquisition and emission of SIP, as well as deprivation-induced drinking.

CHAPTER IV

GENERAL METHODOLOGY

The following chapter describes general procedures used repeatedly throughout the series of experiments. Specific methods will be outlined in the relevant experimental chapters.

HOUSING AND NORMAL REGULATORY BEHAVIOUR

Male hooded Lister rats (bred in-house) were caged individually under a 12 hr light/dark cycle (lights on 8.00 a.m.) and maintained on a diet of *ad libitum* lab chow and tap water, unless stated otherwise.

To monitor recovery from surgery, daily pre- and post-operative measurements were made (to the nearest 0.1 g) of (i) body weight; (ii) weight of food remaining in cage hopper; (iii) food spillage (collected on foil sheets beneath the food hopper) and (iv) amount of water remaining in the water bottle.

ANESTHESIA

Avertin anesthesia (10 ml/kg i.p.) was used during surgery for the majority of experiments. (Avertin concentrate: 100 g 2,2,2, tri-bromo-ethanol/62 ml tertiary-amyl-alcohol; 1.25 ml of this concentrate then added to 5 ml absolute alcohol and 62.5 ml 0.9% saline). However, Avertin was found to induce chronic 'bloat' - gastric distension produced by adhesions in the muscle wall of the gut - in a number of rats following each set of surgery. (Symptoms include a swollen abdomen, aphagia, adipsia and substantial weight loss.) It was therefore decided to change the

anesthetic to a combination of 100 mg/kg ketamine hydrochloride ('Vetlar') i.p. and 20 mg/kg xylazine ('Rompun') i.p. (followed by supplemental injections of ketamine hydrochloride if necessary) for rats receiving 6-OHDA lesions of the N.Acc.. Since ketamine has been reported to act as a non-competitive NMDA antagonist, NMDA lesions were induced under sodium pentobarbitone anesthesia (Sagatal, 60 mg/kg i.p.). However, when making 6-OHDA lesions, the majority of rats receiving ketamine and xylazine prior to surgery died of pulmonary edema (PE) approximately 2 - 4 hr after recovery. This was an entirely unsuspected incident, since ketamine and xylazine anesthesia had been used without problems before. It was then discovered that xylazine-induced PE had previously been reported at doses of 42 mg/kg (Amouzadeh et al, 1991), compared to 20 mg/kg used in the present experiments. It is thus possible that the oedimagenic effects of xylazine were enhanced by the standard pretreatment of rats with pargyline and desimipramine in order to ensure selective depletion of DA terminal fields by 6-OHDA. Both drugs act as hypotensive agents and may have interacted with the oedimagenic properties of xylazine. The anesthetic was therefore changed to sodium pentobarbitone solution for all rats. Minor problems associated with this drug are increased salivation and subsequent choking during post-operative recovery, especially following excitotoxic lesions, and a fall in body temperature particularly in pretreated rats. These problems can be avoided by using a rectal probe and heated blanket to control body temperature during surgery, and by constantly monitoring rats until recovery.

SURGICAL PROCEDURES

The same coordinates were used throughout for injection and excitotoxic lesion sites aimed at the N.Acc. core: anterior-posterior, + 2.00 mm from bregma; lateral, $\pm$ 1.5 mm from midline; vertical, - 7.00 mm from skull surface with level skull (Paxinos and Watson, 1986). Since most previous studies have used different coordinates to produce effective DA terminal depletion in the N.Acc. (Kelly et al, 1975; Koob et al, 1978; Winn and Robbins, 1985), these previous coordinates were used here as well for 6-OHDA lesions: anterior-posterior, + 3.4 mm from bregma; lateral, $\pm$ 1.7 mm from midline; vertical, - 6.2 mm from dura with the incisor bar set 5.0 mm above the intraural line (Pellegrino et al, 1979).

For surgery, anesthetized animals were placed into a Kopf stereotaxic frame fitted with atraumatic earbars. The skull surface was exposed and lamda and bregma located to ensure level skull, if necessary. The frame was adjusted according to the coordinates and holes were drilled in the appropriate locations. Following cannulation or injection of the lesion toxin, skull holes were filled with gelatin foam and the wound was closed with Michel clips, if necessary, and the wound was dressed with wound dressing powder. To aid recovery, all rats received an injection of 6% glucose in 0.9% phosphate buffered saline (5 ml i.p.) before being returned to their home cages.

Cannulation

After holes had been drilled into the skull, 3 small stainless steel screws were inserted into the skull to ensure firm hold of the dental cement. 23 ga stainless steel guide cannulae of 11.0 mm length were made from ends of 0.6 mm x 30 mm 23 ga single use hypodermic needles. The upper ends of the cannulae were roughened with a file to achieve firm

hold in the dental cement. Guide cannulae were implanted bilaterally using 2 parallel cannulae guides consisting of 5 cm of straight 23 ga stainless steel tubing from which 30 ga tubing protruded at a length of exactly 11.0 mm. The cannula guides were firmly attached to the side of the stereotaxic frame, 3.0 mm apart according to the coordinates. The guide cannulae were fitted over the 30 ga tubing and inserted into brain by lowering the vertical arm of the stereotaxic frame to the point 1.5 mm above the vertical coordinate (i.e., 5.5 mm below skull surface). Acrylic dental cement was then applied to the skull surface around the cannulae. As soon as the cement was sufficiently hard, the cannula guides were withdrawn and stylets placed in each implant. Stylets were made of 30 ga stainless steel wire to prevent blocking of the cannulae.

Neurotoxic Lesions

Lesions were made by bilateral stereotaxic infusions of NMDA, quinolinate or 6-OHDA. NMDA (Sigma Chemicals) and quinolinate (Sigma Chemicals) were dissolved in phosphate buffer (pH 7.4) and if necessary the pH was further adjusted with 2 M NaOH; final pH was 7.4. To obtain a 120 nmol solution, 17.652 mg of NMDA were dissolved in 1 ml of phosphate buffer. This stock solution was diluted further for lower concentrations. To obtain a 60 nmol solution of quinolinate, 10.026 mg of the drug were dissolved in 1 ml of phosphate buffer. The doses and concentrations of the excitotoxins varied in some of the experiments, until the most effective drug, dose and concentration had been found.

6-OHDA lesions were induced by infusion of 8 *u*g/2 *u*l 6-hydroxydopamine hydrobromide (Sigma Chemicals). The drug was prepared as the free base in 0.1 mg/ml ascorbate saline and kept on ice throughout. Rats receiving 6-OHDA infusions or the vehicle were

pretreated with 15 mg/kg pargyline hydrochloride (Sigma Chemicals), a monoamine oxidase inhibitor, and 15 mg/kg desimpramine hydrochloride (Sigma Chemicals), a noradrenaline uptake blocker, 30 min prior to surgery.

Injections of excitotoxins or 6-OHDA were made using stereotaxically mounted 30 ga stainless steel cannulae connected via polyethylene tubing to 10 *u*l SGE syringes driven by a Harvard infusion pump. Injection volumes were 1 *u*l/2 min for excitotoxins and their vehicles and 2 *u*l/4 min for 6-OHDA and its vehicle. After infusion of the drug, cannulae were left *in situ* for 2 min and 4 min respectively to allow for diffusion fo the drug away from the cannula tip.

MICROINJECTIONS

Intra-accumbens injections of DA were given bilaterally through 12.5 mm 30 ga stainless steel injection cannulae inserted inside the permanent guide cannulae, thus extending into brain tissue 1.5 mm below the tip of the implant. Injection cannulae were connected via polyethelene tubing to 10 *u*l SGE glass syringes driven by a Harvard infusion pump. Injection volume was 1 *u*l/2 min and cannulae were left *in situ* for a further minute to prevent the drug from being drawn back up into the cannula track. To obtain a 200 nmol solution of DA, 37.92 mg of dopamine hydrochloride (Sigma Chemicals) were dissolved in 1 ml of 0.1 mg/ml ascorbate saline.

LOCOMOTOR ACTIVITY

Locomotor activity was measured in a battery of 18 w x 24 l x 37

h cm wire activity cages with 2 photoelectric cells 2 cm above the grid floor. Interruptions of infrared lightbeams were recorded by a BBC microprocessor with a Eurorack extension unit and a Spider interface system using 'Spider' BASIC programming language (Paul Fray Ltd.). The programme discounted sequential activation of 1 beam only, requiring the 2 beams to be interrupted one after another. Since activity scores are often positively skewed (Robbins, 1977), photocell counts were square-root transformed throughout for statistical analysis.

SCHEDULE-INDUCED POLYDIPSIA

Rats were food-deprived to 80% of their free-feeding weight and maintained at this weight throughout the experiment. Body weight, food and water intake were recorded on a daily basis. Testing was carried out in identical operant chambers measuring 24 w x 24 l x 19 h cm, housed in a larger, sound-attenuating box. The cages were constructed with 3 aluminium walls, a clear perspex front that could be opened, and a metal grid floor. By means of an automatic food dispenser fixed to the outside of the chamber, food pellets (Campden, 45 mg) were delivered to a recessed food tray mounted at floor level in the middle of one of the walls of the chamber. Food pellets could be obtained by pushing open a hinged aluminium flap covering the front of the tray. A graduated burette and aluminium drinking spout filled with tap water were mounted to the outside of the cage and could be reached by the rat through a hole in the wall next to the food tray 5.5 cm above the grid floor. Photocells were attached to both the flap covering the food tray and the drinking spout. Interruptions of infrared lightbeams were recorded by a BBC microprocessor with a Eurorack extension unit and a 'Beetle' interface

using 'Spider' BASIC language (Paul Fray Ltd.) also controlling the delivery of food pellets. Experimental sessions lasted for 30 or 60 min; during each session, rats received 30 or 60 food pellets on a fixed-interval 60 s schedule of food delivery (FI 60). Water was freely available for all rats during the experimental sessions, unless stated otherwise. Numbers of drinking bouts and pellet door presses, latencies of first drinking bouts and first door presses after pellet delivery, and drinking bout lengths were recorded by the microprocessor. The amount of water intake was measured in the beginning by weighing the glass water bottles attached to the test cages before and after each session. Since considerable spillage could not be avoided, this technique proved to be inaccurate. Therefore, graduated plastic burettes were substituted for glass bottles and water intake was read from the burette.

STATISTICAL ANALYSIS

The majority of the data obtained here were analyzed using ANOVA (analysis of variance) to test for differences between and within groups and conditions. ANOVA's were carried out using the SPSS/PC+ statistical package and Sun mainframe. Significant effects were further analyzed using Neuman-Keuls post-hoc tests (Winer, 1971). Stereotypy ratings in response to drugs were examined using the Fisher exact probability (Siegel, 1956). Pearson correlation coefficients were calculated with SPSS/PC+ for linear relationships between lesion volumes and behavioural responses.

HISTOLOGY

On completion of behavioural testing, rats were deeply anaesthetized with 1.5 ml 'Euthatal' i.p. (sodium pentobarbitone, 200 mg/ml, May and Baker) and perfused transcardially with 0.9% saline followed by 10% phosphate buffered formalin. Brains were removed and stored in formalin until sectioning. Lesion and injection sites were verified using standard cresyl violet staining methods (40 *u*m sections every 200 *u*m). The volumes of NMDA-induced lesions were assessed using a Leitz Diaplan microscope fitted with a drawing tube. Silhouettes of lesions were drawn onto a set of 8 appropriate standardized stereotaxic atlas drawings (Paxinos and Watson, 1986). Using a digitized tablet ('Grafpad') connected to a BBC microprocessor, the area of each lesion on each section was calculated and an estimate of lesion volume made on these standardized sections.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was carried out as described previously (Rugg et al, 1992) in rats with 6-OHDA lesions of the N.Acc.. Rats were sacrificed by i.p. injection of 1.5 ml 'Euthatal' after completion of behavioural testing. 30 min prior to the injection, each rat was pretreated with heparin (2500 USP units in 0.5 ml sterile saline i.p.). They were then perfused transcardially with 100 ml of Ca^{++} free Tyrodes solution (Rugg et al, 1992) containing heparin (10 USP units ml) at 28 - 30^{o}C, at a rate of 20 ml/min using a Gilson Minipuls 3 pump fitted with ID 0.125" tubing. The Tyrodes solution was followed by 300 ml fixative (4% paraformaldehyde/0.05% glutaraldehyde in 0.1 M phosphate buffer) at the same rate of delivery. The brains were removed and post-fixed in the same

fixative solution for 30 min at room temperature. The brains were then cut into 50 *u*m sections (horizontal, sagittal or coronal) on a freezing microtome and placed into phosphate buffered saline (PBS). Every third section was stained for tyrosine hydroxylase (TOH), a catecholamine precursor, and Nissl substance. Sections were washed with 30% sucrose for 30 min and then washed 5 times for 5 min in PBS at room temperature.

Incubation with antibodies and other reagents were carried out in 24-well tissue culture plates in a volume of 0.3 - 0.5 ml. Up to 6 sections were incubated per well. For washing sections were transferred to a container on a flat bed shaker, with the exception of initial incubations with the primary antibodies for TOH, which were at $4^{o}C$. Sections were placed in a blocking solution (20% normal goat serum, 0.1% tritron X-100 in PBS) for 60 min and then washed 3 times for 5 min with PBS. They were incubated with anti-TOH (from mouse-mouse hybridomas, [Boehringer]) 1:50 in antibody diluting solution (ADS) overnight (approximately 15 hr). The ADS used was 0.1% normal goat serum and 0.1% tritron X-100 in PBS. Following 5 x 5 min washes with PBS, the sections were then incubated with anti-mouse IgG (1:30 in ADS [sheep, Sera-lab]) for 1 hr and washed 5 x 5 min in PBS. Following the wash, sections were incubated with monoclonal mouse peroxidase anti-peroxidase (1:100 [Sigma] in ADS) for 60 min and washed 5 x 5 min in PBS. The IgG and peroxidase anti-peroxidase incubations were repeated in the same order for the same length of time with PBS washes between each step. Finally, sections were incubated with 0.05% diaminobenzidine (DAB) for 15 min (1 ml/well) followed by the addition of 10 *u*l/well 1% H_2O_2 and a further incubation of 5 - 10 min. The sections were washed 5 x 5 min in PBS, mounted on glass slides, air dried and coverslipped.

CHAPTER V

LOCOMOTOR ACTIVITY AND EXPLORATION

NMDA AND 6-OHDA LESIONS OF THE NUCLEUS ACCUMBENS, LOCOMOTOR ACTIVITY AND EXPLORATION

The effects of depletion of DA from N.Acc. terminal fields on locomotor activity, exploration and the locomotor response to dopaminergic agonists have been investigated in some detail. Briefly, DA depletion in the N.Acc. has been reported to reduce spontaneous locomotion (Evenden and Carli, 1985; Koob et al, 1978; Kubos et al, 1987), although this was not confirmed by others (Koob et al, 1981). The locomotor response induced by low doses of *d*-amphetamine was attenuated (Fink and Smith, 1980a, 1980b; Kelly et al, 1975; Koob et al, 1981; Winn and Robbins, 1985), while apomorphine-induced hyperactivity was enhanced (Kelly et al, 1975; Koob et al, 1981; Winn and Robbins, 1985). Evidence relating to exploratory behaviours is controversial: the reduction in exploratory locomotion and investigatory behaviour demonstrated by Fink and Smith (1980a) following 6-OHDA lesions of the N.Acc. was not observed by other authors (Robbins and Everitt, 1982; Winn and Robbins, 1985).

In order to distinguish the functional significance of dopaminergic innervation of the N.Acc. from the contribution of neurones intrinsic to it, the present series of experiments was aimed at evaluating the behavioural consequences of fibre-sparing NMDA lesions on spontaneous locomotion, exploration and the locomotor and stereotyped responses to *d*-amphetamine and apomorphine. In addition, assessments were made of post-lesion body weight and home cage food and water intake. The

behavioural effects of neuronal loss following NMDA lesions were compared directly with DA depletion induced by 6-OHDA.

METHODS

Surgical procedures

33 rats were taken for surgery at 289.0 g (SD = $\pm$ 25.85) mean body weight and received bilateral infusions of either 1 *u*l 60 nmol NMDA (n = 6), 1 *u*l 90 nmol NMDA (n = 6,) 8 *u*g/2 *u*l 6-OHDA (n = 9), 1 *u*l phosphate buffer vehicle (n = 7), or 2 *u*l ascorbate saline vehicle (n = 5).

Exploratory behaviour and spontaneous locomotor activity

4 weeks after surgery, exploration was tested using an exploration choice box (Carlsson box, Robbins, 1977). This was a large rectangular box (120 w x 45 l x 50 h cm) with 3 aluminium walls, a metal grid floor with an aluminium tray underneath, 2 removable tops, and a transparent perspex front. The box was divided into 2 halves by a removable black partition. A perspex startbox (15 w x 15 l x 8 h cm) attached to the centre of the front wall gave equal access to both halves of the box. The grid floor was marked off into 4 equal quadrants on each side of the divider. Each animal was placed into one half of the box (familiar) for 1 hr, following which it was removed and placed in the startbox. The centre partition was then replaced by one with a hole (8 w x 7 h cm) in the bottom allowing the rat to move from one side of the box to the other. Latency and side (novel or familiar) of emergence from the startbox, number of quadrant crossings and rears, as well as the total amount of time spent on either side of the box were recorded continuously over a 10 min period. A 'quadrant crossing' was recorded when the animal had

crossed one of the transition lines with all four paws, but not necessarily the tail. A 'rear' was recorded when the rat was in a position where the two front paws were lifted above the ground and the back at an angle greater than 45^{o} to the ground. Following the exploration tests, spontaneous locomotor activity during a 60 min period was recorded daily for 2 weeks. Equal numbers of rats were familiarized to the right and left hand sides of the test environment.

Locomotor and stereotyped responses to apomorphine and d-amphetamine

After habituation to the activity cages, (8 weeks post-surgery) rats received a counterbalanced series of injections of apomorphine (0.1, 1.0 and 3.0 mg/kg s.c.; Sigma Chemicals), *d*-amphetamine (1.5 and 5.0 mg/kg i.p.; Sigma Chemicals), or vehicle (0.9% saline) prior to the 60 min test session. Order of administration was determined by a Latin square design to control for the possible additive or potentiating effects of repeated drug administration. Injection days were separated by one rest day. In addition to recording locomotor activity, stereotypy was measured on a scale similar to that described previously (Kelly et al, 1975)

0 - asleep or stationary

1 - active

2 - predominantly active with bursts of stereotyped sniffing or rearing.

3 - stereotyped activity predominantly sniffing and rearing over a larger area of the cage.

4 - stereotyped behaviour maintained in one location

5 - stereotyped behaviour in one location with bursts of gnawing or licking.

6 - continual gnawing or licking of the cage bars.

Sniffing and rearing were labelled 'stereotypies' if the behaviours occurred at high frequencies and were not of exploratory nature, i.e., not specifically directed at parts of the test environment.

RESULTS

Histology

Since statistical analysis (ANOVA) of lesion volumes calculated from cresyl violet stained sections showed no significant differences in total lesion volume following infusion of NMDA at concentrations of 60 nmol or 90 nmol ($F_{1,10} = 1.05$) and there were no apparent qualitative differences, the two groups were collapsed into one ($n = 12$) for analysis of lesion and behavioural data. All rats had sustained some damage to the ventral striatum (N.Acc., olfactory tubercle, ventromedial caudate-putamen, bed nucleus of the stria terminalis), ranging from 13.30% to 98.31% (mean: 51.92%, SE = $\pm$ 8.25) and were included in the statistical analysis. Compared to the previously estimated total volumes of the ventral striatum, accumbens core and shell, and accumbens core only of 10.22 mm^3, 6.47 mm^3 and 3.63 mm^3 respectively (see Chapter II), average lesion volume was 5.31 mm^3 (SE = $\pm$ 0.84), as calculated on the basis of standardized atlas drawings. The accumbens core was substantially damaged in all rats. The average lesion volume resulting from infusion of 60 nmol and 90 nmol NMDA into the accumbens core was 146.36% (SE = $\pm$ 23.2) of core area and 81.53% (SE = $\pm$ 12.87) of accumbens core and shell. Other ventral striatal structures were also

damaged in some cases. The olfactory tubercle was destroyed in 3 rats, 1 rat had sustained damage to the ventromedial caudate-putamen, and in 1 rat both structures were affected. In addition, the channels of ventral pallidal neurones lying between the N.Acc. and olfactory tubercle were damaged in 4 animals. Fig. 5.1 shows the smallest (A) and the largest (B) ventral striatal lesions induced by NMDA.

TOH immunohistochemical staining techniques showed an extensive loss of mesostriatal DA neurones in the VTA in all rats following infusion of 6-OHDA into the accumbens core, while mesostriatal DA neurones in the substantia nigra remained intact (see Fig. 3.3). TOH-positive staining thereby confirmed HPLC data reported previously (Winn and Robbins, 1985), where terminal lesions using 8 *u*g/2 *u*l 6-OHDA in the N.Acc. produced large depletion of DA in this area, but had no effect on DA in the caudate-putamen.

Normal regulatory responses

Repeated measures ANOVA showed a steady increase in body weights, food and water intake in all lesion groups 5 days prior to surgery (body weights: $F_{4,120} = 2.47$, $P < 0.05$; food intake: $F_{4,120} = 60.14$, $P < 0.001$; water intake: $F_{4,120} = 15.18$, $P < 0.001$). No group x day interactions were found confirming that rats in the 3 groups gained weight and consumed more food and water at equal rates (body weights: $F_{8,120} = 0.81$; food intake: $F_{8,120} = 0.89$; water intake: $F_{8,120} = 0.65$).

Average post-operative regulatory responses are shown in Fig. 5.2. Repeated measures ANOVA revealed no differences between lesion groups in post-operative body weights and food intake (body weights: $F_{2,30} = 1.24$; food intake: $F_{2,30} = 0.88$). All rats showed significant increases in body weight, food and water intake during post-lesion weeks

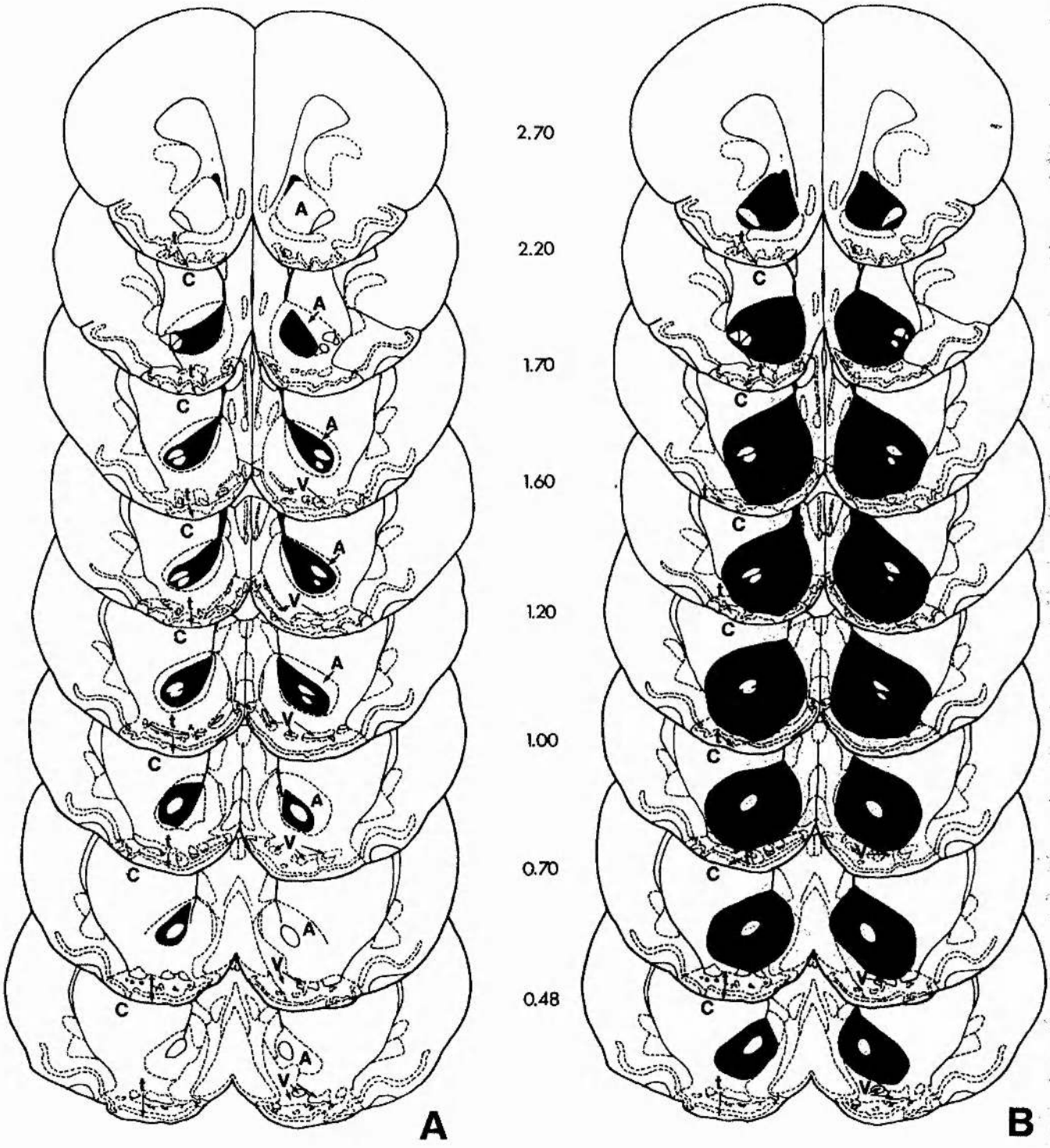
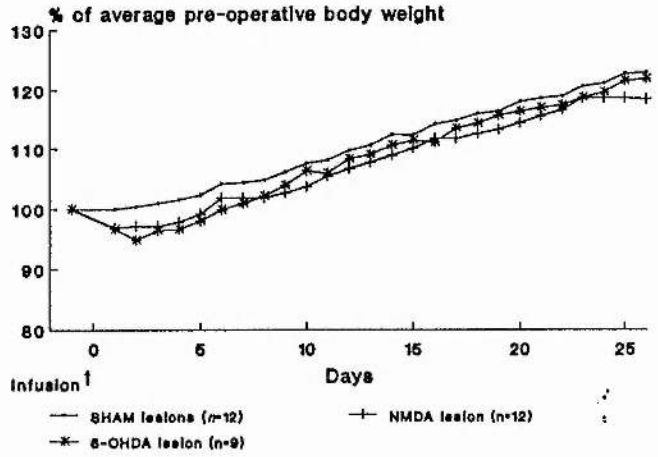
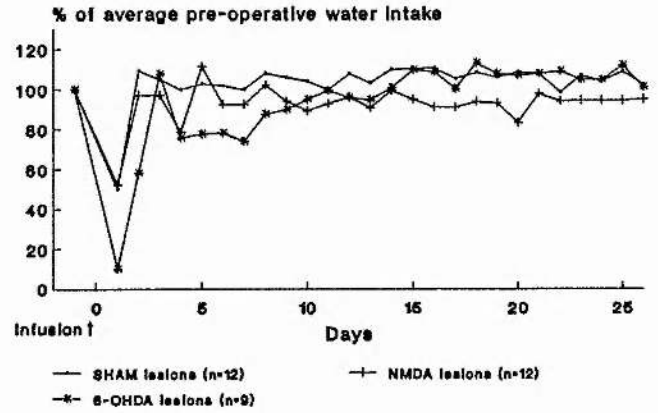
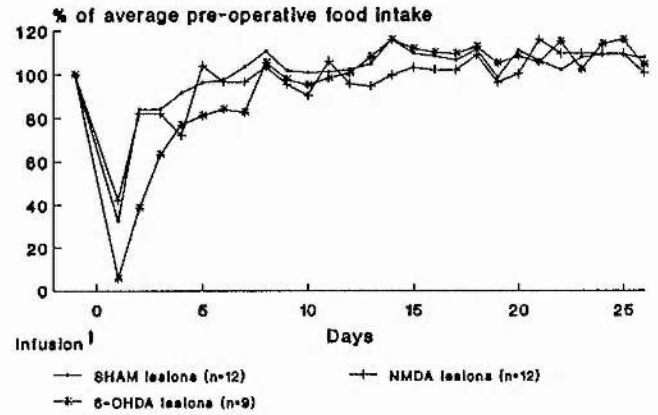


Fig. 5.1 Smallest (A) and largest (B) lesions of the nucleus accumbens induced by 60 nmol and 90 nmol NMDA. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm; A, nucleus accumbens; C, caudate putamen; t, olfactory tubercle; vp, channel neurones of ventral pallidum.

Fig. 5.2 Percentages of average pre-operative body weights, food and water intake measured over 26 days following NMDA, 6-OHDA or sham lesions of the nucleus accumbens.





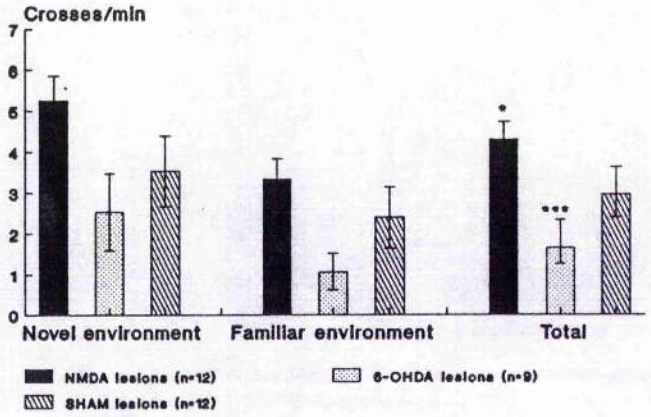


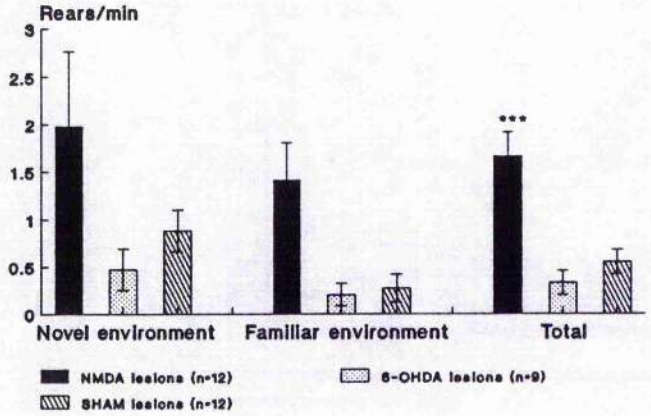
1 to 4 (body weights: $F_{3,90} = 408.31$, $P < 0.001$; food intake: $F_{3,90} = 109.95$, $P < 0.001$; water intake: $F_{3,90} = 42.04$, $P < 0.001$). However, differential patterns of recovery of water intake were observed for the lesion groups (groups: $F_{2,30} = 4.87$, $P < 0.05$). Post-hoc testing showed that while 6-OHDA-lesioned rats drank significantly less water than did NMDA- and sham-lesions animals ($P < 0.001$) during the first post-operative week, but had fully recovered by the end of the second week, NMDA-lesioned rats showed the same initial reduction in water intake as did sham-lesioned animals during post-operative week 1, but drinking levels remained significantly below those of rats with sham lesions until the end of week 3 (week 2: $P < 0.05$; week 3: $P < 0.001$).

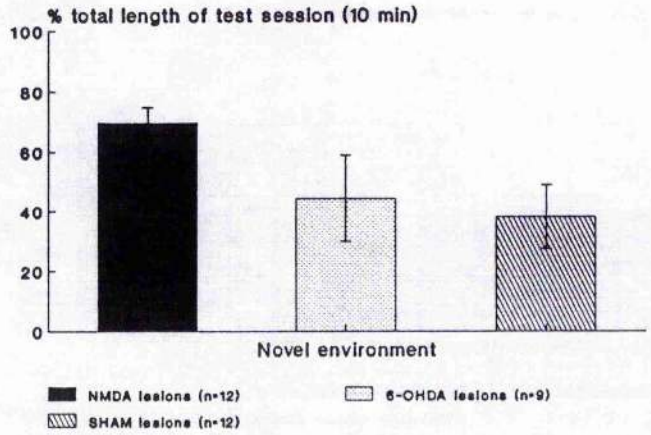
Exploratory behaviour

Data relating to exploration are shown in Fig. 5.3. Multiple ANOVA indicated that rats in all groups engaged in significantly more exploratory behaviours (quadrant crossings and rearing; scores corrected for time spent on novel/familiar side of the test box) in a novel than in a familiar environment (quadrant crossings: $F_{1,60} = 6.74$, $P < 0.05$; rearing: $F_{1,60} = 4.61$, $P < 0.05$). Post-hoc analysis of significant group effects on quadrant crossings ($F_{2,60} = 5.97$, $P < 0.05$) and rearing ($F_{2,60} = 13.89$, $P < 0.001$) showed that NMDA-induced lesions in the N.Acc. significantly enhanced locomotion and rearing in *both* environments compared to sham-lesioned control rats (quadrant crossings: $P < 0.01$; rearing: $P < 0.001$). In addition, 6-OHDA lesions of the N.Acc. were found to attenuate significantly the number of quadrant crossings on both sides of the exploration choice box ($P < 0.001$). The percentage of time spent in the novel environment was not affected by lesion type ($F_{2,30} = 2.36$).

Fig. 5.3 Measures of exploratory behaviour in rats with NMDA, 6-OHDA or sham lesions of the nucleus accumbens. Top panel: average number of quadrant crossings per minute on novel and familiar sides of the Carlsson box, ± SE. Centre panel: average number of rears per minute on novel and familiar sides of Carlsson box, ± SE. Bottom panel: percentage of time (10 min = 100%) spent on novel side of Carlsson box, ± SE. * = $P < 0.05$, *** = $P < 0.001$, compared to sham-lesioned control rats.







Spontaneous locomotor activity

Mean levels of spontaneous locomotor activity are shown in Fig. 5.4. Repeated measures ANOVA on the square root transformed photocell counts per hour revealed no significant between-group differences over 2 weeks of habituation to the locomotor activity cages ($F_{2,30} = 2.95$). Similarly, activity levels did not differ significantly between week 1 and week 2 ($F_{1,30} = 1.68$). However, post-hoc analysis of a group x week interaction ($F_{2,30} = 8.04$, $P < 0.01$) showed that NMDA-lesioned animals were significantly more active than the sham-lesioned group during weeks 1 and 2 ($P < 0.01$). 6-OHDA lesions of the N.Acc. did not induce hypoactivity but led to significant increases in locomotor activity during the second week of testing, compared to the first week ($P < 0.001$).

Locomotor and stereotyped responses to apomorphine and d-amphetamine

Fig. 5.5 shows locomotor activity in response to administration of different doses of apomorphine and *d*-amphetamine. ANOVA with repeated measures on the square root transformed photocell counts per hour showed a significant effect of administration of different doses of apomorphine on locomotor activity ($F_{3,90} = 17.21$, $P < 0.001$). Post-hoc analysis of a significant group x drug interaction ($F_{6,90} = 4.72$, $P < 0.001$) indicated that activity was significantly attenuated by the low dose of apomorphine (0.1 mg/kg) as compared to injection of vehicle in 6-OHDA- and sham-lesioned rats ($P < 0.05$), while administration of a high dose of the drug (3.0 mg/kg) did not alter activity levels in either of the groups. Post-hoc tests further revealed that activity was reduced by the medium dose (1.0 mg/kg) of apomorphine in NMDA-lesioned rats ($P <$

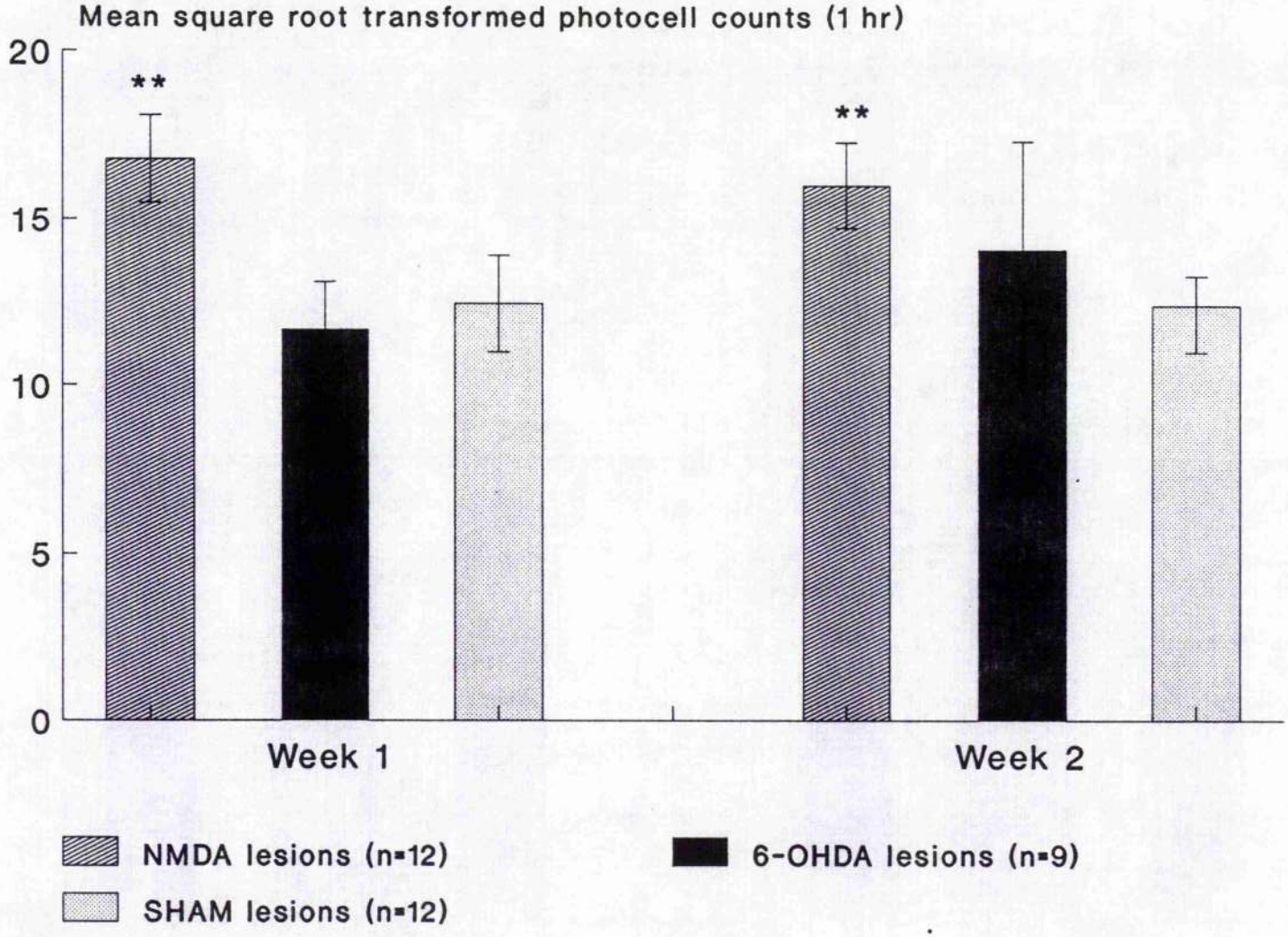


Fig. 5.4 Average of photocell counts (expressed as square roots of total values) during daily 1 hr tests in activity cages over 2 weeks, following NMDA, 6-OHDA or sham lesions of the nucleus accumbens $\pm$ SE. $^{**} = P < 0.01$, compared to sham-lesioned control rats.

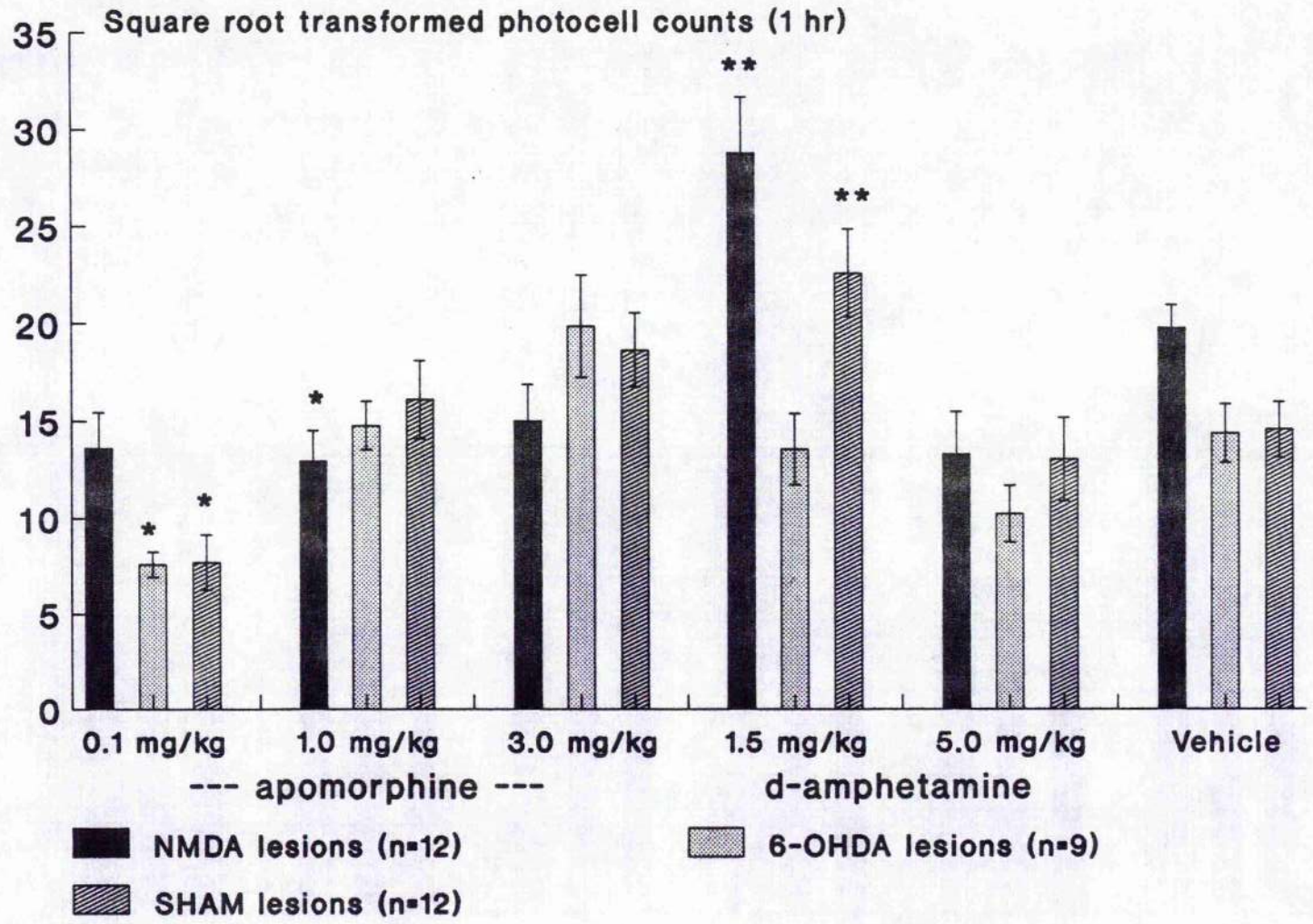


Fig. 5.5 Measures of the locomotor response to dopaminergic agonists in rats with NMDA, 6-OHDA or sham lesions of the nucleus accumbens. Average of photocell counts (expressed as square roots of total values) during 1 hr tests in activity cages following systemic administration of apomorphine, *d*-amphetamine or vehicle, $\pm$ SE. $^{*} = P < 0.05$, $^{**} = P < 0.01$, compared to injection of vehicle.

0.05), while this dose had no effect on locomotion in the other 2 groups. The high dose of apomorphine tended to enhance activity in 6-OHDA- and sham-lesioned animals and attenuate locomotion in NMDA-lesioned rats, but this trend did not reach statistical significance. Repeated measures ANOVA of the effects of systemic injections of *d*-amphetamine at different doses showed a significant drug effect ($F_{2,60} = 19.43$, $P < 0.001$). Post-hoc analysis indicated that activity was increased by administration of the low dose of *d*-amphetamine (1.5 mg/kg, $P < 0.001$) and remained unaffected by the high dose of the drug, as compared to injection of vehicle. Post-hoc analysis of a significant group x drug interaction showed that the locomotor response to the low dose of *d*-amphetamine observed in NMDA- and sham-lesioned animals (NMDA: $P < 0.01$; sham: $P < 0.01$) was attenuated in rats with 6-OHDA lesions of the N.Acc.. ANOVA with repeated measures showed no significant day effect on locomotor activity, ruling out the possibility of potentiating effects of drug administration ($F_{5,150} = 1.40$).

On the basis of their median stereotypy ratings, rats' responses to each drug were divided into a 'stereotyped' (median > 3) and a 'non-stereotyped' (median < 3) group (see Table 5.1). Comparisons using Fisher exact probability tests indicated that compared to administration of saline, stereotyped behaviours were displayed significantly more often following injections of 1.0 mg/kg apomorphine ($X^2(3) = 51.39$, $P < 0.001$), 3.0 mg/kg apomorphine ($X^2(3) = 62.06$, $P < 0.001$) and 5.0 mg/kg *d*-amphetamine ($X^2(3) = 39.66$, $P < 0.001$). No significant differences were found for comparisons between lesion groups.

Correlations of lesion volume and behaviour

Lesion volumes were not correlated to exploratory behaviours

Table 5.1 Measures of median stereotypy ratings (Kelly et al, 1975) following administration of dopaminergic agonists in rats with NMDA, 6-OHDA or sham lesions of the nucleus accumbens. Numbers of rats responding with stereotypy (median > 3) or non-stereotypy (median < 3) to different doses of apomorphine and *d*-amphetamine. APO 0.1, 0.1 mg/kg apomorphine; APO 1.0, 1.0 mg/kg apomorphine, APO 3.0, 3.0 mg/kg apomorphine; AMPH 1.5, 1.5 mg/kg *d*-amphetamine; AMPH 5.0, 5.0 mg/kg *d*-amphetamine.

	NMDA		6-OHDA		SHAM	
	Median		Median		Median	
	< 3	> 3	< 3	> 3	< 3	> 3
Vehicle	12	0	9	0	12	0
APO 0.1	12	0	9	0	12	0
APO 1.0	0	12	0	9	3	9
APO 3.0	0	12	0	9	0	12
AMPH 1.5	11	1	9	0	12	0
AMPH 5.0	0	12	2	10	5	4

(quadrant crossings: r = 0.11; rearing: r = 0.05), spontaneous locomotion (r = - 0.21), or the locomotor response to different doses of apomorphine, *d*-amphetamine or vehicle (0.1 mg/kg apomorphine: r = 0.17; 1.0 mg/kg apomorphine: r = - 0.30; 3.0 mg/kg apomorphine: r = 0.24; 1.5 mg/kg *d*-amphetamine: r = - 0.06; 5.0 mg/kg *d*-amphetamine: r = 0.20; vehicle: r = 0.01).

DISCUSSION

Comparison of the behavioural effects of DA depletion following intra-accumbens administration of 6-OHDA and neurone-specific NMDA lesions in the N.Acc. showed no significant differences in normal feeding, water intake and body weights up to four weeks after surgery. However, animals that had sustained selective excitotoxic destruction of neurones in the N.Acc. took longer to return to normal drinking patterns than did sham-lesioned rats. This between-group difference was small but significant, and since recovery did occur after three weeks it may simply reflect slower recovery from surgery, rather than a genuine functional effect. In line with previous reports (Kelly et al, 1975; Winn and Robbins, 1985), hypophagia or hypodipsia were not observed following DA-depleting lesions of the N.Acc..

As expected, all rats engaged in exploratory activities (locomotion and rearing) more frequently in a novel than in a familiar environment. Exploration of both novel and familiar environments was enhanced by NMDA lesions in the N.Acc.. By contrast, exploratory locomotion (quadrant crossings) in both environments was attenuated by dopaminergic terminal depletion in the N.Acc.. A tendency for reductions in the number

of rears per minute on both sides was also observed in this group, but the effect did not reach statistical significance. These results provide strong evidence for an involvement of neurones within the N.Acc. in exploratory behaviour. However, since increases in exploratory activities occurred in both novel and familiar environments compared to the control group, it is possible to argue that they reflect enhanced levels of overall activity, rather than increased exploration. With regard to lesions of DA terminal fields in the N.Acc. the attenuation of exploration may be secondary to a locomotor deficit since only exploratory locomotion was significantly affected.

Six to seven weeks after surgery, NMDA-lesioned rats were significantly more active than sham-lesioned animals during repeated recordings of spontaneous locomotor activity. In contrast to exploratory locomotion, hypoactivity was not observed in 6-OHDA-lesioned rats, indicating that recovery of normal motor functions had taken place by this time.

Administration of a low dose of apomorphine attenuated activity in 6-OHDA and sham lesion groups, while not affecting the response in NMDA-lesioned rats. At the medium dose only rats with NMDA lesions showed a reduced locomotor response; the high dose did not affect activity levels in either group, although there was a tendency for increased activity in 6-OHDA- and sham-lesioned rats. These results contrast with previously described 'supersensitivity' to administration of low and medium doses of apomorphine leading to increased activity following 6-OHDA lesions (Fink and Smith, 1980b; Kelly et al, 1975; Koob et al, 1981). The failure to observe significant supersensitivity to a direct DA agonist may indicate a partial recovery of post-synaptic DA receptors at the time of drug administration seven to eight weeks post-lesion. (Note

that the response to *d*-amphetamine was still blocked at this time.) In rats that had sustained NMDA lesions in the N.Acc., recovery of post-synaptic receptors at dopaminergic neurones could not have occurred, which may explain the apparent discrepancies of responses to all doses of apomorphine observed in rats with 6-OHDA lesions and controls on one hand and rats with excitotoxic N.Acc. lesions on the other. Systemic administration of a low dose of *d*-amphetamine confirmed previous reports of an attenuation of its locomotor-stimulating effects by 6-OHDA lesions of the N.Acc.. By contrast, the response remained intact in NMDA-lesioned rats; similar to the control group, these animals showed substantial increases in locomotion proportional to baseline levels of activity. The high dose of *d*-amphetamine normally associated with stereotyped behaviours led to small, statistically not significant reductions in locomotion in all three groups reflecting the increase in stereotyped behaviours obtained with this dose. The stereotyped response to medium and high doses of apomorphine and high doses of *d*-amphetamine remained unaffected by either lesion type. This result is in accordance with previous experiments reporting attenuation of amphetamine-induced stereotypy following 6-OHDA lesions of the caudate-putamen, but not the N.Acc., and further underlines the differentiation drawn between dorsal and ventral DA systems (Jones et al, 1989; Joyce and Iversen, 1984; Kelly et al, 1975).

To summarize the experiments reported here, depletion of DA from terminals in the N.Acc. and fibre-sparing lesions of neurones intrinsic to the nucleus produced differential patterns of behavioural responding. Terminal-depleting lesions did not interfere with normal regulatory behaviour but induced hypoactivity from which rats recovered during the course of the experiments. As had been expected, the

locomotor response to a low dose of *d*-amphetamine was attenuated, indicating that DA release from pre-synaptic terminal fields was still blocked seven to eight weeks after infusion of 6-OHDA. In contrast, selective excitotoxic destruction of neurones within the nucleus induced a slight but significant delay in post-operative recovery of home cage drinking. NMDA lesions were found to enhance general levels of activity as well as more specific exploratory locomotion and rearing, while leaving intact the locomotor-stimulating effects of *d*-amphetamine. Locomotion was attenuated by a medium dose of apomorphine and stereotyped behaviours induced by a high dose of *d*-amphetamine remained unaffected.

These observations correspond to theories of the N.Acc. functioning as a control mechanism in the ventral striatal motor system (Mogenson et al, 1980; Swerdlow and Koob, 1987b). Removal of accumbens neurones by infusion of excitotoxic substances would cause overactivity in output pathways and result in inappropriately high locomotor activity, as well as a deficit in the ability to channel activity levels appropriately.

CHAPTER VI

SCHEDULE-INDUCED POLYDIPSIA (1)

Dopamine activity in the ventral striatum is implicated as an important neuronal substance involved in the acquisition of schedule-induced polydipsia (SIP). Depletion of DA from terminal fields in the N.Acc. has repeatedly been shown to prevent acquisition of the response (Koob et al, 1978; Mittleman et al, 1990; Robbins and Koob, 1980; Robbins et al, 1983; Wallace et al, 1983), whereas selective lesions of dopaminergic terminals in the lateral septum (Taghzouti et al, 1985) and excitotoxic lesions of lateral hypothalamic neurones (Winn et al, 1992) have the opposite effect. Hippocampal lesions have also been demonstrated to affect acquisition of the response, although the evidence in this area is conflicting: according to Devenport (1978), SIP acquisition is enhanced following electrolytic lesions of the hippocampus, while aspiration lesions of the structure induced by Mittleman and colleagues (1990) resulted in a reduced consumption of water.

It is further argued that the frequently observed individual differences in the propensity to develop SIP may reflect differences in the responsiveness of forebrain dopamine systems (Dantzer et al, 1988; Mittleman et al, 1986; Tazi et al, 1988). Thus, reduced activity of dopaminergic afferents to the ventral striatum may facilitate adjunctive behaviours and increased activity of these projections may allow selection between alternative behaviours.

The series of experiments in this and the following two chapters aims to investigate this hypothesis in more detail by manipulating dopaminergic activity in the N.Acc., by lesioning N.Acc. intrinsic neurones and by measuring extracellular DA levels in the N.Acc. during

SIP. In this chapter, Experiments 1 to 3 examine the effects of intra-accumbens administration of DA on locomotor activity and the acquisition and emission of SIP. Experiment 4 in Chapter VII was designed to assess the effects of fibre-sparing NMDA lesions in the N.Acc. on SIP acquisition. In Chapter VIII, Experiment 5 investigates the relationship between DA efflux in the N.Acc. and SIP acquisition using electrochemical techniques.

EXPERIMENT 1: INTRA-ACCUMBENS ADMINISTRATION OF DOPAMINE

Prior to analyzing the effects of intra-accumbens DA injections on SIP acquisition and emission, it was necessary to test the feasibility of such injections *per se*, as well as establish a dose-response curve to the drug, if behavioural effects were observed.

Since DA injected into brain tissue is rapidly metabolized or taken up into dopaminergic terminals, it is generally assumed that behavioural consequences can only be obtained by giving the drug in conjunction with a re-uptake blocker, such as reserpine, and/or a monoamine oxidase (MAO) inhibitor, such as nialamide or pargyline. Most studies report intracranial DA application after pretreatment with nialamide, alone or in combination with reserpine (Anden and Jackson, 1975; Costall and Naylor, 1976; Pijnenburg et al, 1973). However, the effects of the pretreatment itself on behavioural responding are unclear and may confound the data. In addition, pretreatment interferes with quantification of the amounts of intra-accumbens DA that lead to a specific alteration in behaviour. Controversial results have been obtained after DA

administration without pretreatment: Pijnenburg and co-workers (1976) report significant increases in locomotor activity lasting 30 - 45 min after injections of 5 *u*g and 10 *u*g DA into the N.Acc.; Cador and colleagues (1991) showed that responding for conditioned reinforcement was significantly enhanced by intra-accumbens administration of 20 to 100 *u*g DA. By contrast, Cools (1986) observed significant increases in locomotion only after administration of 5 *u*g DA, while the drug remained ineffective at doses of 1 and 10 *u*g.

This initial experiment was therefore designed to test whether it is possible to induce significant behavioural changes by injecting different doses of DA into the N.Acc., without pretreatment. In addition, the dose-response relationship between drug and behaviour and the additive or potentiating effects of multiple injections were investigated. It was decided to examine alterations in locomotor activity, since the stimulating effects of such injections with pretreatment are well documented (Anden and Jackson, Costall and Naylor, 1976; Pijnenburg et al, 1973, 1976). Behavioural changes are also easy to monitor and can be quantified accurately.

METHODS

Prior to surgery, 7 rats were habituated to the test environment by placing them in the activity cages (see General Methodology, Chapter IV) for 3 hr daily over an 8-day period, until activity levels had stabilized. At 249.6 g mean body weight (SD = $\pm$ 21.5), rats were taken for surgery and implanted bilaterally with permanent 23 ga stainless steel guide cannulae in the accumbens core. Following a 1-week recovery period after

surgery, rats were re-habituated to the test cages, as well as being familiarized with the microinjection procedure - removing and replacing stylets in the same manner as the microinjection needles - for another 8-day period, until activity levels had stabilized. Each rat was then assigned to receive a series of counterbalanced single 1 *ul*/2 min injections of DA (50, 100, 150 and 200 nmol) and its vehicle. The 100 nmol dose contained 18.96 mg DA dissolved in 1 ml of 0.1 mg/ml ascorbate saline vehicle. Order of administration was determined by a 5 x 5 Latin square to control for the possible additive or potentiating effects of repeated drug administration. Immediately after infusion of the drug, rats were placed into activity cages and tested for 3 hr. To ensure complete metabolism of the drug and recovery from drug effects, the 5 injection sessions were separated by 3 non-drug days. To prevent rats from establishing an association between drug administration and activity testing, locomotor activity without drug was recorded on every second intervening day.

RESULTS AND DISCUSSION

Histology

Cresyl violet staining of coronal sections of the N.Acc. showed that all of the bilateral injections aimed at the accumbens core had been placed correctly. Individual placements are shown in Fig. 6.1. One rat had a blocked cannula and was therefore not included in the statistical analysis.

Locomotor activity

Repeated measures ANOVA of square root transformed photocell counts revealed no significant drug effect over the entire period of testing

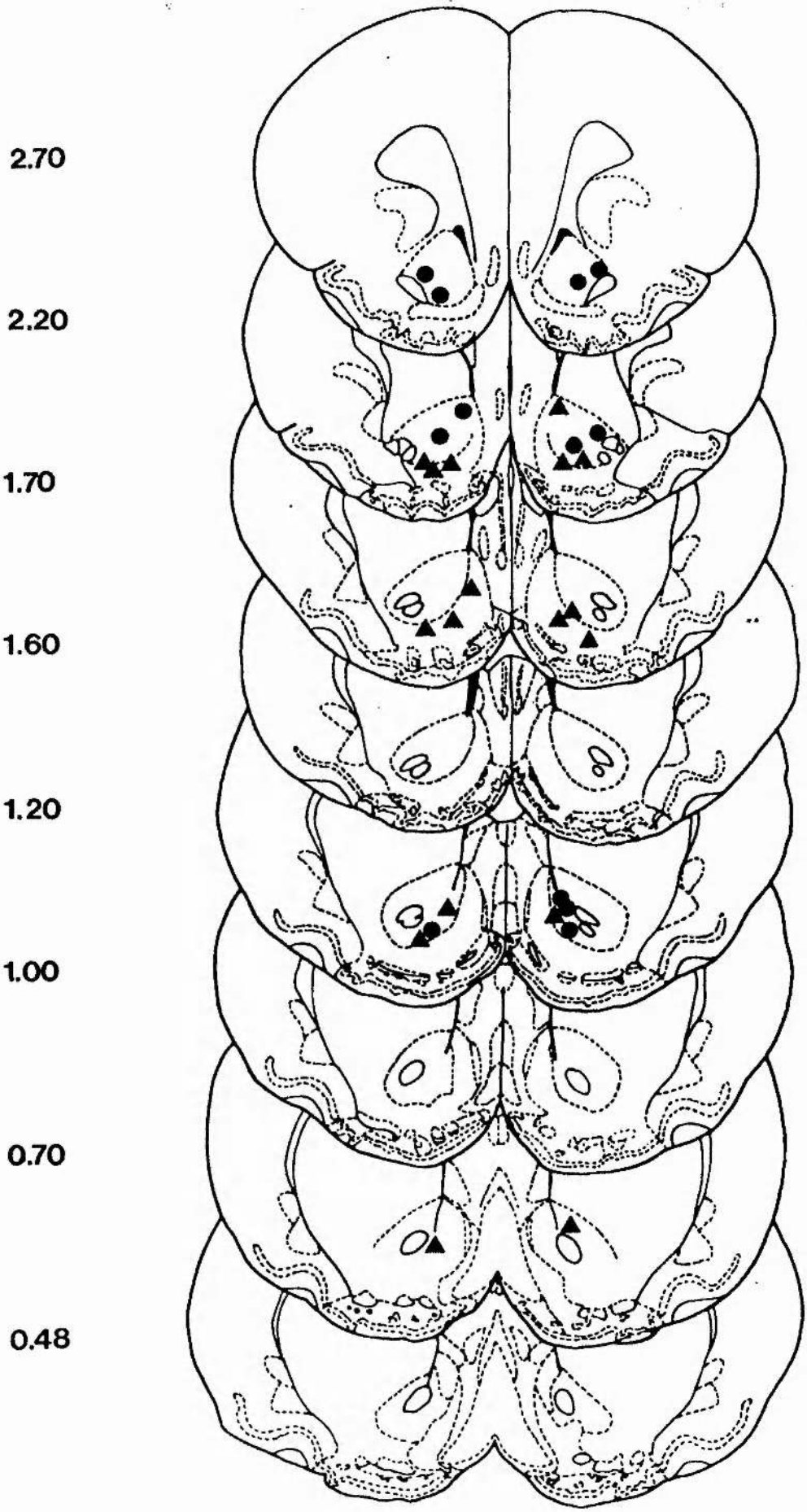


Fig. 6.1 Cannula placements for intra-accumbens injections of dopamine in rats tested for locomotor activity (circles) and schedule-induced polydipsia (triangles). Sections drawn from the atlas of Paxinos and Watson (1986); numbers represent distances from bregma in mm.

($F_{4,20} = 1.02$) but activity levels were found to change over time ($F_{5,25} = 32.07$, $P < 0.001$). Post-hoc analysis showed that rats were significantly more active during the first two half-hour test periods following drug administration, compared to the 4 remaining half-hour periods ($P < 0.001$; $P < 0.05$). Post-hoc analysis of a significant drug x time interaction ($F_{20,100} = 2.81$, $P < 0.001$) further revealed that doses of 150 nmol and 200 nmol DA microinjected into the accumbens core enhanced locomotor activity during the first 30 min of testing, compared to administration of vehicle ($P < 0.001$) or smaller doses of DA (50 nmol: $P < 0.01$; 100 nmol: $P < 0.05$). Fig. 6.2 represents the locomotor response to different doses of intra-accumbens DA during the first half-hour segment after drug administration. Following the first segment, no significant drug effects were observed. ANOVA with repeated measures showed no significant influence of drug administration day on locomotor activity, ruling out the possibility of additive or potentiating effects of repeated local administration of DA ($F_{4,20} = 0.98$).

The data presented here therefore confirm previous research by Pijnenburg and colleagues (1976) demonstrating significant enhancement of locomotor activity in response to intra-accumbens administration of DA without pretreatment with agents inhibiting MAO or blocking re-uptake. Further, a dose-response relationship was identified, where activity levels were enhanced by increasing doses of the drug. No additive or potentiating effects of repeated injections were observed. In order to obtain maximum effects with a relatively small dose of drug, a dosage of 150 nmol DA was selected for further experiments assessing the effects of intra-accumbens DA on SIP acquisition and emission.

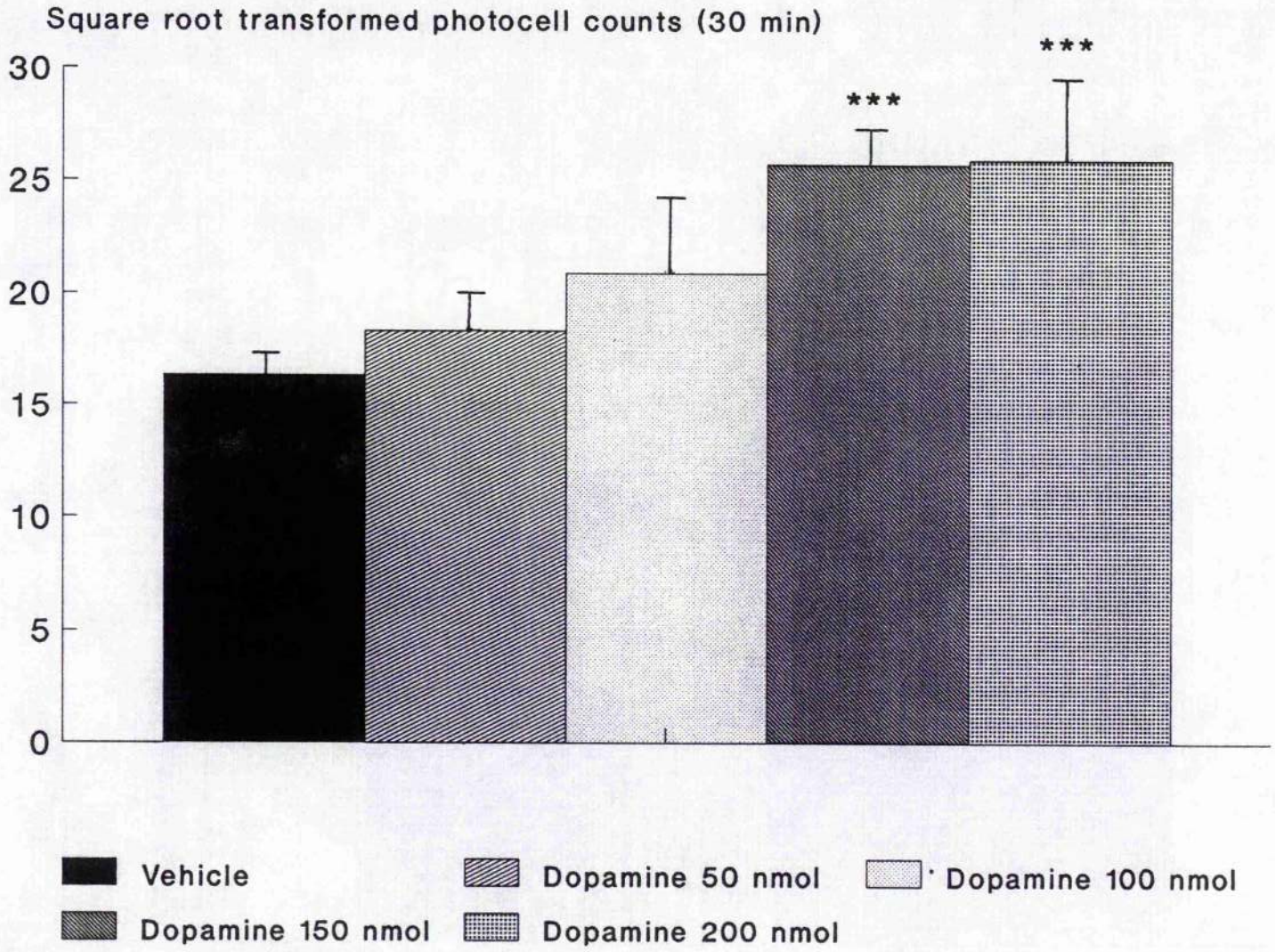


Fig. 6.2 Average of photocell counts (expressed as square roots of total values) in response to intra-accumbens administration of dopamine (DA), ± SE. *** = $P < 0.001$, compared to administration of vehicle.

EXPERIMENT 2: ACQUISITION OF SCHEDULE-INDUCED POLYDIPSIA IN UNOPERATED CONTROL RATS

The aim of the second control study was to obtain a detailed picture of SIP acquisition in unoperated rats. Since SIP performance has been found to vary between strains of rats and may differ according to the apparatus used, it was necessary to test normal performance on the parameters associated with the behaviour, such as the number of exposures to the FI schedule necessary for SIP development, water intake, the number of door presses and drinking bouts, door press and drinking bout latencies after pellet delivery and drinking bout lengths. It was further hoped to establish a baseline level for the percentage of rats developing the response, as well as a possible threshold of water intake that reliably predicts SIP acquisition. In order to replicate previous data by Tazi et al (1988) suggesting differential responding to the stimulating effects of *d*-amphetamine in polydipsic and non-polydipsic rats, activity levels were measured following systemic administration of different doses of the drug.

METHODS

Eight unoperated rats (291.3 g mean body weight, SD = $\pm$ 7.6) were food-deprived and maintained at 80% free-feeding body weight. On alternate days, rats were exposed to 30 min sessions of the FI 60 food-reinforcement schedule. Testing was discontinued after session 18. Following SIP testing, rats were allowed to regain free-feeding body weights and habituated to the locomotor activity cages for 3 hr. Every other day, all rats then received 3 counterbalanced i.p. injections of 1.5

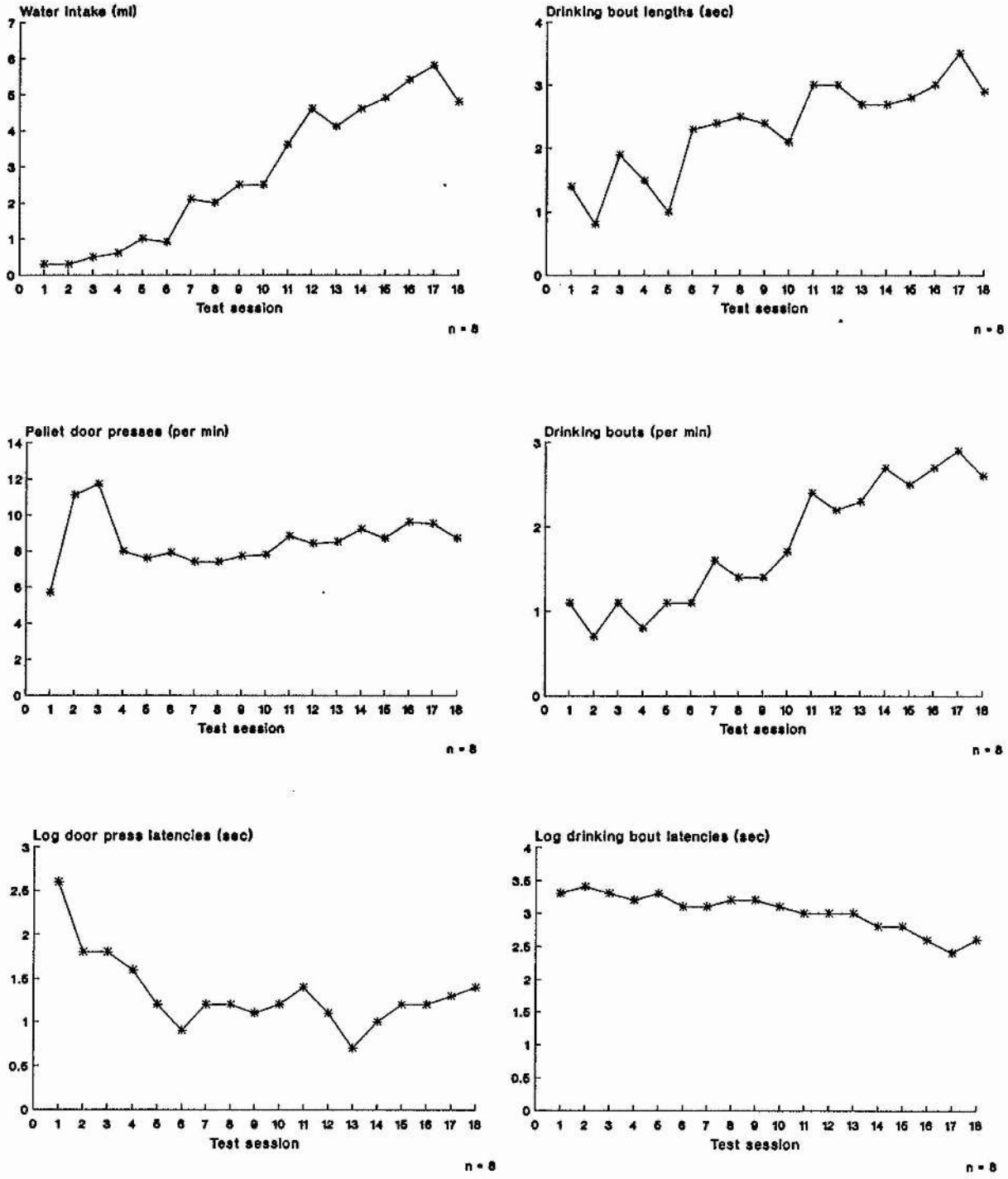
mg/kg *d*-amphetamine, 5.0 mg/kg *d*-amphetamine or vehicle before being placed into the cages. Activity levels were recorded for 60 min.

RESULTS AND DISCUSSION

Schedule-induced polydipsia

Fig. 6.3 shows measures of SIP acquisition in unoperated control rats over 18 test days. ANOVA with repeated measures confirmed that water intake increased significantly across 18 test sessions ($F_{17,119}$ = 9.2, $P < 0.001$). Levels of home cage drinking increased during the test period ($F_{17,119} = 2.2$, $P < 0.01$). With regard to the other parameters of SIP acquisition, significant increases were found in drinking bout lengths ($F_{17,119} = 2.2$, $P < 0.01$), the number of drinking bouts per minute ($F_{17,119} = 10.0$, $P < 0.001$). The average number of door presses per minute ($F_{17,119} = 1.77$, $P < 0.05$), and log latencies of the first drinking bout ($F_{17,119} = 6.68$ $P < 0.001$) and the first door press after pellet delivery ($F_{17,119} = 5.99$, $P < 0.001$) were significantly reduced. Post-hoc tests showed that compared to the first test session, water intake and the number of drinking bouts were consistently enhanced from session 11 onwards (water intake: session 11, $P < 0.05$; sessions 12 to 18, $P < 0.01$; average number of drinking bouts per minute: sessions 11 and 13 to 18, $P < 0.01$; session 12, $P < 0.05$). Drinking bout log latencies were significantly reduced from session 14 onwards, compared to the first test day (sessions 14 to 18: $P < 0.001$), while drinking bout lengths did not change significantly. By contrast, the average number of door presses per minute had stabilized after the third test day, door press latencies after the fourth session. Since rats respond appropriately to intermittent food reinforcement long before SIP acquisition occurs, this

Fig. 6.3 Measures of schedule-induced polydipsia in unoperated control rats.

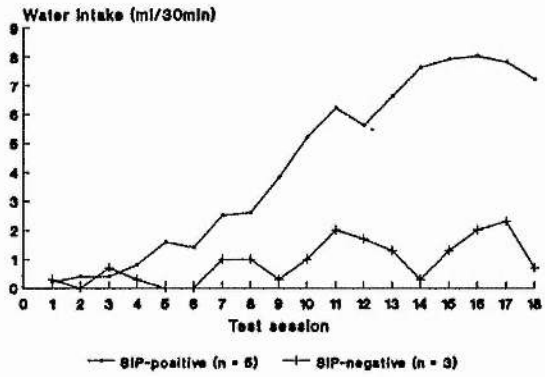


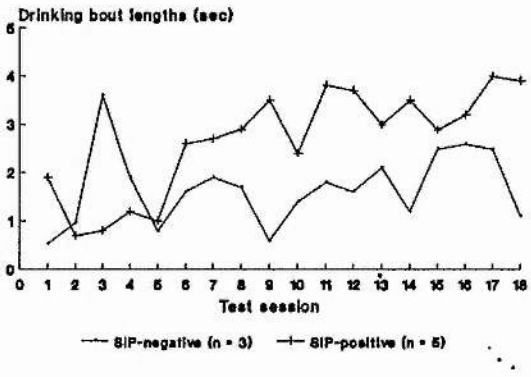
pattern supports the claim that excessive drinking is not induced by the reinforcement schedule itself.

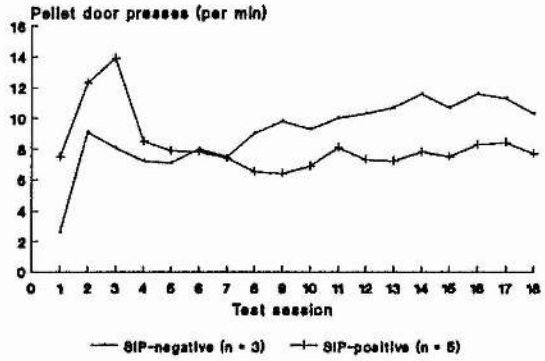
Since pronounced individual differences in SIP acquisition had become apparent during testing, all parameters of SIP were correlated in all 8 animals. Pearson correlation coefficients were significant for water intake and the average number of drinking bouts per minute ($r = 0.938$, $P < 0.001$), and water intake and drinking bout lengths ($r = 0.697$, $P < 0.05$), indicating that excessive water intake was a result of increases in both the number of drinking bouts and their lengths. In addition, a significant correlation was found for the average number of drinking bouts and door presses per minute ($r = - 0.807$, $P < 0.01$) - a larger number of drinking bouts being associated with a smaller number of door presses. No other correlation was statistically significant.

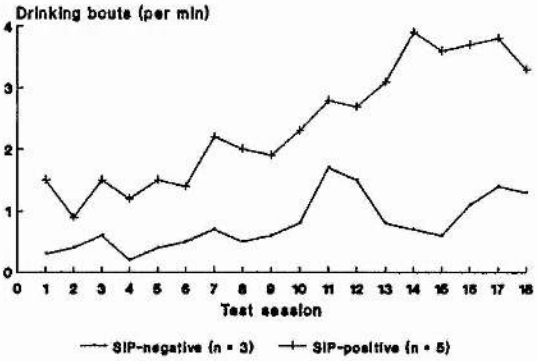
It was noted that 5 rats drank excessive amounts of water in response to intermittent food-reinforcement, while 3 rats failed to acquire the response. The threshold level of water intake distinguishing the two groups was found to lie at approximately 5 ml of water consumed during each of 5 consecutive 30 min test sessions. To analyze further the individual differences in the propensity to develop SIP, it was therefore decided to divide rats into 'SIP-positive' and 'SIP-negative' groups, depending on water intake above and below threshold level. SIP onset was reached during sessions 5, 7, 10, 13 and 14 respectively by the 5 SIP-positive rats. After acquisition, water intake in different rats ranged between 5 and 12 ml during a 30 min test period. Within subjects, amounts of water consumed changed by up to 3 ml during consecutive sessions, but always remained > 5 ml. Group means of SIP-positive and SIP-negative rats were compared using ANOVA and are shown in Fig. 6.4.

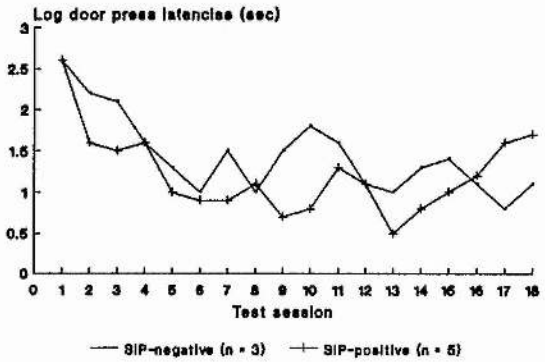
Fig. 6.4 Measures of schedule-induced polydipsia (SIP) in SIP-positive and SIP-negative control rats.

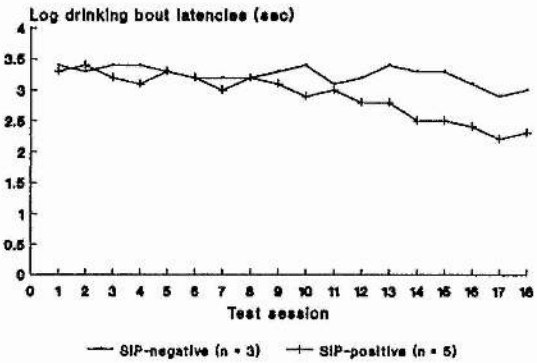












WATER INTAKE. As expected, ANOVA with repeated measures revealed that SIP-positive rats drank significantly more water during test sessions than did SIP-negative rats ($F_{1,6} = 6.08$, $P < 0.05$). Post-hoc analysis of a significant group x day interaction ($F_{17,102} = 5.06$, $P < 0.001$) indicated that this difference developed across time and was consistently present from session 10 onwards (sessions 10 to 12: $P < 0.05$; sessions 13 to 18: $P < 0.001$). On average, SIP-positive rats drank 7.2 ml (SE = $\pm$ 0.66) of water during the last test session, compared to 0.20 ml (SE = $\pm$ 0.2) during the first session. By contrast, SIP-negative rats drank an average of 0.67 ml (SE = $\pm$ 0.67) during session 18 and 0.33 ml (SE = $\pm$ 0.33) during session 1.

AVERAGE NUMBER OF DRINKING BOUTS PER MINUTE. While the number of drinking bouts increased during the course of testing ($F_{17,102} = 10.24$, $P < 0.001$), SIP-positive rats engaged in more drinking bouts than SIP-negative animals ($F_{1,6} = 10.0$, $P < 0.05$), the difference developing over time ($F_{17,102} = 3.49$, $P < 0.001$). Post-hoc testing showed that SIP-positive rats engaged in more drinking bouts from session 13 onwards (sessions 13 to 18: $P < 0.001$).

DRINKING BOUT LENGTHS. Drinking bout lengths did not differ between groups ($F_{1,6} = 1.54$) but showed a small increase across test sessions ($F_{17,102} = 1.92$, $P < 0.05$). A group x day interaction indicated that SIP-positive rats engaged in longer drinking bouts than did SIP-negative rats in the course of testing ($F_{17,102} = 1.98$, $P < 0.005$). Post-hoc analysis, however, did not show any significant differences between means. On average, SIP-positive rats made 2.35 drinking bouts per minute (SE = $\pm$ 0.63) lasting 2.9 sec (SE = $\pm$ 0.63), while SIP-negative subjects engaged in 0.9 drinking bouts per minute (SE = $\pm$ 0.46), lasting 1.8 sec (SE = $\pm$ 1.15).

AVERAGE LATENCY OF FIRST DRINKING BOUT AFTER PELLET DELIVERY (LOG). Responding differed significantly between groups ($F_{1,6}$ = 13.55, $P < 0.01$) and across time ($F_{17,102}$ = 5.85, $P < 0.001$). Post-hoc analysis of a group x day interaction ($F_{17,102}$ = 2.35, $P < 0.01$) indicated that log latencies were reduced in SIP-positive rats from test session 14 onwards ($P < 0.05$). Average latencies were 19.99 sec (SE = $\pm$ 2.12) in SIP-positive and 26.16 sec (SE = $\pm$ 2.83) in SIP-negative rats.

AVERAGE NUMBER OF DOOR PRESSES PER MINUTE. Responding decreased significantly over time ($F_{17,102}$ = 2.33, $P < 0.05$), while no group effect ($F_{1,6}$ = 0.62) was observed. Post-hoc analysis of a significant group x day effect ($F_{17,102}$ = 2.33, $P < 0.01$) showed no consistent interaction during the course of testing.

AVERAGE LATENCY OF FIRST DOOR PRESS AFTER PELLET DELIVERY (LOG). Both groups responded significantly faster to pellet delivery across test sessions ($F_{17,102}$ = 6.11, $P < 0.001$). Group differences ($F_{1,6}$ = 1.71) and group x day interaction ($F_{17,102}$ = 1.72) did not reach statistical significance. Door press latencies in session 1 were 12.3 sec (SE = $\pm$ 0.63) and 14.4 sec (SE = $\pm$ 3.23) for SIP-positive and SIP-negative rats respectively, while in session 18, latencies were 6.4 sec (SE = $\pm$ 1.34) and 3.16 sec (SE = $\pm$ 0.75) respectively. Results of the last two parameters of SIP indicate that since both SIP-positive and SIP-negative rats learn to respond appropriately to intermittent food reinforcement at the same rate. Differential responding during SIP is therefore selective to excessive drinking during intermittent food reinforcement and does not extend to the reinforcement schedule itself.

HOME CAGE WATER INTAKE. During the remainder of the day, no differences in water intake were observed between SIP-positive and SIP-

negative rats ($F_{1,6} = 3.14$). SIP-positive rats drank 16.1 ml (SE = $\pm$ 2.01) of water on average in the home cage, while SIP-negative rats consumed 19.2 ml (SE = $\pm$ 2.54) on average.

Locomotor response to d-amphetamine

ANOVA with repeated measures showed a significant drug effect ($F_{2,14} = 35.77$, $P < 0.001$) and post-hoc analysis revealed that activity levels were significantly enhanced by the low dose of *d*-amphetamine, compared to administration of vehicle ($P < 0.001$). The locomotor response to different doses of *d*-amphetamine and saline vehicle was not significantly correlated to the amount of water drunk during SIP (1.5 mg/kg *d*-amphetamine: r = 0.07; 5.0 mg/kg *d*-amphetamine: r = 0.16; saline: r = 0.35).

On the basis of the above results, it is possible to draw the following conclusions about the course of SIP acquisition:

(1) excessive water intake during SIP is a result of an increased average number of drinking bouts per minute from session 11 onwards, and increased drinking bout lengths;

(2) the average number of door presses per minute and door press latencies are stabilized after the third or fourth test day;

(3) individual rats vary in their propensity to develop SIP, which needs to be taken into account when designing and analyzing SIP studies;

(4) the propensity to develop SIP does not correlate with the locomotor response to *d*-amphetamine

(5) water intake varies substantially between as well as within subjects;

(6) the time of SIP onset differs greatly between subjects;

(7) in the above experiment, the threshold level of water intake predicting SIP acquisition was 5 ml during a 30 min FI 60 food reinforcement session; however, variances in strains of rats and equipment used may make alterations of the criteria of SIP onset necessary.

These findings clearly underline the importance of taking into account individual differences in the propensity to develop SIP. Only 62.5% of subjects acquired the behaviour, thereby confirming previous reports of SIP-positive and SIP-negative rats (Dantzer et al, 1988; Tazi et al, 1987; Tazi et al, 1988). Rats that acquire SIP, and rats that do not, differ with regard not only to water intake, but also to the number and length of drinking bouts and possibly drinking bout latencies after pellet delivery. These differences appear to be selective to excessive drinking in response to intermittent food reinforcement, since home cage drinking as well as the number and latency of door presses are unrelated to SIP. Before attempting to manipulate the acquisition and emission of SIP, it would therefore be best to identify those animals that are more likely than others to consistently engage in the behaviour. However, although SIP-positive rats have been shown to differ from SIP-negative rats in their response to administration of *d*-amphetamine, aversive stimuli and threat, so far, no reliable and easily administered screening method for the propensity to show SIP has been reported (Dantzer et al, 1988; Tazi et al, 1987; Tazi et al, 1988). It should be noted that in the present experiment, water intake during SIP did not predict the locomotor response to *d*-amphetamine. However, this discrepancy with previous results may be due to small subject numbers or insufficient habituation to activity cages.

In summary, this study has demonstrated the differential acquisition of responding to the reinforcement schedule and excessive drinking in response to the schedule. Schedule-induced drinking was

accompanied by a distinct response pattern including increases in the number and lengths of drinking bouts, as well as reductions in the number of door presses and in latencies of the first drinking bout and door press after pellet delivery. Time course of acquisition and the amount of water consumed were found to vary considerably between individual rats.

EXPERIMENT 3: INTRA-ACCUMBENS ADMINISTRATION OF DOPAMINE AND SCHEDULE-INDUCED POLYDIPSIA

As mentioned above, dopaminergic depletion of terminal fields in the N.Acc. has repeatedly been reported to abolish the acquisition of SIP (Koob et al, 1978; Mittleman et al, 1990; Robbins and Koob, 1980; Wallace et al, 1983), but the consequences of dopaminergic overactivity in the N.Acc. have not been investigated. The following experiment was devised to test the effects of intra-accumbens infusion of DA on SIP acquisition. On the basis of data reported in the previous experiment using normal control rats, and following some preliminary experiments not reported here showing an interference of intra-accumbens DA *and* vehicle injections with SIP acquisition, the present study was designed to take into account individual differences in the propensity to develop the behaviour and to minimize DA administration to avoid unnecessary tissue damage. Rats were exposed to the reinforcement schedule without treatment for 13 days, or until they developed a drinking response, before DA was administered. On the basis of the previous control study, the criterion of SIP onset was set at 5 ml of water consumed during a 30 min test session. Only rats that had met this threshold criterion and were likely to develop the drinking response further were therefore included in the drug or

vehicle groups, and the effects of DA on acquisition and emission of SIP after onset of the response were examined.

METHODS

At an average body weight of 290.4 g (SD = $\pm$ 17.7), 24 rats were taken for surgery and received bilateral cannulation with 11.0 mm permanent 23 ga stainless steel guide cannulae aimed at the accumbens core.

Two weeks after surgery, when all rats had regained their pre-operative body weights, they were food-deprived to 80% of free-feeding body weight and maintained at this weight throughout the experiment. Subsequently, rats were placed into the operant boxes on alternate days and exposed to the FI 60 food-reinforcement schedule for 30 min periods. All animals were tested without injections, until water intake was equal to or exceeded 5 ml. When the criterion had been met, rats received intra-accumbens injections of either 150 nmol DA (n = 4) or its vehicle (n = 4) prior to the following 5 test sessions. The 150 nmol dose of DA was made up of 24.88 mg of DA dissolved in 1 ml 0.1 mg/ml ascorbate saline. Testing was discontinued after a rat had received 5 injections of drug or vehicle, or after it had completed 13 test sessions without having met the criterion for SIP onset.

RESULTS AND DISCUSSION

Histology

Cresyl violet staining of coronal sections of the N.Acc. showed that injections sites were located correctly within the accumbens core in all of the animals receiving intra-accumbens DA or vehicle (see Fig. 6.1).

Schedule-induced polydipsia

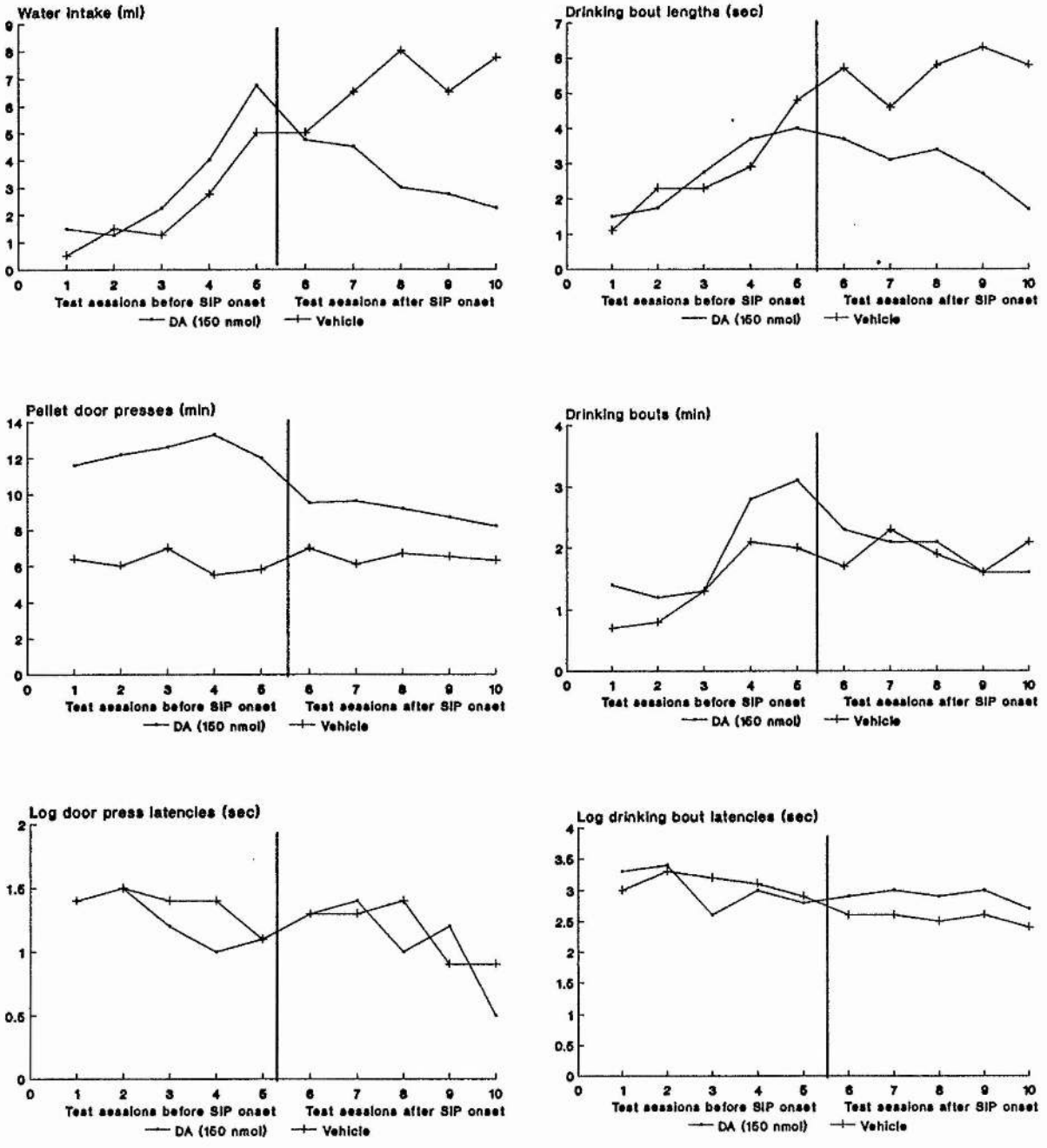
Fig. 6.5 shows responses in drug and control rats during the 5 SIP sessions before and after microinjections started.

WATER INTAKE. Repeated measures ANOVA of water intake during the 5 test sessions before and after the start of microinjections revealed no significant effects of drug group ($F_{1,6} = 0.94$) or start of injections ($F_{1,6} = 0.51$) on water intake during SIP testing. However, water intake was significantly enhanced over time ($F_{4,24} = 6.16$, $P < 0.001$). During the 5 test sessions preceding and following the start of injections, rats in the DA group drank 3.15 ml (SE = $\pm$ 1.25) and 3.65 ml (SE = $\pm$ 1.70) of water on average. By comparison, rats in the vehicle group consumed 2.20 ml (SE = $\pm$ 0.9) and 6.75 ml (SE = $\pm$ 1.95) respectively.

AVERAGE NUMBER OF DRINKING BOUTS PER MINUTE. In both groups, a tendency to engage in more drinking bouts over time was observed, but did not reach statistical significance ($F_{4,24} = 2.57$, $P = 0.064$). Group or group x day effects were not significant (group: $F_{1,6} = 0.23$; group x day: $F_{4,24} = 0.53$).

DRINKING BOUT LENGTHS. Confirming responses of unoperated control subjects, drinking bout lengths increased in both groups over time ($F_{4,24} = 4.06$, $P < 0.05$) but did not differ between groups ($F_{1,6} =$

Fig. 6.5 Measures of schedule-induced polydipsia before and after microinjection of dopamine or vehicle into the nucleus accumbens. Drugs were administered immediately prior to sessions 6 - 10.



2.04) or were differentially affected by time ($F_{4,24} = 2.12$)

AVERAGE LATENCY OF FIRST DRINKING BOUT AFTER PELLET DELIVERY (LOG). No decrease in drinking bout log latencies was observed across test sessions ($F_{4,24} = 1.62$) and responding was not affected by lesion group ($F_{1,6} = 0.32$) or a group x day interaction ($F_{4,24} = 0.72$).

AVERAGE NUMBER OF DOOR PRESSES PER MINUTE. The number door presses were unaffected by drug group ($F_{1,6} = 4.57$), test day ($F_{4,24} = 0.41$) or an interaction of both factors ($F_{4,24} = 0.49$).

AVERAGE LATENCY OF FIRST DOOR PRESS AFTER PELLET DELIVERY (LOG). Door press log latencies did not differ between groups ($F_{1,6} = 0.12$) or injection days ($F_{4,24} = 1.43$). No group x day interaction was found ($F_{4,24} = 0.17$). Although the two latter findings contrast with data from unoperated control rats, they can be explained by the fact that rats typically respond appropriately to the food reinforcement schedule after only a few exposures to the schedule - for most animals, data from these first test sessions was not analyzed here.

HOME CAGE WATER INTAKE. During the remainder of the day, drinking was unaffected by drug administration, since repeated measures ANOVA showed no significant effect of drug groups, start of injections or test days on home cage drinking (drug group: $F_{1,6} = 1.05$; start of injections: $F_{1,6} = 0.35$; test days: $F_{4,24} = 0.79$).

The data reported here show that addition of exogenous DA to the N.Acc. did not affect schedule-induced drinking after SIP onset. Although a tendency for dopaminergic suppression of SIP was observed, this effect did not reach statistical significance. This finding is clearly unexpected on the basis of (1) a previous experiment outlined above, where strong behavioural responses (increased locomotor activity) were obtained with intra-accumbens administration of a similar dose of DA and (2) extensive

research reporting changes in SIP, displacement activities and behavioural switching after manipulation of dopaminergic activity in the N.Acc. (Koob et al, 1978; Mittleman et al, 1990; Robbins et al, 1983; Robbins and Koob, 1980; Wallace et al, 1983).

The data correspond to previous suggestions that it may be necessary to distinguish the effects of dopaminergic activation on SIP acquisition and already established polydipsia. The dose of DA administered in this experiment may not have induced sufficient increases in dopaminergic activity to abolish SIP after its onset, confirming the proposal by Robbins and colleagues (1983) that prior acquisition of SIP may protect it from the disruptive effects of 6-OHDA lesions of the N.Acc.. It could further be argued that since SIP is a very persistent response, it is slowly extinguished over time and effects of DA may only be observed after more than the 5 injections administered here. Alternatively, the results may represent a more fundamental problem questioning the importance of dopaminergic activity in the N.Acc. for SIP emission. The failure to alter schedule-induced drinking by overstimulation of dopaminergic transmission in the N.Acc. may indicate that this structure is less involved than previously thought in mediating the response once it has been established. Finally, chronic implantation of guide cannulae into the N.Acc. appears to have had some effect on responding since only one third of the operated animals developed the behaviour, as opposed to almost two thirds of unoperated control rats.

To summarize, intra-accumbens administration of DA at doses that significantly enhance spontaneous locomotion did not affect acquired polydipsia in response to intermittent food-reinforcement. DA in the N.Acc. may therefore be involved in the *acquisition* of SIP, while other, related structures, such as the lateral hypothalamus, hippocampus and

lateral septum may be responsible for mediating the established response.

CHAPTER VII

SCHEDULE-INDUCED POLYDIPSIA (2)

EXPERIMENT 4: NMDA LESIONS IN THE NUCLEUS ACCUMBENS AND SCHEDULE-INDUCED POLYDIPSIA

It has previously been mentioned that dopaminergic depletion of terminal fields in the N.Acc. has been shown to disrupt the acquisition of SIP (Koob et al, 1978; Mittleman et al, 1990; Robbins and Koob, 1980; Robbins et al, 1983; Wallace et al, 1983) and that selective lesions of dopaminergic terminals in the lateral septum (Taghzouti et al, 1985) or excitotoxic lesions of lateral hypothalamic neurones (Winn et al, 1992) enhance acquisition of the response. Different types of hippocampal lesion have been reported to either disrupt or enhance the response (Devenport, 1978; Mittleman et al, 1990). In addition, terminal-depleting and fibre-sparing lesions in the N.Acc. have been found to have differential effects on normal regulatory behaviour, locomotor activity, exploration and the locomotor response to *d*-amphetamine (Weissenborn and Winn, in press; see Chapter V). The present study was designed to investigate the effects of fibre-sparing, excitotoxic lesions in the N.Acc. on the response pattern typically associated with SIP.

METHODS

Surgical procedures

At mean body weight of 383.9 g (SD = $\pm$ 20.2), 19 rats were taken for surgery and received bilateral stereotaxic infusions of either 1 *u*l

60 nmol NMDA (n = 11) or 1 ul phosphate buffer vehicle (n = 8) into the accumbens core. The 60 nmol dose contained 8.826 mg NMDA dissolved in 1 ml of phosphate buffer.

Locomotor response to d-amphetamine

After recovery from surgery, rats were habituated to locomotor cages for 90 min daily for 3 days. Subsequently, rats received a series of counterbalanced injections of different doses of *d*-amphetamine (1.5 mg/kg, 5.0 mg/kg), or vehicle after a 30 min habituation period and locomotor activity was recorded for a further 60 min. Order of administration was determined by a Latin square design to control for the possible additive or potentiating effects of repeated drug administration. Injection days were separated by 1 test day on which all rats received an injection of saline after the habituation period.

Schedule-induced polydipsia

Following completion of the locomotor tests, rats were food-deprived to 80% of their current, post-operative free-feeding body weights and maintained at these weights throughout the experiment. Rats were exposed to daily 60 min FI 60 food-reinforcement sessions as described previously. The length of test sessions was increased to 60 min (as compared to 30 min in previous experiments) in order to establish consistently high levels of water intake in control groups.

Physiological challenges

Following the SIP experiment, a free-feeding schedule was reinstated. After 2 weeks, when rats had regained normal levels of body weight, they were water-deprived for 23 hr, after which drinking bottles

were returned. Water intake during the first 30 min period after deprivation was recorded. This procedure was repeated after 24 hr. In another test, rats received counterbalanced injections of either 10 ml physiological saline (0.9%) i.p. or 10 ml hypertonic saline (5.0%) i.p. and water intake was recorded after 60 min and 180 min. Injections were separated by a 24 hr rest period.

RESULTS

Histology

Analysis of cresyl violet stained coronal sections showed that 2 rats had no visible lesion of the N.Acc., while another animal had sustained damage to the entire ventral striatum, as well as adjacent areas such as the lateral septum, caudate-putamen and ventral pallidum. One rat fell ill and had to be put down before the completion of testing. Only the data of the remaining 7 subjects was included in the analysis. The smallest (A) and largest (B) lesions obtained by infusion of 60 nmol NMDA into the accumbens core are outlined in Fig. 7.1. Average lesion volume was 59.33% (SE = $\pm$ 8.05) of the accumbens core area and 33.32% (SE = $\pm$ 4.52) of the accumbens core and shell. Other ventral striatal structures were not damaged.

Normal regulatory behaviour

Fig. 7.2 shows body weight, food and water intake measured 1 week pre- and 3 weeks post-operatively. ANOVA with repeated measures confirmed that regulatory responses measured over the 7-day period prior to surgery did not differ significantly between groups (body weight: $F_{1,13}$

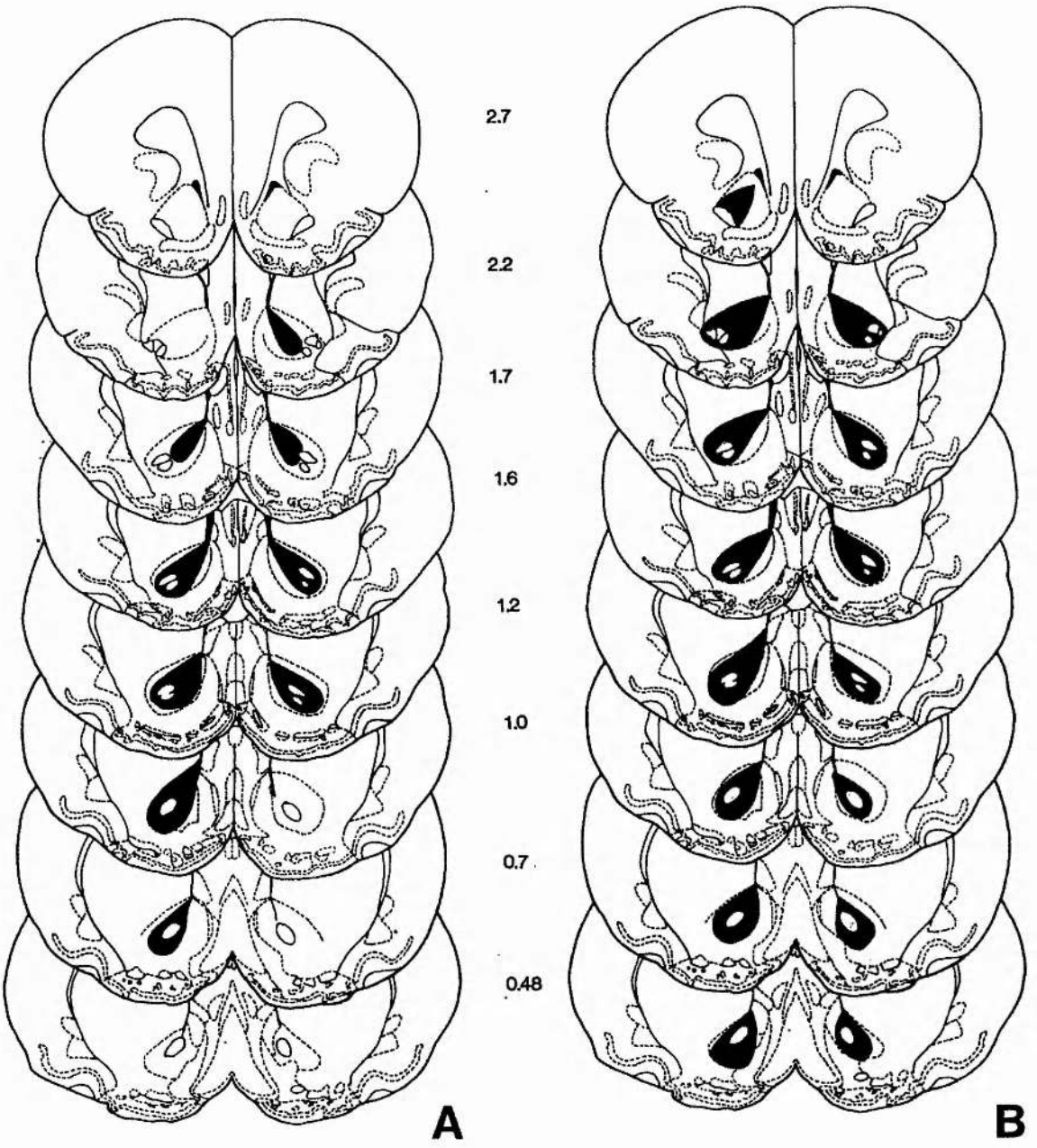
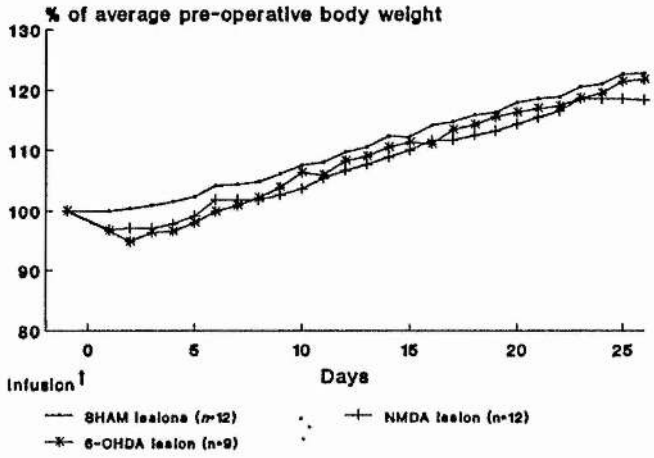
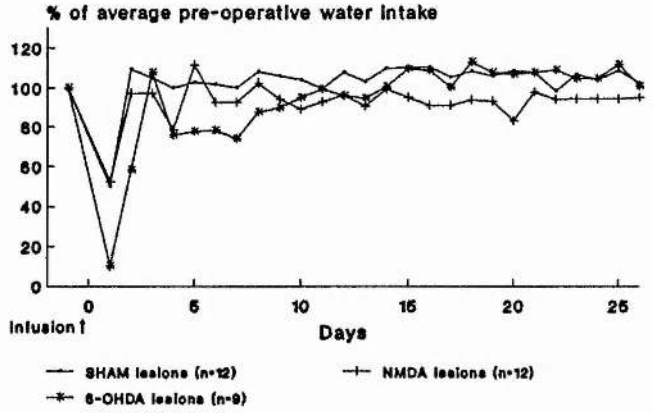
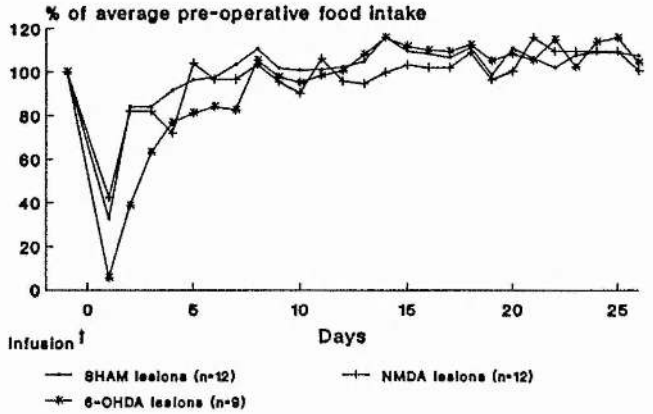


Fig. 7.1 Smallest (A) and largest (B) lesions obtained by infusion of 60 nmol NMDA into the nucleus accumbens. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm.

Fig. 7.2 Average pre- and post-operative body weights, food and water intake following NMDA or sham lesions in the nucleus accumbens.





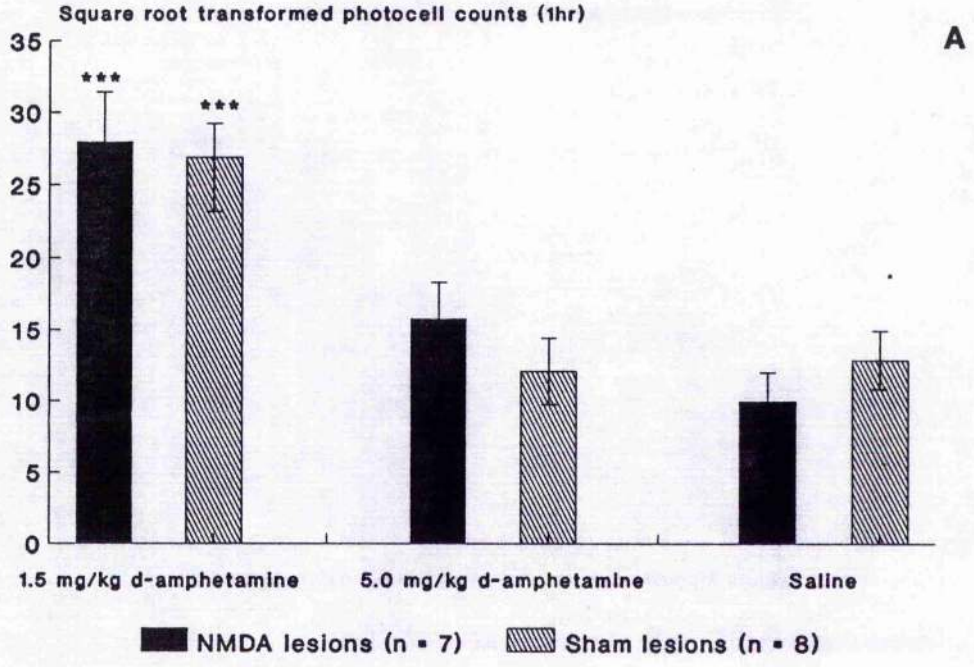


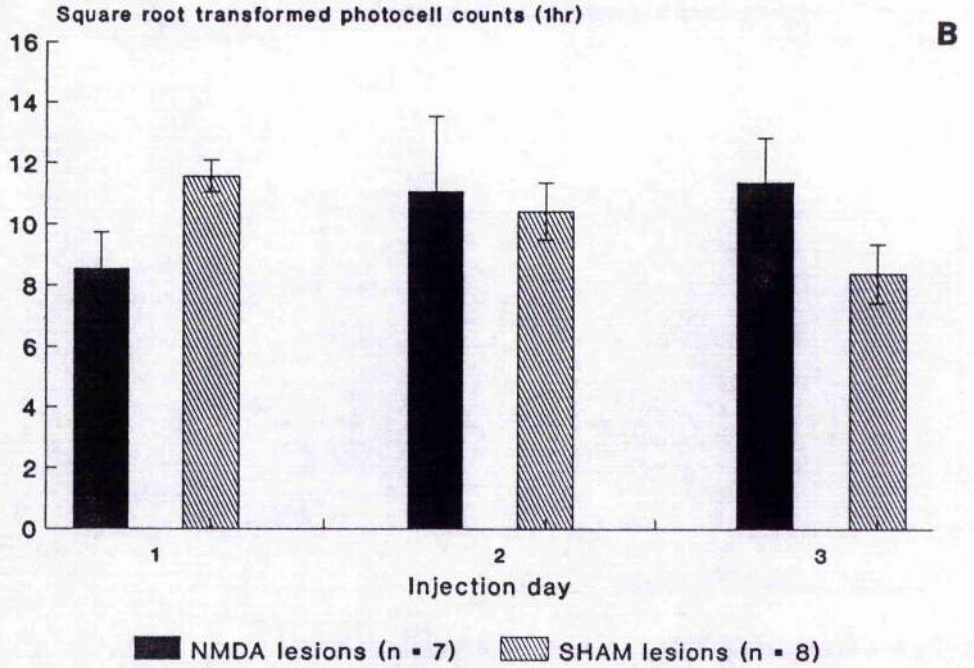
= 1.18; food intake: $F_{1,13}$ = 1.68; water intake: $F_{1,13}$ = 0.46). Repeated measures ANOVA of regulatory responses during the 3 weeks following surgery revealed the following: body weight, food and water intake were found to increase significantly across weeks (body weight: $F_{2,26}$ = 41.97, $P < 0.001$; food intake: $F_{2,26}$ = 96.47, $P < 0.001$; water intake: $F_{2,26}$ = 34.58, $P < 0.001$). Post-hoc testing showed significant reductions in all regulatory responses during the first post-operative week and subsequent recovery (body weights: $P < 0.001$; food intake: $P < 0.001$; water intake: $P < 0.001$). However, post-hoc analysis of a group x week interaction indicated that the deficit in post-operative body weight in NMDA-lesioned rats present in week 1 ($P < 0.05$) became more pronounced during the following weeks (week 2: $P < 0.01$; week 3: $P < 0.001$). In addition, a small but significant group effect on food intake was found ($F_{1,13}$ = 7.02, $P < 0.05$), with NMDA-lesioned rats consuming on average 18.46 g (SE = $\pm$ 1.22) of food per day, compared to 19.89 g (SE = $\pm$ 1.03) in sham-lesioned rats.

Locomotor response to different doses of d-amphetamine

Locomotor activity following injection of different doses of *d*-amphetamine is shown in Fig. 7.3A. ANOVA with repeated measures on the square root transformed photocell counts per 60 min showed a significant effect of administration of different doses of *d*-amphetamine and vehicle on locomotor activity ($F_{2,26}$ = 19.0, $P < 0.001$). Post-hoc testing indicated that locomotor activity was significantly increased following the low dose of *d*-amphetamine ($P < 0.001$), compared to administration of vehicle. The high dose of *d*-amphetamine did not alter activity levels. Administration of drugs did not differentially affect groups ($F_{1,13}$ = 0.19), and no significant group x drug interaction was observed

Fig. 7.3 Measures of locomotor activity in response to different doses of d-amphetamine (A) and saline (B) expressed as square roots of photocell counts during 1 hr tests in activity cages, ± SE. *** = $P < 0.001$, compared to administration of vehicle.





($F_{2,26} = 0.77$). Repeated measures ANOVA of the locomotor response to saline on alternative days showed no significant differences between groups ($F_{1,13} = 0.03$) or between days ($F_{2,26} = 0.30$), and the group x day interaction did not reach statistical significance ($F_{2,26} = 3.33$) (see Fig. 7.3B). ANOVA with repeated measures revealed no significant effect of drug administration day on locomotor activity, ruling out the possibility of potentiating effects of drug administration ($F_{2,26} = 0.1$).

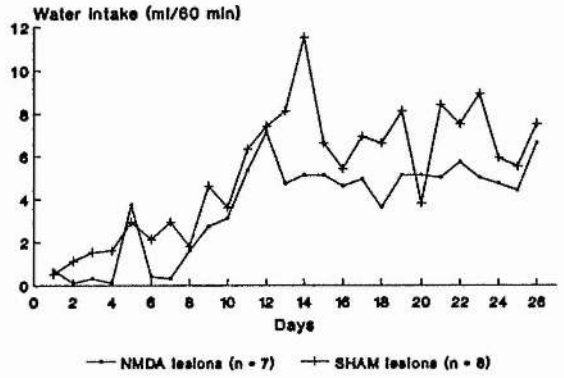
The locomotor response to different doses of *d*-amphetamine and saline vehicle was not correlated to lesion volume (1.5 mg/kg *d*-amphetamine: r = 0.16; 5.0 mg/kg *d*-amphetamine: r = 0.16; saline: r = - 0.03). Further, no correlation of locomotor activity following administration of *d*-amphetamine to average water intake during the last 5 SIP sessions was found (1.5 mg/kg *d*-amphetamine: r = - 0.37; 5.0 mg/kg *d*-amphetamine: r = 0.19; saline: r = - 0.32).

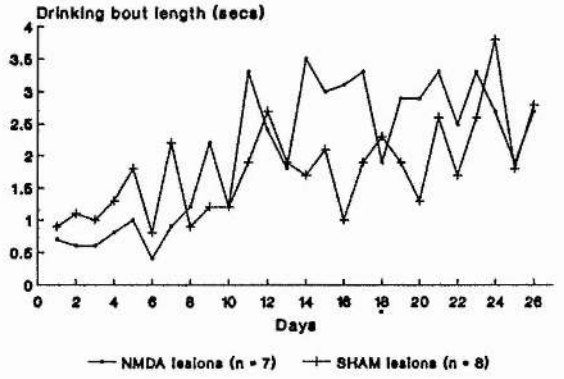
Schedule-induced polydipsia

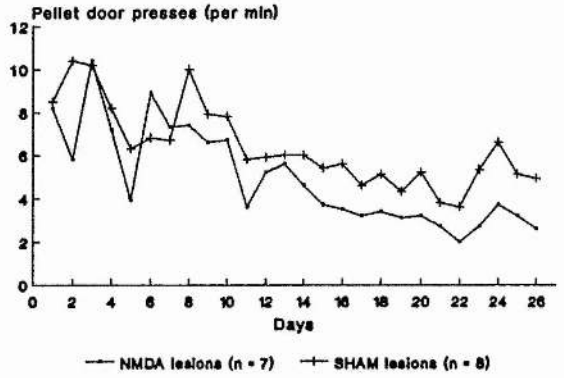
Results relating to SIP acquisition are shown in Fig. 7.4.

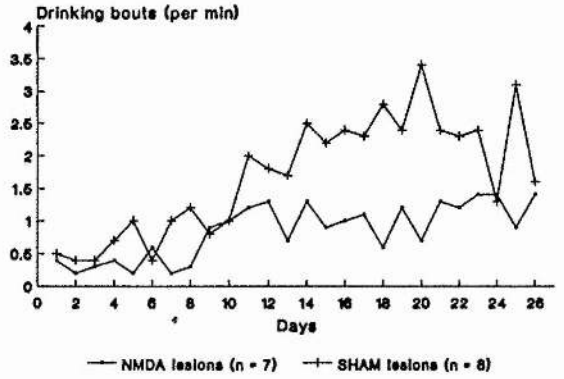
WATER INTAKE. ANOVA with repeated measures showed a significant increase in water intake across days ($F_{25,325} = 34.58$, $P < 0.001$), but no difference between lesion groups ($F_{1,13} = 0.94$) or an interaction between groups and days ($F_{25,325} = 0.60$). Post-hoc analysis of the day effect showed that compared to the first test session, rats tended to drink more water from session 11 onwards (sessions 11, 15 and 17: $P < 0.05$; sessions 12 to 14, 19, 21 to 23 and 26: $P < 0.01$). On average, NMDA-lesioned rats consumed 3.66 ml of water (SE = $\pm$ 1.07) during a 60 min test session, compared to 5.31 ml (SE = $\pm$ 1.96) in sham-lesioned rats.

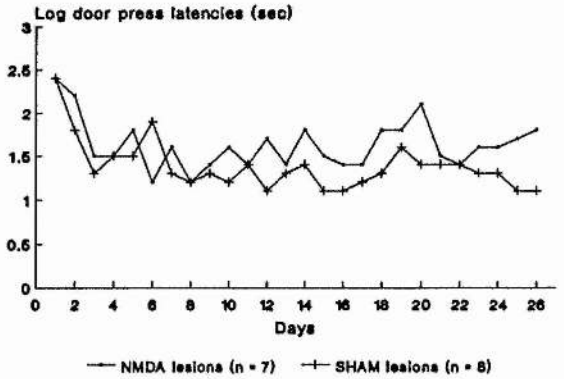
Fig. 7.4 Measures of schedule-induced polydipsia following NMDA lesions in the nucleus accumbens.

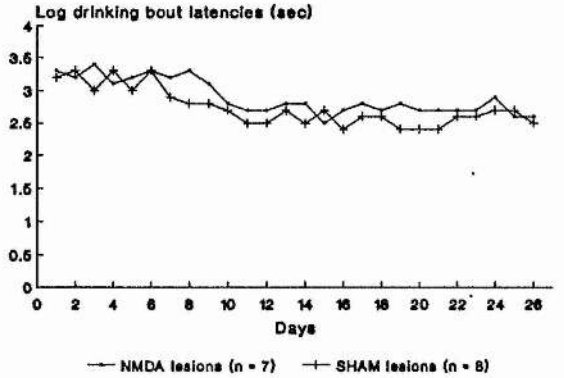












AVERAGE NUMBER OF DRINKING BOUTS PER MINUTE. The number of drinking bouts per minute differed significantly across test sessions ($F_{25,325} = 5.37$, $P < 0.001$). However, although the rate of drinking tended to increase over time, post-hoc tests did not reveal a consistent effect. Although there was a tendency for lesioned rats to approach the drinking spout less often than did control rats, no lesion group effect or group x day interaction was observed ($F_{1,13} = 2.81$; $F_{25,325} = 1.39$) On average, rats with NMDA lesions in the N.Acc. engaged in 0.85 drinking bouts/min (SE = $\pm$ 0.26), compared with 1.66 drinking bouts/min (SE = $\pm$ 0.58) in sham-lesioned controls.

LATENCY OF FIRST DRINKING BOUT AFTER PELLET DELIVERY (LOG). Log latencies of drinking bouts decreased significantly across test sessions ($F_{25,325} = 6.24$, $P < 0.001$). Post-hoc analysis showed that compared to the first test, this effect was consistently present from test session 10 onwards (sessions 10 and 24: $P < 0.05$; sessions 11 to 23, 25 and 26: $P < 0.01$). Lesion groups did not differ significantly in log latencies of drinking bouts ($F_{1,13} = 2.53$) and were not differentially affected by time ($F_{25,325} = 0.67$).

DRINKING BOUT LENGTHS. Average drinking bout lengths differed significantly across test sessions ($F_{25,325} = 3.09$, $P < 0.001$). Although there was a tendency for bout lengths to increase with time, post-hoc tests did not reveal a consistent effect. No differences between lesion groups were observed ($F_{1,13} = 0.22$) and no group x day interaction was found ($F_{25,325} = 0.98$).

AVERAGE NUMBER OF DOOR PRESSES PER MINUTE. Door press frequencies decreased significantly over time ($F_{25,325} = 11.07$, $P < 0.001$). Post-hoc tests showed that this effect persisted until session 10, following which the rate of door presses stabilized. Rats with NMDA

lesions engaged in significantly less door presses than did sham-lesioned animals ($F_{1,13} = 6.14$, $P < 0.05$). On average, NMDA-lesioned rats pressed the pellet door 4.94 times per minute (SE = $\pm$ 0.66), compared to 6.39 times per minute (SE = $\pm$ 0.99) for sham-lesioned rats. No group x day interaction was found ($F_{25,325} = 1.18$).

Latency of first door press after pellet delivery (log). Log latencies of door presses decreased significantly across test sessions ($F_{25,325} = 2.31$, $P < 0.001$). Post-hoc analysis established that compared to the first test period, log latencies were significantly reduced from session 3 onwards (sessions 3 and 4, 6 to 13, 15 to 19, 21 to 26: $P < 0.01$; sessions 5, 14 and 20: $P < 0.05$) and remained at the same levels subsequently. No effects of lesion group or group x day interaction were observed ($F_{1,13} = 2.01$; $F_{25,325} = 0.74$).

Correlations. To test for the effects of lesion volume on SIP acquisition, Pearson correlation coefficients were calculated for individual lesion volumes and performance on the parameters of SIP. Sham-operated animals (lesion volume = 0) were included in the analysis. It was found that only the average number of door presses per minute were inversely correlated to lesion volume (r = - 0.64, $P < 0.01$), while all other tests were not significant (water intake: r = - 0.27; average number of drinking bouts per minute: r = - 0.32; log latency of first door press after pellet delivery: r = 0.37; log latency of first drinking bout after pellet delivery: r = 0.27). In line with previous results, water intake and the average number of drinking bouts per minute (r = 0.71, $P < 0.01$), as well as water intake and drinking bout lengths (r = 0.58, $P < 0.05$) were significantly correlated. In addition, inverse relationships of water intake and log latencies of drinking bouts (r = - 0.58, $P < 0.05$) and of drinking bout lengths and log latencies (r = - 0.666, $P < 0.01$) were found.

Physiological challenges

Data relating to the responses to hypertonic saline (A) and water deprivation (B) are shown in Fig. 7.5.

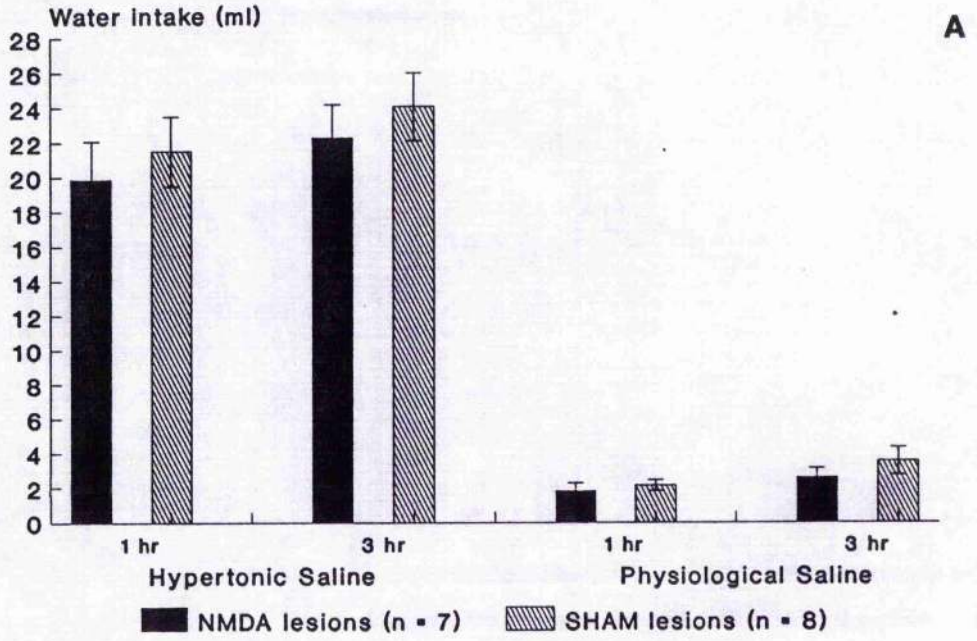
HYPERTONIC SALINE. Repeated measures ANOVA of the data for all animals indicated that rats drank significantly more water in response to an injection of hypertonic saline, than after administration of the same volume of physiological saline after 1 hr ($F_{1,13} = 151.11$, $P < 0.001$) and after 3 hr ($F_{1,13} = 229.07$, $P < 0.001$). Lesion group did not significantly affect drinking 1 hr post-injection ($F_{1,13} = 0.42$) or 3 hr post-injection ($F_{1,13} = 0.73$). No group x dose interactions were observed (1 hr: $F_{1,13} = 0.18$; 3 hr: $F_{1,13} = 0.10$).

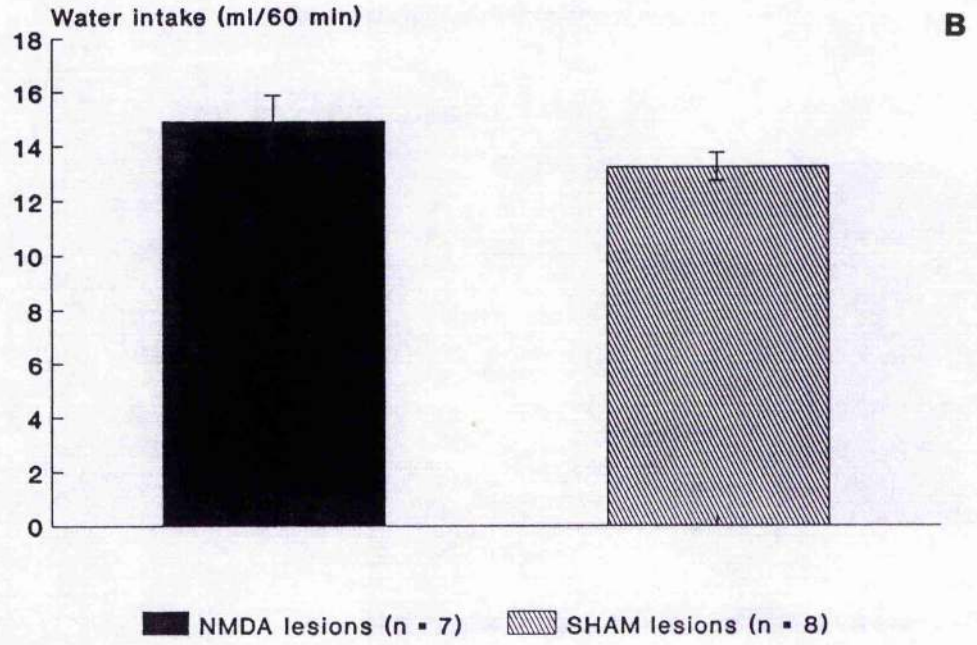
WATER DEPRIVATION. ANOVA with repeated measures showed that NMDA lesions of the NAS did not significantly affect levels of deprivation-induced drinking ($F_{1,13} = 2.35$).

DISCUSSION

In contrast to terminal depletion of DA in the N.Acc., excitotoxic lesions of intrinsic N.Acc. neurones did not disrupt the acquisition of an excessive drinking response to intermittent food reinforcement. Similarly, neurone-specific lesions in the N.Acc. did not interfere with responding to physiological challenges. NMDA-induced lesions resulted in a small but statistically significant deficit in post-operative body weights, although normal recovery of regulatory responses with time was observed in both lesion and control groups. The SIP response pattern in both lesion groups confirmed previous observations (see Experiment 2 in Chapter VI),

Fig. 7.5 Responses to physiological challenges in rats with NMDA or sham lesions in the nucleus accumbens, ± SE. Water intake following (A) systemic administration of hypertonic saline and (B) 23 hr water deprivation.





including significant increases in water intake during test sessions and reductions in log latencies of drinking bouts after sessions 10 or 11, as well as significant decreases in log latencies of door presses at an earlier point in the experiment. These observations indicate that both experimental groups acquired SIP within the normal time course. The rate of door pressing was reduced as well, although this change corresponded with increases in drinking and therefore occurred at a later point in time than was reported in the control experiment. Both the number and the lengths of drinking bouts tended to increase with time, but, in contrast to the control experiment, these observations did not reach statistical significance. In line with the preliminary study, average water intake during the last 5 test sessions was positively correlated with rate of drinking and drinking bout lengths in these sessions. In addition, negative correlations of log latencies of drinking bouts with water intake and drinking bout lengths were observed.

With regard to lesion group differences, Fig. 7.4 suggests that responding on some of the parameters measured during SIP (water intake, the average number of drinking bouts per minute, log latencies of drinking bouts and door presses) may have been impaired in NMDA-lesioned rats, whereas drinking bout lengths appeared to be enhanced. However, due to substantial within-group and within-subject variances none of these effects reached statistical significance. Several explanations can be put forward for the failure of NMDA lesions to disrupt SIP. First, the absence of consistent lesion effects needs to be considered in conjunction with the fact that although recovery was normal, small deficits in food intake and body weights were observed in NMDA-lesioned rats. Since the food deprivation regime during SIP was based on post-operative regulatory behaviour, it may be possible that NMDA-lesioned rats were actually *more* food-

deprived than the control group, resulting in enhanced acquisition of the response. Second, some of the lesion volumes produced by injection of 60 nmol NMDA into the accumbens core may have been too small to affect SIP acquisition. Indeed, an average lesion volume of 33% of accumbens core and shell was induced here, compared with 82% in the previous lesion study (Chapter V). However, the correlation of water intake and lesion size that would have been expected on the basis of this explanation was not observed here. Finally, neurones intrinsic to the N.Acc. may not be involved in the acquisition of excessive drinking in response to intermittent food-reinforcement, so that differential behavioural effects are obtained with terminal-depleting and fibre-sparing lesions in the N.Acc.. Instead, the acquisition of SIP may be mediated by other structures anatomically related to the N.Acc., such as the lateral septum (Taghzouti et al, 1985), hippocampus (Devenport, 1978; Mittleman et al, 1990) or lateral hypothalamus (Winn et al, 1992). It is important to bear in mind that the above structures provide input to distinct aspects of the N.Acc.. Afferents from the hippocampus, for example, project to a well-defined area of the accumbens shell (Kelley and Domesick, 1982); efferents from the accumbens core innervate a region where the entopeduncular nucleus extends into the lateral hypothalamus, while the accumbens shell has more diffuse synapses in the rostrocaudal extent of the lateral hypothalamus (Groenewegen and Russchen, 1984). Thus, due to the pronounced heterogeneity of the N.Acc., more localized neuronal damage in the N.Acc. may affect acquisition of SIP, depending on the projection systems associated with the area affected.

Lesion groups were found to differ significantly only with regard to the average number of door presses per minute, which was attenuated in NMDA-lesioned rats and inversely correlated to lesion volume. This

observation cannot be interpreted as a failure to respond to the food-reinforcement schedule appropriately, since door press latencies were reduced at the same rate in both lesion groups. Similarly, this effect was not the result of a gross motor deficit as levels of locomotor activity tested in photocell cages are unimpaired in rats with excitotoxic N.Acc. lesions (Weissenborn and Winn, in press; see Chapter V). Instead, the reduction in door presses may represent a deficit in the ability to switch appropriately between different activities. With the exception of the drinking response to scheduled reinforcement, excitotoxic N.Acc. lesions induced a response pattern similar to that obtained following DA terminal depletion, i. e., an attenuation in the number of door presses and longer drinking bouts (Robbins and Koob, 1980; Robbins et al, 1983). It is argued that 6-OHDA lesions of the N.Acc. reduce activation and thereby lead to 'behavioural focusing' and the inability to switch between or away from strongly motivated forms of behaviour (Evenden and Carli, 1985; Koob et al, 1978; Robbins and Koob, 1980; Robbins et al, 1983). However, in conjunction with enhanced locomotion following excitotoxic lesions in the N.Acc., it seems more likely that reductions in pellet door presses represent a control deficit of general motor outflow and are secondary to competing locomotor activity.

In summary, NMDA-induced, partial lesions of neurones intrinsic to the N.Acc. did not abolish the response pattern typically associated with SIP acquisition. However, on the basis of the heterogeneity of afferent and efferent projections in the N.Acc., it can be hypothesized that more localized excitotoxic lesions would affect the acquisition of SIP, depending on the N.Acc. area damaged. Excitotoxic lesions in the N.Acc. were shown to attenuate the rate of door pressing and therefore may possibly enhance behavioural focusing or impair the rat's ability to terminate a

specific behaviour. This claim would lend further support to Mogenson's hypothesis of the N.Acc. as a 'limbic-motor' interface (Mogenson et al, 1980). According to the model of the N.Acc. motor system proposed by Swerdlow and Koob (1987a), lesions of neurones intrinsic to the N.Acc. would remove one of the control mechanisms of the motor system, leading to overactivity of descending pathways and a subsequent inability to change firing patterns according to information about the direction of change.

CHAPTER VIII

SCHEDULE-INDUCED POLYDIPSIA (3)

EXPERIMENT 5: *IN VIVO* VOLTAMMETRIC RECORDINGS OF DOPAMINE LEVELS IN THE NUCLEUS ACCUMBENS DURING SCHEDULE-INDUCED POLYDIPSIA

In previous chapters, the role of the N.Acc. in mediating motivated responding was examined by stimulating or blocking dopaminergic activity in the nucleus, or by selectively lesioning intrinsic neurones. However, a causal association between N.Acc. DA and behaviour can only be assumed on the basis of these manipulations. More conclusive evidence about the relationship between neurotransmitter activity and behavioural responding could be gained from techniques allowing one to monitor changes in transmitter efflux in the behaving animal, such as *in vivo* voltammetry (Adams, 1978; Lane et al, 1979; Stamford, 1989).

The main purpose of the present study was to assess the relationship between extracellular DA levels in the N.Acc. and acquisition and emission of SIP using rapid-scan semidifferential voltammetry. To be able to dissociate possible results from excessive water intake *per se*, an additional experiment was conducted measuring DA efflux in the N.Acc. during deprivation-induced drinking. Since the experiments were carried out during a research visit to Dr A.G. Phillips FRSC and Dr C.D. Blaha at the University of British Columbia, Vancouver (Canada), a detailed description of the methodology will be given, although most procedures do not differ greatly from those outlined previously.

METHODS

Animals

The subjects were 5 male Long Evans hooded rats (Charles River Canada, Inc.) housed individually at 22°C under a 12 hr light/dark cycle (lights on 9.00 a.m.). All tests were carried out during the light period of the cycle. Unless otherwise stated, all rats had free access to tap water and standard lab chow pellets.

Surgical procedures

Rats were anesthetized with 100 mg/kg ketamine hydrochloride i.p. and 20 mg/kg xylazine i.p. and if necessary given supplemental injections of ketamine hydrochloride to maintain a constant level of anaesthesia. Mean body weight at the time of surgery was 333.2 g (SD = $\pm$ 40.3). Stearate-modified graphite paste recording electrodes were constructed from Teflon insulated stainless steel wire with a tip diameter of 200 *u*m. 1.5 g of graphite powder were thoroughly mixed with a mixture of 100 mg of stearic acid (99.9% purity) dissolved in 1 ml of liquid paraffin warmed to approximately 40° (Blaha and Lane, 1983). As outlined previously (see Chapter III, page 55) these electrodes allow the *in vivo* measurement of changes in DA efflux without interference from other oxidizable compounds in brain extracellular fluid. Recording electrodes were implanted bilaterally into the N.Acc.. The coordinates were 2.0 mm anterior to bregma, 1.5 mm lateral to midline, and 6.5 mm below dura, with level skull (Paxinos and Watson, 1986). An Ag/AgCl (0.9% NaCl) reference electrode was implanted into cortical tissue and a stainless-steel auxiliary electrode attached to one of the skullscrews. An example of a typical voltammetric implant is shown in Fig. 8.1.

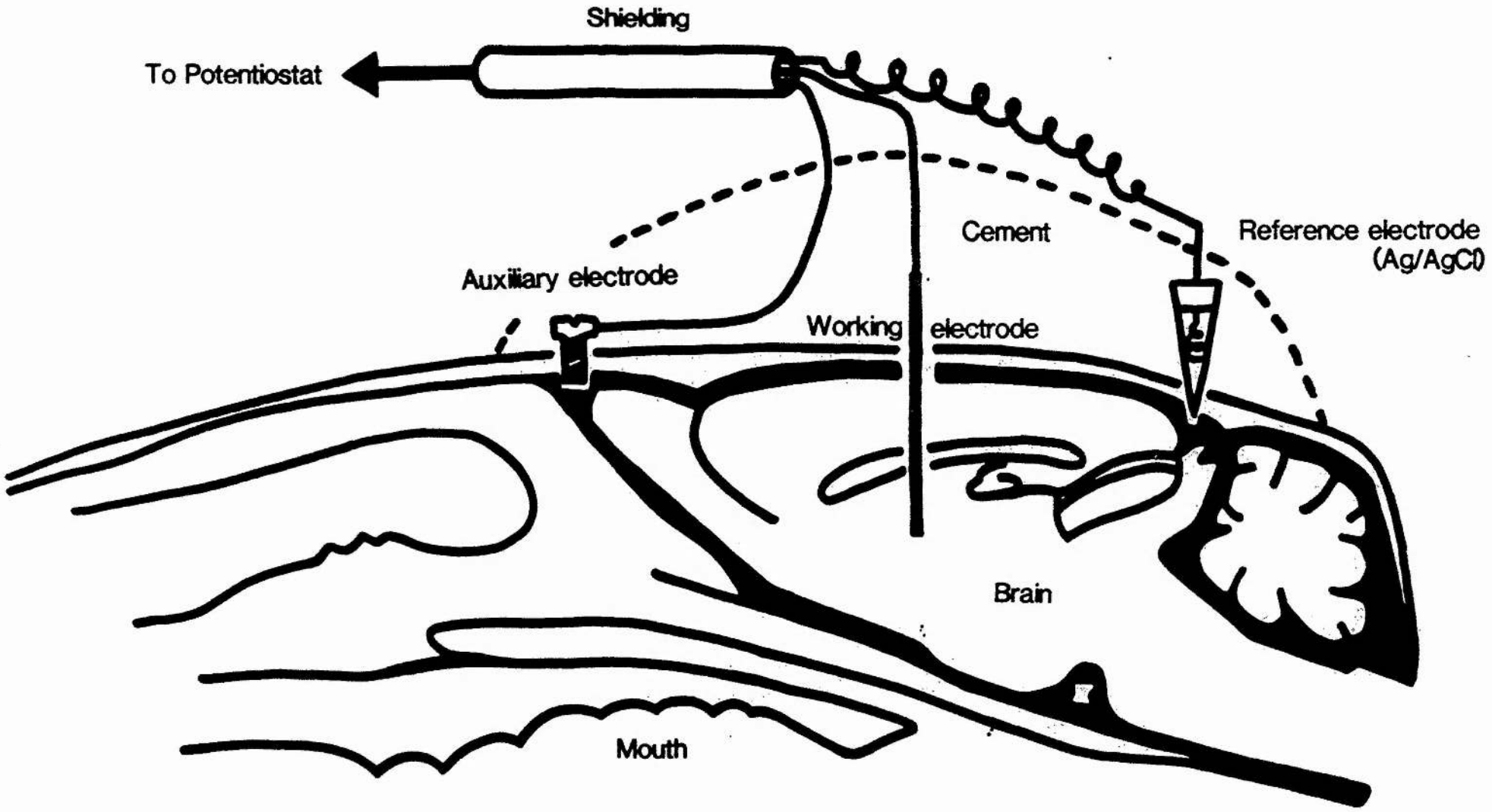


Fig. 8.1 Schematic representation of an *in vivo* voltammetric experiment (Stamford, 1989).

Electrochemical recordings

Extracellular DA concentration were measured with rapid-scan (sawtooth) semidifferential voltammetric procedures applying voltage ramps from -50 mV to +600 mV at a 200 mV/s scan rate to either the left or the right working electrode every 180 s. Semi-differential voltammetry was used since the peaks obtained with this technique are more defined and more clearly separated than those obtained with linear scanning methods. Voltage application, amplification, A/D translation and storage of the current were controlled by a custom-made electronic device; the resulting data were analyzed off-line on a PC.

Schedule-induced polydipsia

On the basis of previous experiments (see Chapter VI) indicating that the threshold levels of water intake predicting SIP acquisition is approximately 5 ml during a 30 min FI 60 food reinforcement session, the onset of SIP was defined here as the first day on which a rat drank more than 5 ml of water during the 30 min SIP period.

At least one week prior to surgery, rats were food-deprived to 80% of their free-feeding body weights and maintained at this level throughout the experiment. SIP testing began after rats (n = 5) had regained 80% of their free-feeding body weights after surgery. Tests were conducted in 4 identical perspex operant boxes (23 l x 29 w x 20 h cm) with grid floors and a food tray located in the middle of the short wall of each box. A graduated plastic burette and aluminium drinking spout was mounted to the outside of the cage, with the spout protruding into the cage through a hole 4 cm to the side of the food tray and 5 cm above the grid floor. All 4 test cages were contained within sound-attenuating boxes and ventilation fans masked any outside noise. Two operant boxes were additionally

placed inside a faradaic wire-mesh cage connected to the electrochemical instrument.

Tests were carried out twice daily (separated by at least 4 hr) to ensure fast acquisition of schedule-induced drinking. Each 60 min session consisted of a 15 min baseline period, a 30 min SIP test during which rats received a food pellet (Bioserv Inc., 45 mg) every 60 sec and had free access to tap water, and a 15 min post-test period. The delivery of food pellets was controlled by an INI computer using the Manx programming language. Water intake was read from the burette. Changes in extracellular DA levels were monitored at the same time each day either during the morning or the evening session. Testing of individual animals was discontinued after the rat had consistently displayed schedule-induced drinking over 5 consecutive days (i.e., water intake $\geq$ 5 ml during a 30 min test session).

The quality of the electrochemical signal permitted 3 rats to be tested further in a 'no water' condition one day after the last SIP session. Animals underwent the standard procedure, with the exception that water was not made available during presentation of food pellets.

Deprivation-induced drinking

One week after surgery, rats (n = 2) were habituated to the test box for 1 hr on 2 consecutive days with water freely available. After the second habituation period, water bottles were removed from home cages. Following a 23 hr deprivation period, rats were placed into test boxes and extracellular DA levels were monitored during 30 min baseline, 30 min access to water and 30 min post-drinking periods.

Calibration of dopamine oxidation potentials

Responses of brain-treated stearate-modified electrodes *in vitro* were evaluated in solutions of 0.5, 1, 2 and 4 *u*mol DA and 1, 2, 4, 8 and 10 *u*mol serotonin (5-HT), using the same parameters used *in vivo*. Oxidation potentials of 5-HT were measured *in vitro* to rule out a confounding of results by serotonergic projections to the N.Acc.. All solutions were prepared in 0.1 M phosphate-buffered saline (pH 7.4) and were deoxygenated with prepurified nitrogen prior to and during use.

Statistical analysis

Extracellular DA concentrations in the N.Acc. were measured as changes in absolute oxidation peak heights calculated from *in vivo* rapid-scan voltammetric recordings. For statistical purposes, each test session in Experiment 1 was divided into 4 15 min intervals: pre-SIP, SIP I, SIP II and post-SIP. Repeated measures ANOVA's were carried out on levels of water intake and absolute DA peak heights during test sessions on the 3 days preceding and the 3 days following the onset of SIP (as defined above). ANOVA with repeated measures was carried out on DA peak heights during the 3 30 min test periods in the water-deprivation experiment. Newman-Keuls tests were used for post-hoc comparison of individual means throughout, given ANOVA results were significant.

Histology

On completion of behavioural testing, rats were dispatched in a CO_2 chamber, brains were quickly removed and postfixed in formalin solution. 40 *u*m sections every 200 *u*m were cut on a bench microtome and Nissl substance stained with cresyl violet. Electrode placements were verified using a Leitz Diaplan microscope.

RESULTS

Histology

Fig. 8.2 confirms that all electrodes from which recordings were taken were correctly placed within the accumbens core.

Calibration of dopamine oxidation potentials

Fig. 8.3 illustrates the oxidation potentials of different concentrations of DA (A) and 5-HT (B) at stearate-modified electrodes measured *in vitro* using rapid-scan semi-differential voltammetry (200 mV/s). Comparison with typical oxidation potentials of DA measured *in vivo* using the same parameters confirmed that the DA oxidation peak occurs at approximately +200 mV in both situations. By contrast, the maximum peak height of 5-HT was found to occur at a more positive value of approximately +350 mV.

Schedule-induced polydipsia

SIP onset was defined post-hoc as the first occasion on which rats drank 5 ml of water or more during a single 30 min SIP session, provided that the water intake was 5 ml or more during all consecutive sessions. Fig. 8.4 shows the average oxidation peak heights (amps times 10e-8) of N.Acc. DA before, during and after SIP sessions at different stages of acquisition (before/after). Not surprisingly, ANOVA with repeated measures showed that rats drank significantly more water after than before SIP onset ($F_{1,4} = 34.25$, $P < 0.01$). On average, rats consumed 1.73 ml of water (SE = $\pm$ 0.47) during a 30 min SIP session before the onset of SIP, compared to 10.6 ml (SE = $\pm$ 1.3) after they had developed the behaviour. ANOVA with repeated measures of the absolute DA peak

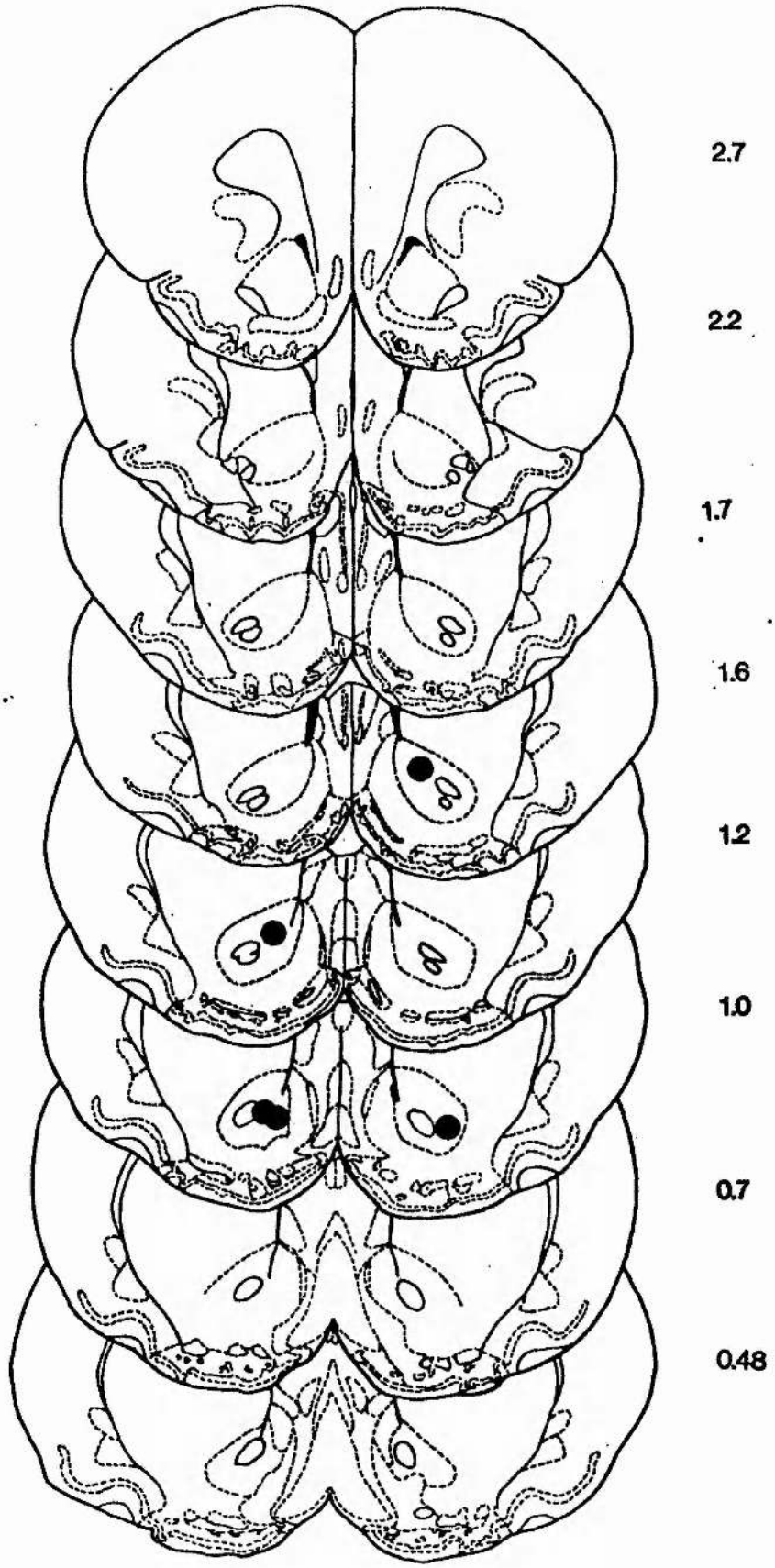
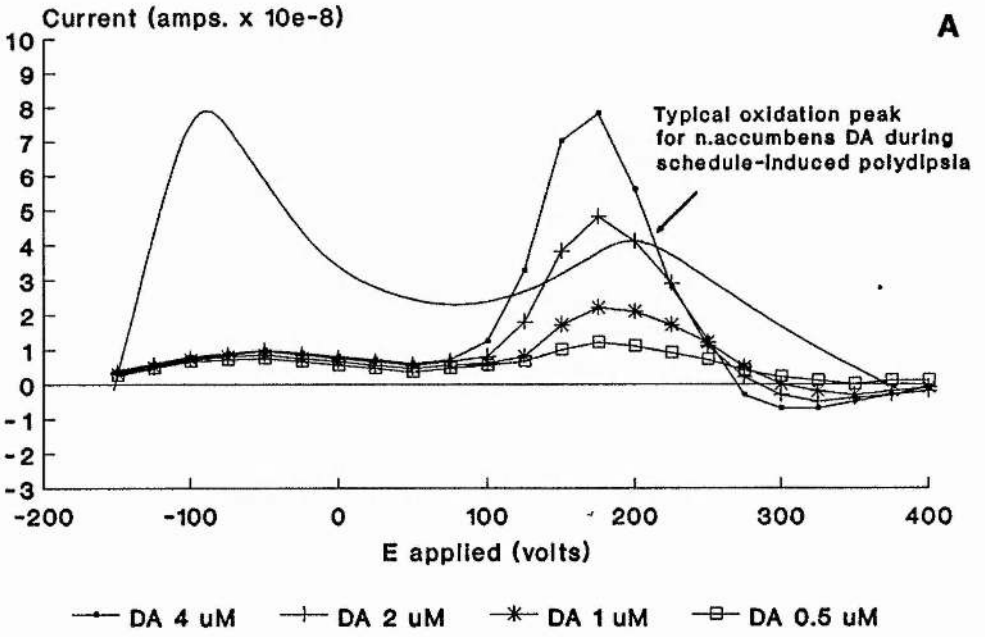
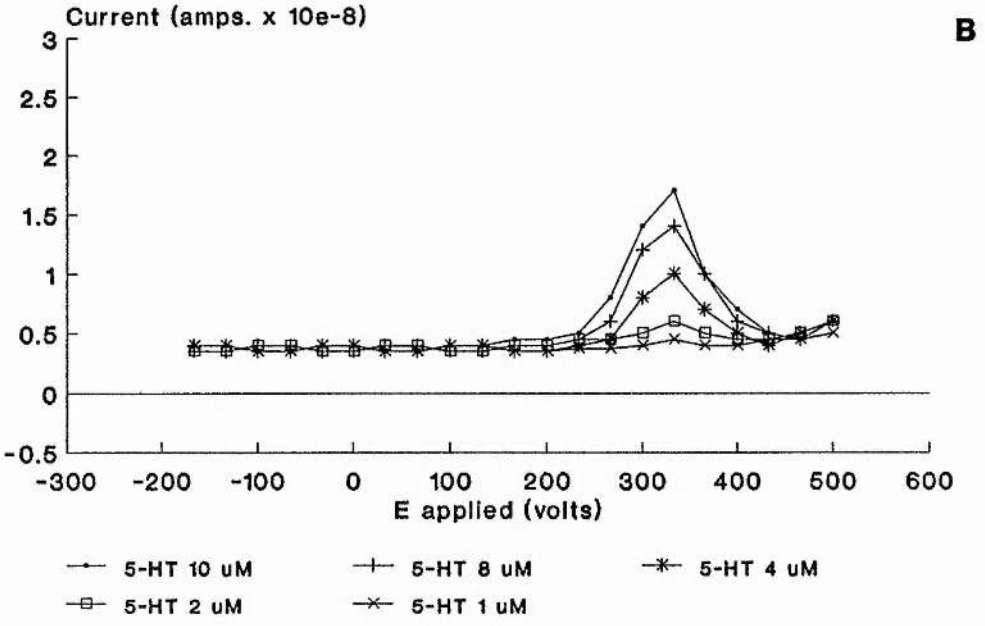


Fig. 8.2 Electrode placements in the nucleus accumbens. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicated the distance from bregma in mm.

Fig. 8.3 Oxidation potentials of different concentrations of dopamine (DA) and serotonin (5-HT) at stearate-modified electrodes measured *in vitro* using rapid-scan semi-differential voltammetry (200 mV/sec).





heights before, during and after 30 min SIP sessions on the 3 days preceding and the 3 days following the onset of SIP indicated significant differences between the 4 15 min intervals ($F_{3,12} = 39.13$, $P < 0.001$) and differences between sweeps within intervals ($F_{4,16} = 15.91$, $P < 0.001$). However, no changes in DA peak heights were observed across days ($F_{5,20} = 1.02$). Post-hoc testing showed that peak heights increased during the session and were significantly greater during the SIP I ($P < 0.001$), SIP II ($P < 0.001$) and post-SIP ($P < 0.001$) intervals compared to the pre-SIP recordings. The same pattern was obtained looking at individual sweeps, where peak heights for sweeps 2 to 5 were all greater than those for sweep 1 ($P < 0.001$). Repeated measures ANOVA's carried out for individual days show that this increase in peak height did not occur on day 3 before the onset of SIP but was consistently present subsequently. On average, DA peak heights increased by 24.17% (SE = $\pm$ 3.1), or 11.0 nA (SE = $\pm$ 1.20) during the post-SIP intervals, compared to baseline levels during the pre-SIP intervals (100%).

Separate ANOVA's with repeated measures were carried out for the 3 rats tested in the 'no water' condition where water was not available during scheduled pellet delivery. DA peak heights were found to increase significantly within each session ($F_{3,6} = 41.58$, $P < 0.001$), as well as within intervals ($F_{4,8} = 8.99$, $P < 0.01$). The average increase in DA peak heights during the post-SIP interval was 25.09% (SE = $\pm$ 4.27), or 11.0 nA (SE = $\pm$ 1.50). Again, no differences were found between days ($F_{6,12} = 1.14$). Similarly, ANOVA with repeated measures comparing the third SIP day to the 'no water' condition showed significant within-session increases in peak height ($F_{3,6} = 6.02$, $P < 0.05$) but no differences between the two days ($F_{1,2} = 0.84$).

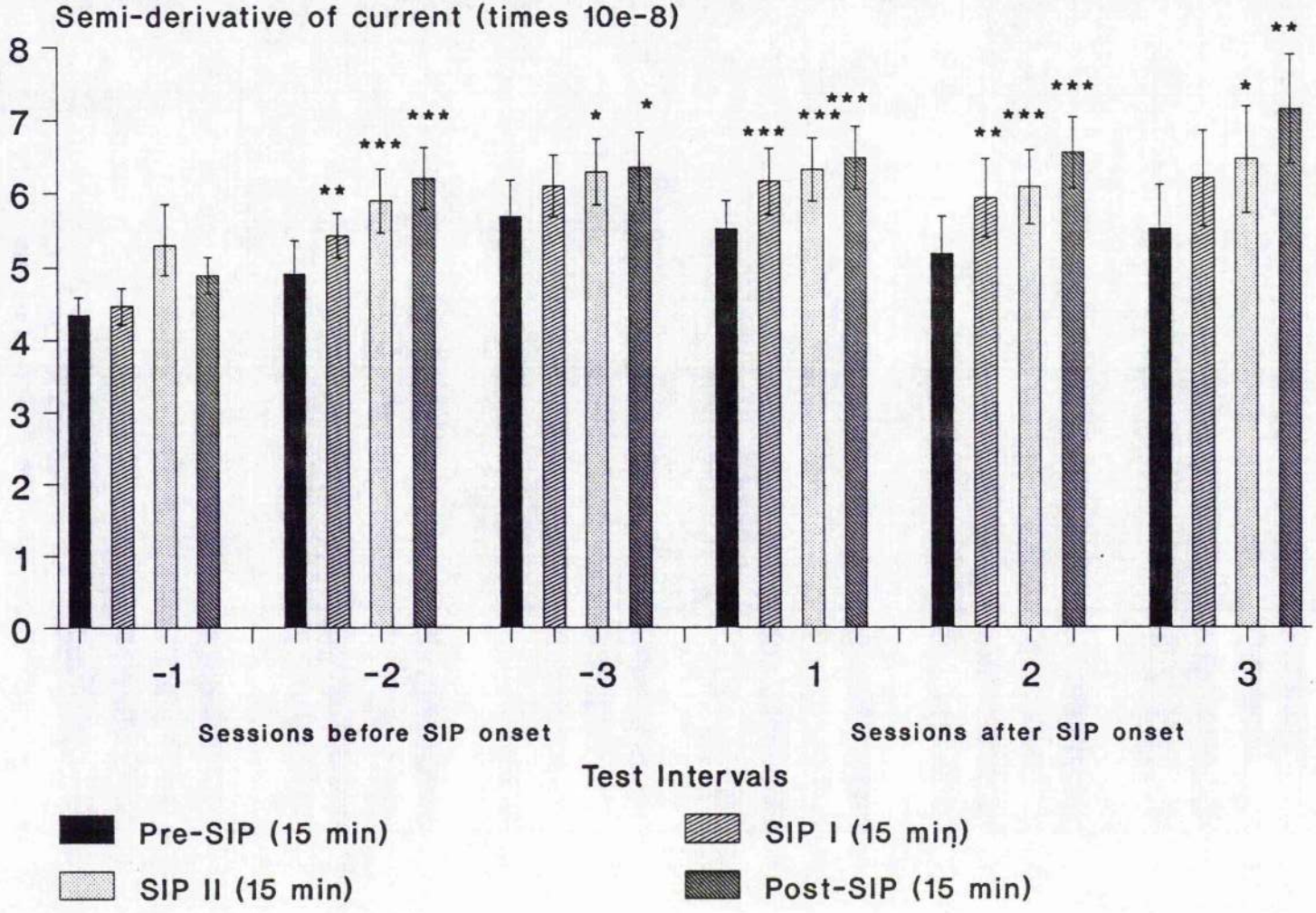


Fig. 8.4 Oxidation peak heights of nucleus accumbens dopamine before and after the onset of schedule-induced polydipsia (SIP), ± SE. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Water deprivation

Fig. 8.5 shows average oxidation peak heights (amps times 10e-8) of N.Acc. DA before, during and after access to water following a 23 hr period of water deprivation. After 23 hr water deprivation, rats consumed 9.5 ml (SE = $\pm$ 0.71) during 30 min free access to water. Repeated measures ANOVA of 3 30 min intervals showed that the differences in DA peak heights between as well as within intervals were significant ($F_{2,2}$ = 44.66, $P < 0.05$; $F_{9,9}$ = 6.08, $P < 0.01$). Post-hoc testing indicated an increase in peak heights during the session, with recordings being significantly greater after access to water than before ($P < 0.05$). Peak heights were increased by 50.51% (SE = $\pm$ 15.83) or 10.4 nA (SE = $\pm$ 3.30) on average in the post-water interval compared to the pre-water baseline. However, no statistical differences were observed whether or not rats had access to water during deprivation ($F_{1,1}$ = 3.58).

DISCUSSION

The electrochemical data presented here show increases in extracellular DA levels in the N.Acc. during the course of a SIP session while, contrary to what had been expected, changes were not observed comparing DA efflux before and after the acquisition of SIP. Similarly, when water was not available to polydipsic rats during scheduled food-reinforcement, extracellular DA levels in the N.Acc. increased during test sessions but did not significantly differ from those recorded when the animal was able to drink. Results of a second experiment showed that N.Acc. DA efflux increased following water-deprivation, regardless of whether or not rats had access to water during the test session.

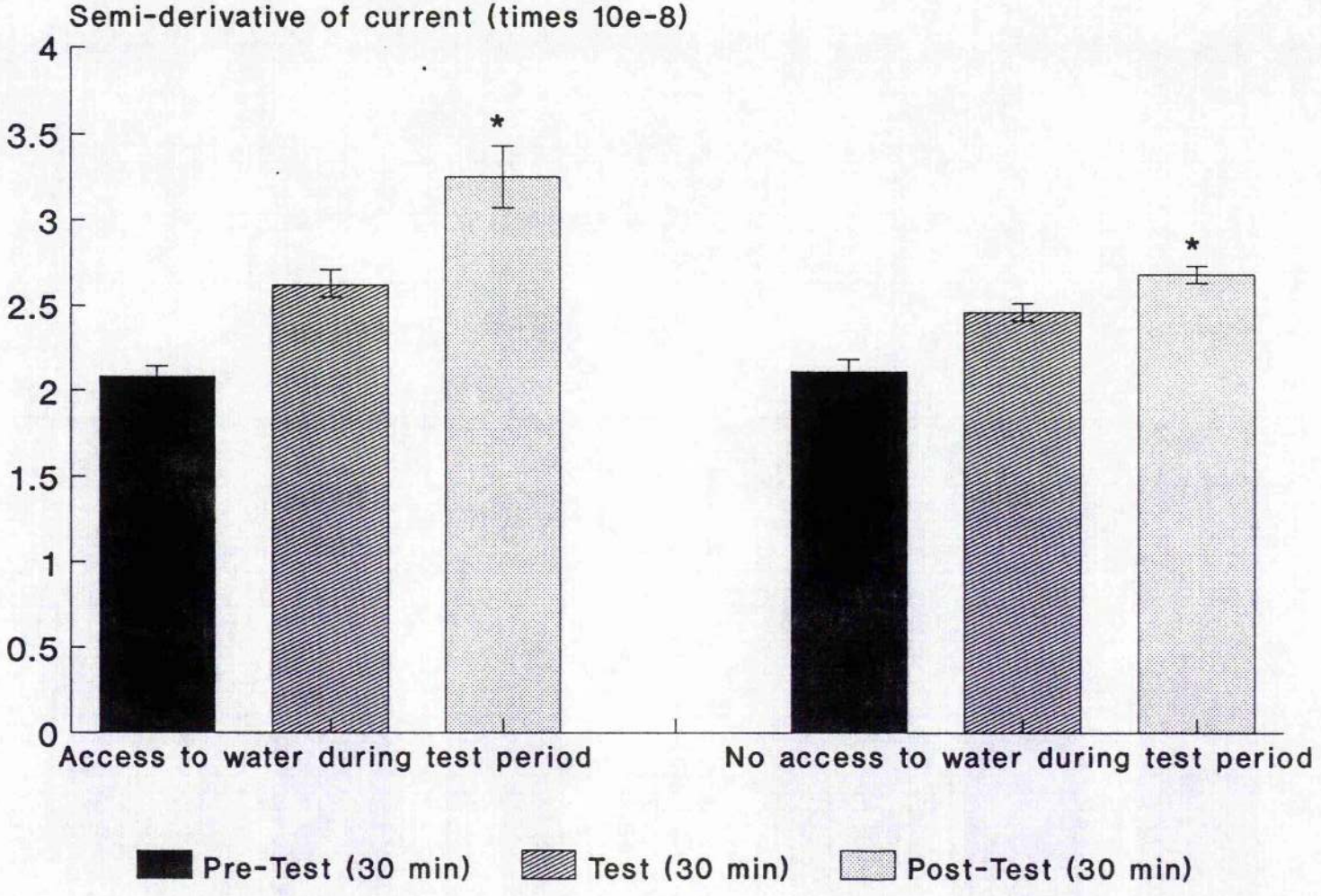


Fig. 8.5 Oxidation peak heights of nucleus accumbens dopamine before, during and after access to water following a 23 hr period of deprivation, $\pm$ SE. * = $P < 0.05$.

The fact that extracellular N.Acc. DA levels increased during individual SIP sessions before as well as after polydipsia was established suggests that DA efflux in the N.Acc. and excessive drinking are not directly related. In the light of the data presented here, it is necessary to re-evaluate claims that N.Acc. DA is one of the major neuroanatomical substrates involved in the mediation of SIP. In conjunction with previous experiments reporting blockade of SIP acquisition following 6-OHDA lesions of the N.Acc. (Koob et al, 1978; Mittleman et al, 1990; Robbins and Koob, 1980; Robbins et al, 1983; Wallace et al, 1983) and attenuation following administration of the dopamine receptor agonists *d*-amphetamine and apomorphine (Robbins et al, 1983), the present series of experiments suggest that although DA in the N.Acc. may be necessary for drinking to occur initially in response to intermittent food-reinforcement, it is not directly involved in the mediation of excessive drinking during sessions. Moreover, the data presented here lead to the hypothesis that different substrates are involved in the mediation of SIP. Dopaminergic activity in the N.Acc. may be necessary for drinking to occur initially but the excessive water intake in response to scheduled food-reinforcement itself is unrelated to DA efflux. This finding supports the data obtained in a microinjection experiment reported in Chapter VI, where increased concentrations of DA in the N.Acc. did not significantly affect the expression of SIP. Thus, rather than examining isolated areas of brain, more valuable information may be gained by looking at interactions of different neuroanatomical structures typically associated with SIP, such as the lateral hypothalamus (Winn et al, 1992), hippocampus (Devenport, 1978; Mittleman et al, 1990) and lateral septum (Taghzouti et al, 1985), all of which are anatomically related to the N.Acc.. Electrochemical methods allow recordings from multiple sites or could be used in

conjunction with lesion techniques to investigate interactive processes involved in the mediation of schedule-induced behaviours.

The DA oxidation peaks obtained using rapid-scan semidifferential voltammetry were clearly distinguishable, without interference from 5-HT and dopaminergic or serotonergic metabolites. *In vitro* calibration of DA oxidation potentials at stearate-modified electrodes measured with the same parameters used in the *in vivo* experiments confirmed that rapid-scan semidifferential voltammetry is a valid tool for assessing changes in DA oxidation peak heights both within and across test sessions. These observations are supported by previous data indicating that stearate-modified electrodes selectively measure extracellular levels of DA *in vitro*, in brain homogenate solutions and *in vivo* in striatal tissue without interference from DOPAC or AA (Blaha and Lane, 1983; Blaha and Jung, 1991; Broderick, 1989; Lane et al, 1987).

Significant within-session increases in DA efflux were consistently observed throughout SIP sessions and during deprivation-induced drinking. These results appear to be supported by recent reports of increases in DA release in the N.Acc. during drinking in thirsty rats measured using microdialysis (Young et al, 1992). The authors further found a temporal delay in maximum DA efflux which typically occurred 10-20 min after the maximum rate of licking; a similar temporal delay might explain the within-session increases in extracellular DA observed in the present experiment. However, since enhanced DA release occurred even if water was not presented during SIP or deprivation-induced drinking sessions it seems unlikely that enhanced DA efflux in the N.Acc. before and after development of SIP is related to drinking. An alternative interpretation of the data obtained here is based on previous reports of an involvement of N.Acc. DA in behavioural switching (Evenden and Carli,

1985; Robbins and Koob, 1980), and enhanced switching in response to dopaminergic stimulation by *d*-amphetamine (Evenden and Robbins, 1983). Thus, baseline levels of DA may be necessary for drinking to occur initially in response to intermittent food-reinforcement. By contrast, elevated levels of extracellular DA may reflect the animal's need to change between different activities in a situation characterized by high levels of motivation that possibly decline in the course of an experimental session.

In summary, *in vivo* voltammetric recordings of extracellular DA in the N.Acc. indicate that a straightforward causal relationship between N.Acc. DA and SIP does not exist, although extracellular DA in the nucleus may be necessary for drinking to occur initially. Enhanced DA efflux may be involved in mediating the need to switch between behaviours. In conjunction with the microinjection data presented in Chapter VI, the series of experiments presented here suggest that it is not possible to identify a single neuronal correlate of adjunctive behaviours; instead, investigation of interactive processes between anatomically related substances seems to be a more constructive approach to the question.

CHAPTER IX

CONDITIONED PLACE-PREFERENCE

NMDA LESIONS IN THE NUCLEUS ACCUMBENS AND AMPHETAMINE-INDUCED PLACE PREFERENCE

Dopaminergic projections to the ventral striatum have been identified as important neuronal correlates for the mediation of place preference conditioning which is regarded as an indicator of the rewarding effects of drugs of abuse. CPP is reliably induced by intra-accumbens injections of psychostimulant drugs such as *d*-amphetamine and cocaine (Aulisi and Hoebel, 1983; Carr and White, 1983, 1986), as well as by central injections of morphine and heroin into the N.Acc. or VTA (Bozarth and Wise, 1981; Phillips and LePiane, 1980; Spyraki et al, 1983; van der Kooy et al, 1982). Conversely, amphetamine and heroin CPP is blocked by DA receptor antagonists or dopaminergic depletion of N.Acc. terminal fields using 6-OHDA (Aulisi and Hoebel, 1983; Spyraki et al, 1982a, 1983). Other brain sites associated with the mediation of reinforcement and reward include the basolateral amygdala (Everitt et al, 1991), ventral pallidum (Hubner and Koob, 1990) and PPTg (Bechara and van der Kooy, 1989). Several studies suggest that different mechanisms may be activated during the processing of information about different rewarding drugs (Carr et al, 1989; Pettit et al, 1984; Spyraki et al, 1982b), and during the acquisition and expression of conditioned responses (Bechara and van der Kooy, 1989; Brown and Fibiger, 1992). The following experiment aimed to investigate the effects of fibre-sparing excitotoxic lesions in the N.Acc. on the acquisition of amphetamine-induced CPP.

METHODS

Surgical procedures

At 229.8 g average body weight (SD = $\pm$ 9.55), 24 rats received bilateral simultaneous infusions of 1 *u*l 60 nmol NMDA (n = 12) or 1 *u*l phosphate buffer vehicle (n = 12) into the N.Acc.. Body weight, food and water intake were recorded daily for 1 week before and 3 weeks after surgery.

Conditioned place preference

Following 10 days of post-operative recovery, rats were trained and tested in a rectangular (60 h x 100 w x 30 l cm) wooden box subdivided into 3 compartments, 2 measuring 60 h x 80 w x 30 l and 1 60 h x 20 w x 30 l cm, with clear Perspex fronts and covered by metal lids. For clarification, a schematic drawing of the test apparatus is shown in Fig. 9.1. The 2 large end-compartments were painted in black or white and separated from the small grey choice area by appropriately coloured metal guillotine doors (12 x 12 cm). In each of the larger compartments, transition lines running parallel to the short sides of the test box divided the floor area into 3 equal parts. Appropriately shaded screens (18 x 14 cm) were placed 10 cm in front of the doors to prevent rats from visually monitoring the areas from the grey compartment. To differentiate the compartments with regard to tactile and olfactory, as well as visual cues, the black compartment had a metal grid floor and a few drops of vinegar (1 ml 2% acetic acid) were placed on the floor and walls, while the white compartment had a wire mesh floor and neutral odour. The grey compartment had a grid floor and neutral odour.

Behavioural testing consisted of three consecutive main phases: a

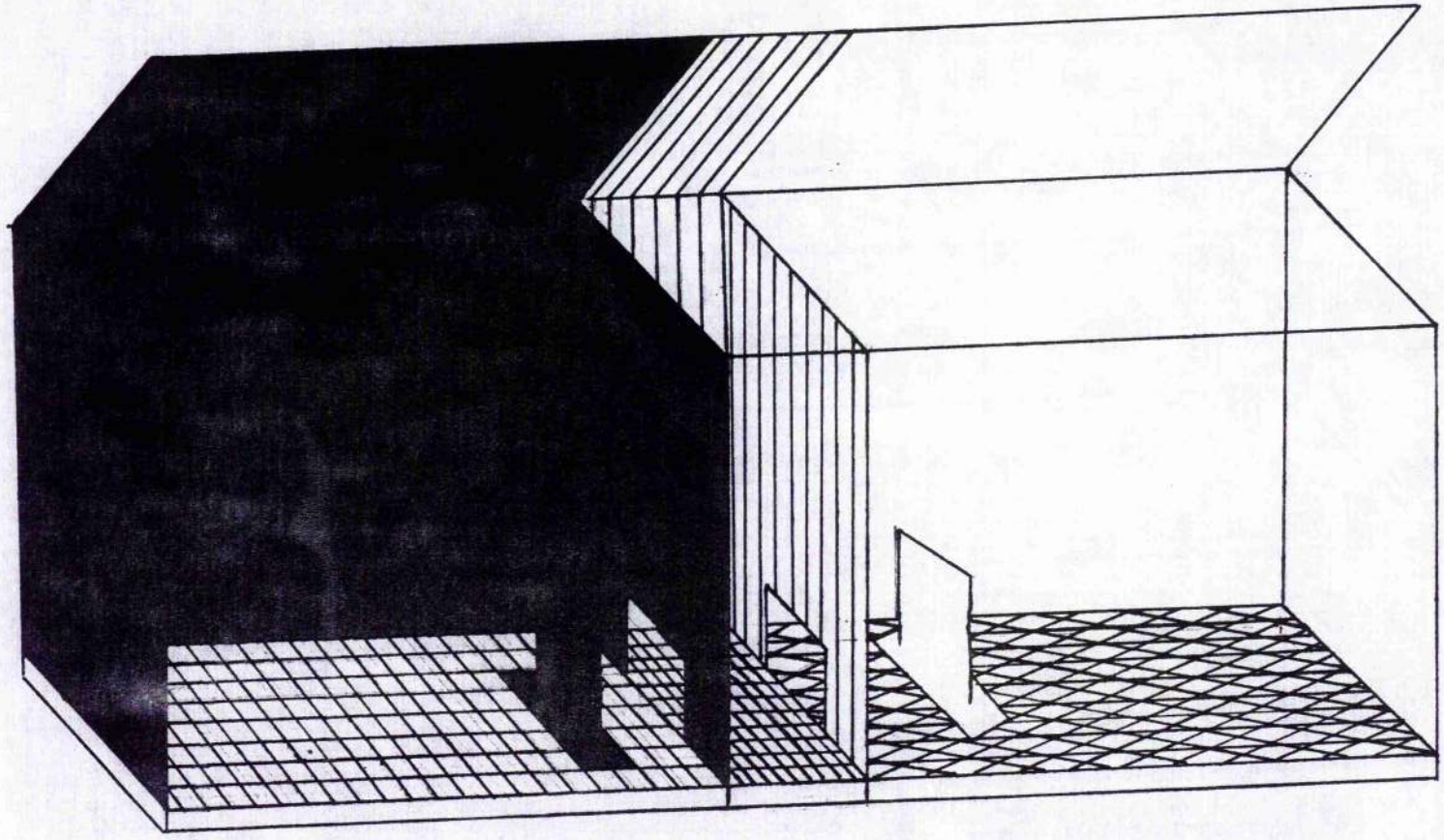


Fig. 9.1 Schematic representation of the conditioned place-preference apparatus.

pre-exposure phase (3 days), a conditioning phase (8 days), and a test phase (1 day).

Pre-exposure phase. Every day, each animal was placed in the grey compartment for 1 min, following which the guillotine doors were raised and the rat was allowed access to the entire test box for a further 15 min. On the third day, preference for the black or white compartment was assessed by recording the time spent in each of the two large compartments during a 15 min period. In addition, transitions between the 3 areas within compartments, as well as rearing behaviour were recorded as indicators of exploratory activity. A 'transition' was recorded when the entire head, shoulders and both forepaws of the rat had crossed a transition line. A 'rear' was recorded when the animal moved from a stance with all four paws on the floor to one where the body axis was raised above a 45^{o} angle to the floor. The data were recorded manually, with the 15 min test subdivided into 10 sec time-bins. On the basis of this initial preference test, rats were assigned to 2 counterbalanced groups for conditioning, matched for type of lesion (NMDA or sham) and time spent on each side.

Conditioning phase. During this phase, place preference was conditioned by compartment-specific administration of *d*-amphetamine. The compartment in which rats were placed after injection of drug was referred to as the 'paired' side of the apparatus, while administration of saline was followed by exposure to the 'unpaired' compartment. On the even-numbered days (2, 4, 6 and 8), each rat received an injection of 1.5 mg/kg *d*-amphetamine i.p. and was placed in the assigned compartment for 30 min. On alternate days (1, 3, 5 and 7), rats were restricted to the 'unpaired' side of the apparatus for the same length of time after administration of physiological saline. The order in which rats were exposed to the test box was reversed after every 2 days.

Test phase. A 15 min preference test (during which rats had access to the entire test box) identical to the one described in the pre-exposure phase was carried out to assess post-conditioning preference.

RESULTS

Histology

Analysis of cresyl violet stained coronal sections showed an inaccurate lesion site in one animal which was excluded from statistical analysis. All of the remaining 11 rats had sustained at least some damage to the accumbens core and shell, while other areas were not affected. The smallest and largest lesions obtained by infusion of 60 nmol NMDA into the N.Acc. are outlined in Fig. 9.2. Average lesion volume was 105.64% (SE = $\pm$ 10.60) of the accumbens core area and 59.33% (SE = $\pm$ 5.95) of the accumbens core and shell.

Normal regulatory behaviour

Fig. 9.3 shows body weight, food and water intake measured 1 week before and 3 weeks after surgery. ANOVA with repeated measures indicated that pre-operative regulatory behaviour did not differ between groups (body weight: $F_{1,21} = 0.1$; food intake: $F_{1,21} = 1.24$; water intake: $F_{1,21} = 0.12$). Repeated measures ANOVA of post-operative regulatory responses revealed a significant difference in body weight, food and water intake across weeks (body weight: $F_{2,42} = 114.55$, $P < 0.001$; food intake: $F_{2,42} = 21.67$, $P < 0.001$; water intake: $F_{2,42} = 24.01$, $P < 0.001$). Post-hoc testing showed that all regulatory responses were significantly enhanced in post-operative weeks 2 and 3, compared to week 1 (body weights: $P < 0.001$; food intake: $P < 0.001$; water intake:

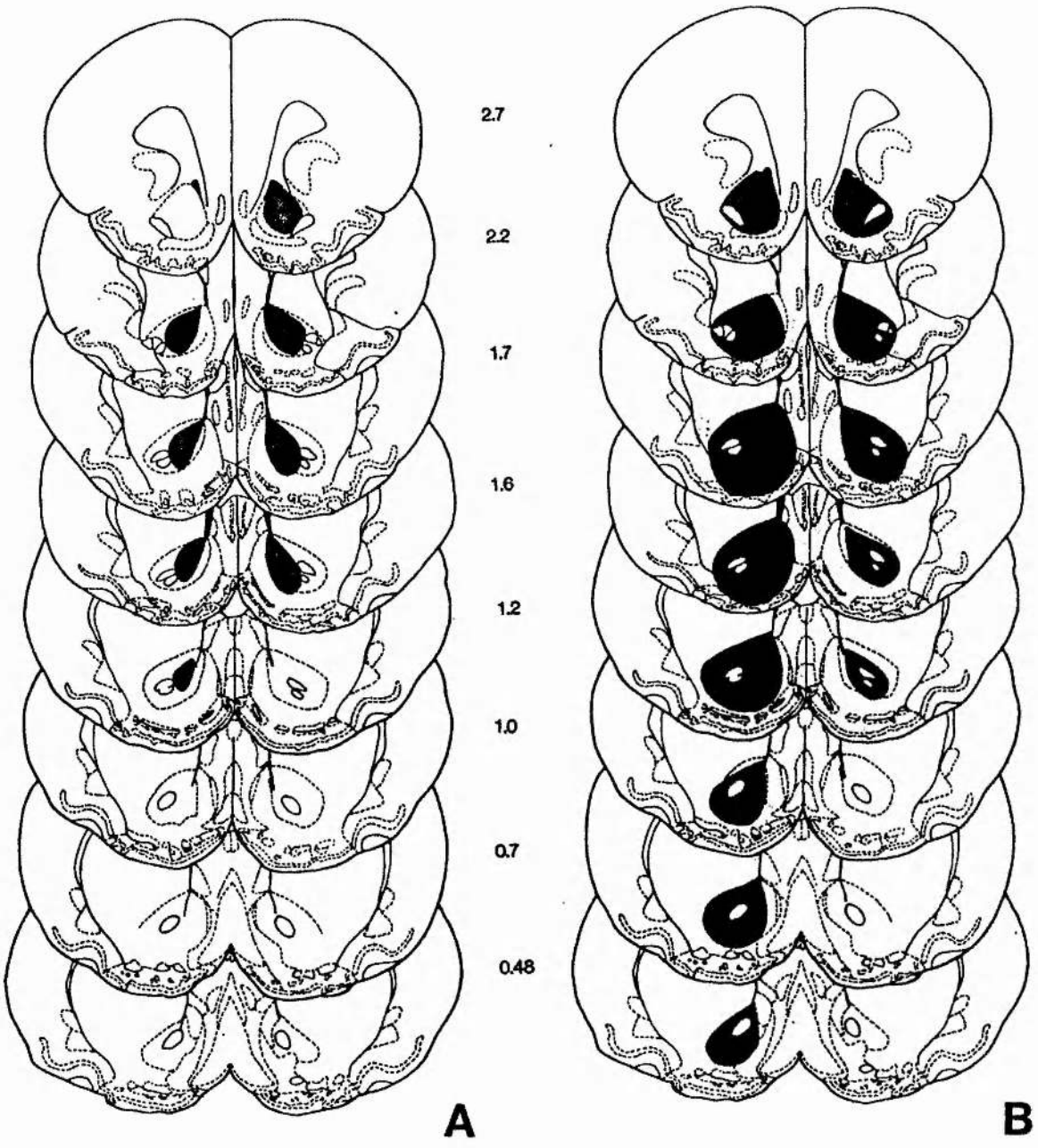
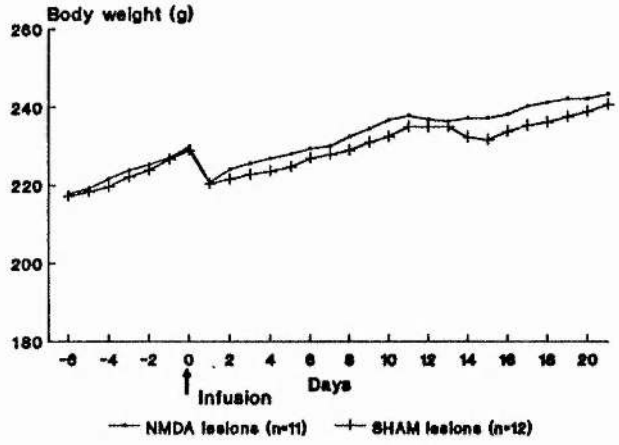
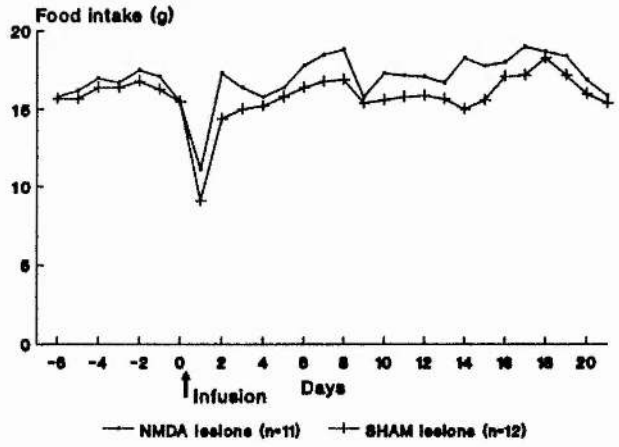
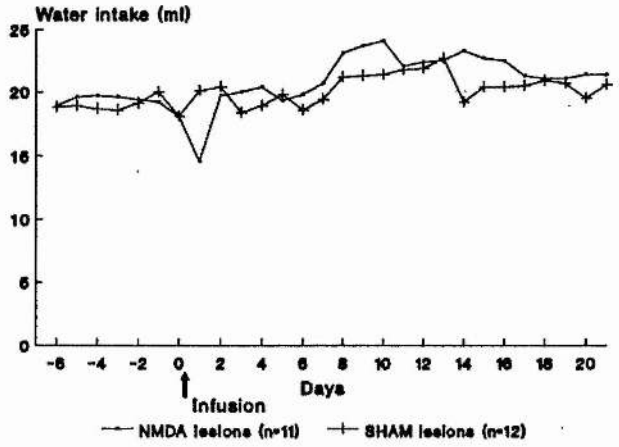


Fig. 9.2 Smallest (A) and largest (B) lesions following infusion of 60 nmol NMDA into the nucleus accumbens. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm.

Fig. 9.3 Average pre-and post-operative body weights, food and water intake in rats with NMDA or sham lesions in the nucleus accumbens.





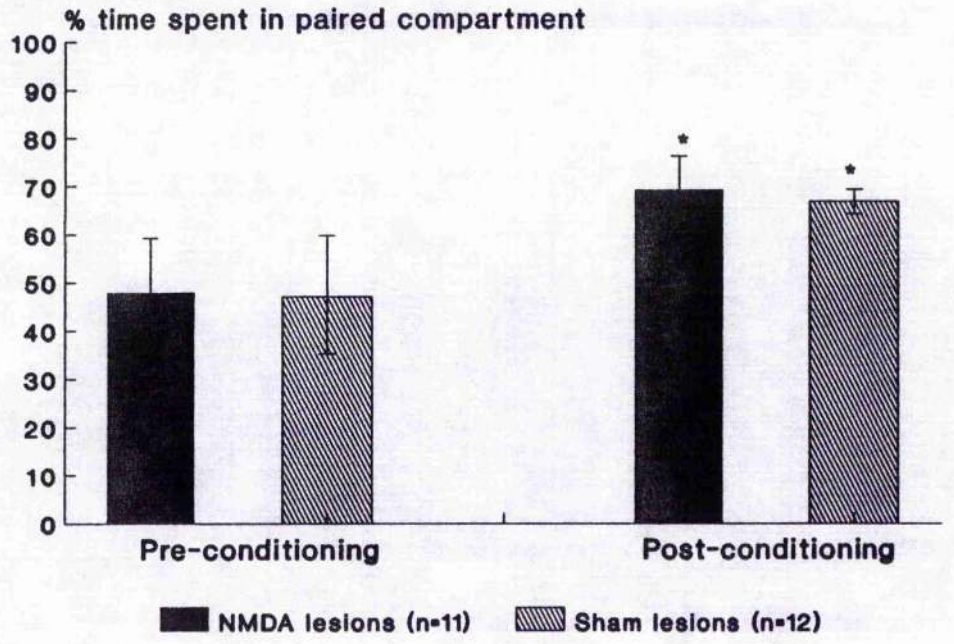


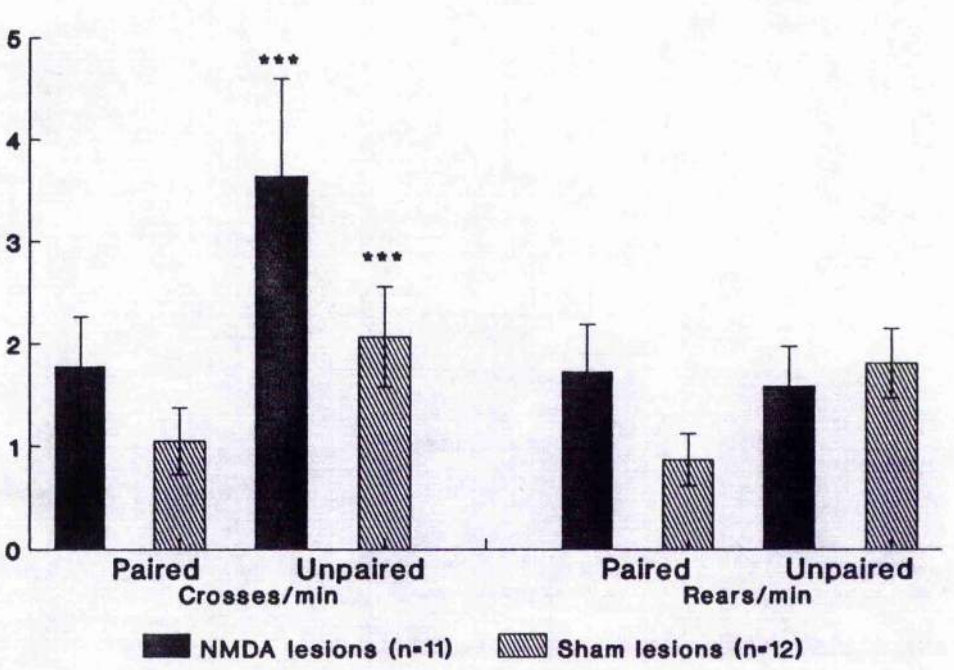
$wP < 0.001$). No group x week interactions were observed (body weights: $F_{2,42} = 0.56$; food intake: $F_{2,42} = 0.35$; water intake: $F_{2,42} = 2.39$). Finally, food intake was significantly affected by lesion group ($F_{1,21} = 10.72$, $P < 0.01$), with NMDA-lesioned rats consuming 17.11 g (SE = $\pm$ 0.80) of food per day on average, compared to 15.76 g (SE = $\pm$ 0.54) in the control group.

Conditioned place preference

Fig. 9.4 shows the percentage of time rats spent in the paired compartment before and after conditioning (A), as well as exploratory locomotion and rearing during the post-conditioning preference test (B) (scores were adjusted to length of time spent on each side). Preferences were expressed as percentages to account for individual differences in the time spent in neither the paired or the unpaired compartment, i.e., the neutral compartment. ANOVA with repeated measures revealed a significant effect of preference tests before and after drug pairings ($F_{1,21} = 5.65$, $P < 0.05$), while excitotoxic lesions in the N.Acc. were found to have no effect on place preference conditioning ($F_{1,21} = 0.03$). No interaction between lesion groups and preference tests was observed ($F_{1,21} = 0.01$). On average, NMDA-lesioned rats spent 47.78% (SE = $\pm$ 11.44) of time in the paired compartment before conditioning, and 69.08% (SE = $\pm$ 7.15) afterwards. Control rats spent 47.11% (SE = $\pm$ 11.90) of time on the paired side before, 66.80% (SE = $\pm$ 2.51) after conditioning. Differences in time spent in the paired vs the unpaired compartment were assessed using untransformed raw data. ANOVA with repeated measures additionally revealed an interaction of preference before and after conditioning with paired and unpaired compartments ($F_{1,21} = 9.96$, $P < 0.01$). No compartment effect ($F_{1,21} = 1.03$) or group x

Fig. 9.4 Measures of amphetamine-induced conditioned place preference following NMDA or sham lesions in the nucleus accumbens. Percentage of time spent in the paired compartment before and after conditioning (A), and exploratory locomotion (crosses/min and rears/min) in paired and unpaired compartments after conditioning (B), ± SE. * = $P < 0.05$, ***





compartment interaction was observed ($F_{1,21} = 0.07$).

Exploratory locomotion was not affected by lesion groups ($F_{1,21} = 2.26$), but both groups crossed transition lines significantly more often in the unpaired compartment, compared to the paired side during the post-conditioning preference test ($F_{1,21} = 13.76$, $P < 0.001$). No group x compartment interaction was found ($F_{1,21} = 1.23$). The number of rears did not differ with regard to lesion group or compartment, and no interaction was found (group: $F_{1,21} = 0.55$; compartment: $F_{1,21} = 2.06$; group x compartment: $F_{1,21} = 3.78$). Finally, no between-group difference in the total time spent in both paired and unpaired compartments was observed ($F_{1,21} = 0.08$). On average, NMDA-lesioned rats spent 569.18 sec (SE = $\pm$ 61.32), sham-lesioned rats 525.67 sec (SE = $\pm$ 39.73) on the paired or unpaired sides. Calculation of the Pearson correlation coefficient indicated that there was a very small but significant positive correlation between lesion volumes and the number of transitions per min in the paired compartment ($r = 0.36$, $P < 0.05$). No other linear relationship between lesion volume and side preference or exploratory behaviour was observed (% time spent in paired compartment: $r = 0.04$; % time spent in unpaired compartment: $r = - 0.06$; total time spent in both compartments: $r = 0.21$; transitions/min in unpaired compartment: $r = 0.20$; rears/min in paired compartment: $r = - 0.07$; rears/min in unpaired compartment: $r = 0.08$).

DISCUSSION

Repeated pairings of a distinct environment with *d*-amphetamine were found to lead to preference of this environment over one paired with administration of saline. Analysis of exploratory behaviour indicated that

following conditioning, rats engaged in more exploratory locomotion in the *unpaired* environment, while displaying similar levels of exploratory rearing in both test compartments. Specific neuronal lesions in the N.Acc. did not interfere with the acquisition of place preference or exploratory activities. At first sight, these data appear unexpected on the basis of considerable evidence suggesting that dopaminergic mechanisms in the ventral striatum and its interactions with other brain sites are important processes underlying conditioned place preference, as well as drug self-administration and electrical self-stimulation (Phillips and Fibiger, 1989; Koob and Goeders, 1989; Koob, 1992). Specifically, Spyraki and colleagues (1982a) found that depletion of DA terminal fields in the N.Acc. using 6-OHDA interfered with the acquisition of amphetamine-induced place conditioning. The failure to observe deficits in amphetamine-induced incentive-learning following neuronal degeneration in the N.Acc. further supports the hypothesis that depletion of DA terminals in the N.Acc. and fibre-sparing lesions of neurones intrinsic to the nucleus produce differential patterns of behavioural responding. If CPP is regarded as a measure of drug reward, then the reinforcing properties of *d*-amphetamine may depend on ventral tegmental DA projections to the N.Acc. or DA-independent mechanisms, rather than on the integrity of neuronal functions within the nucleus itself. Similar differential effects of depletion of terminal fields and neuronal degeneration in the N.Acc. have previously been observed in experiments investigating exploratory behaviour, locomotor activity and the acquisition of schedule-induced polydipsia (Weissenborn and Winn, in press; see Chapters V and VII). The present study further reports a small but significant correlation between lesion volume and exploratory locomotion in the paired environment, as well as increased exploratory locomotion in

rats with NMDA lesions in the N.Acc. compared to control rats. Although this effect did not reach statistical significance, the data confirm previous data of enhanced locomotion in rats with excitotoxic N.Acc. lesions (Weissenborn and Winn, in press; see Chapter V). Differences in the strength of lesion effects on locomotor activity are likely to reflect greater average lesion volumes of more than 80% total N.Acc. area induced in the earlier series of experiments, compared to 60% reported here. This argument may also account for the small but significant increase in post-operative food-intake described here, which contrasts with observations of small but significant reductions in feeding and body weight and slower recovery of normal drinking levels in rats with NMDA lesions in the N.Acc. reported previously (Weissenborn and Winn, in press; see Chapter V).

Data published by Everitt and colleagues (1991) showing that *established* sucrose CPP was abolished by bilateral quisqualate lesions of the ventral striatum were not replicated here. Several explanations may account for these discrepancies: first, it may be necessary to distinguish between the mechanisms underlying different stages of acquisition and expression of reward-related activities (Bechara and van der Kooy, 1989). Thus, neuronal activity within the N.Acc. may be involved during the display of already established incentive-learning, but not during acquisition stages of the response. Second, comparisons of lesion volumes in the two experiments are difficult since Everitt and colleagues provide no detailed assessment of the extent of their lesions, but the authors may have induced greater damage affecting large areas of accumbens shell, whereas the damage was restricted mainly to the accumbens core area in the present study. Due to the pronounced heterogeneity of the ventral striatum and N.Acc. expressed in distinct core and shell subdivisions and patch-

matrix compartmentalization, small differences in lesion volumes or cannulae placements are likely to have led to discrepancies in behavioural data. Third, it is possible that the rewarding effects of sucrose and *d*-amphetamine are not mediated through the same mechanisms and are therefore differentially affected by excitotoxic N.Acc. lesions. There is evidence to suggest that opiate reward involves two distinct processes; one mediated through DA-containing neurones in the VTA, the other through activation of opiate receptors within the N.Acc. (Koob, 1992; Pettit et al, 1984). Different DA-dependent and -independent mechanisms may similarly be responsible for the rewarding effects of *d*-amphetamine and sucrose.

Although tegmentostriatal DA activity has been attributed a critical role in the mediation of reinforcement and reward, other brain sites also appear to be involved. Everitt and colleagues (1991) demonstrated that established sucrose CPP was abolished by excitotoxic lesions of the basolateral amygdala projecting to the ventral striatum, as well as by asymmetric lesions of the basolateral amygdala and ventral striatum and ventral striatal lesions. In line with previous work investigating reward-related processes (Cador et al, 1989; Everitt et al, 1989; Mogenson et al, 1980; Robbins et al, 1989), this would suggest that the two structures interact in the mediation of CPP; the amygdala being involved in associating environmental stimuli and reward, and the ventral striatum controlling motor responses directed towards those stimuli. Excitotoxic lesion data by Hubner and Koob (1990), although based on drug self-administration experiments rather than CPP, suggests the ventral pallidum as an important site mediating the reinforcing effects of cocaine and heroin. Finally, Bechara and van der Kooy (1989) have identified the PPTg as a critical area of drug reward. Excitotoxic lesions of mostly non-

cholinergic neurones in the ventromedial part of this nucleus abolished acquisition of morphine and amphetamine CPP, but did not interrupt the response once it had been established. In line with this, nicotine administered into the PPTg has been reported to induce CPP (Iwamoto, 1990).

The failure of excitotoxic lesions in the N.Acc. to attenuate amphetamine CPP therefore leads to the following hypotheses: differential response patterns are obtained after DA terminal depletion and after specific neuronal damage. Alternatively, neuronal activity in both subregions of the N.Acc. or in the accumbens shell only may need to be disrupted to abolish CPP. In addition, amphetamine reward may be mediated through more than one mechanism; other structures likely to be involved include basolateral parts of the amygdala, the ventral pallidum and PPTg.

CHAPTER X

DISCUSSION

The role of the ventral striatum for the expression of a number of behaviours, such as locomotion, exploration, schedule-induced drinking or responding to reward has been of considerable interest in the past. The majority of studies, however, have employed techniques manipulating dopaminergic activity through administration of receptor agonists or antagonists, or through depletion of terminal fields in the N.Acc., thereby inducing damage to both axons and axon terminals. The work presented here was therefore designed to extend previous research in two directions: one investigating the functional significance of neurones intrinsic to the nucleus, the other specifically examining dopaminergic activity within the N.Acc.. Using these methods, the aim was to obtain further information about the role of the N.Acc. for a variety of behavioural responses that could then be integrated into a general model of N.Acc. function.

The same stereotaxic N.Acc. coordinates were used throughout for infusions of excitotoxins, microinjections of DA and electrochemical measurements. Comparison of the histological results transferred to standardized sections drawn from the stereotaxic atlas indicated that placements of injection cannulae were typically located within the accumbens core, medial to the anterior commissure, or in the ventromedial shell area 1.0 - 2.7 mm anterior to bregma. Lesion boundaries varied with volume but tended to extend medially, laterally and ventrally around the anterior commissure between 0.5 and 2.7 mm anterior to bregma in most cases (see Fig. 3.1, 3.2, 5.1, 6.1, 7.1, 8.2, 9.1). Since placements of injection cannulae and electrochemical sensors varied within each experiment, comparisons between the behavioural

effects of different types of intervention appear justified, although the problem of error margins during stereotaxic surgery should be borne in mind.

Excitotoxic lesions in the nucleus accumbens

The behavioural consequences of lesions of terminal fields in the N.Acc. are well documented and include the attenuation of amphetamine-induced hyperactivity (Fink and Smith, 1980b; Kelly et al, 1975; Koob et al, 1981; Winn et al, 1985), the abolition of schedule-induced behaviours (Koob et al, 1978; Mittleman et al, 1990; Robbins and Koob, 1980; Robbins et al, 1983; Wallace et al, 1983) and reductions in the rewarding properties of drugs (Koob, 1992; Phillips and Fibiger, 1989; Spyraki et al, 1982a, 1983). In order to allow comparisons between the functional deficits induced by depletion of DA from the N.Acc. and removal of neurones within the nucleus, one of the aims of the experiments reported here was the development of an effective excitotoxic lesion technique. It was found that infusion of NMDA at doses of 60 nmol and 90 nmol reliably produced lesions (identified by significant cell loss and gliosis) in the accumbens core and shell, as well as in other ventral striatal structures in some cases. A striking characteristic of excitotoxic N.Acc. lesions reported here was a substantial variance in lesion volume induced by the same toxin dose. Although some differences would be expected, the extent of the lesions ranged from 21.04% to 111.80% of accumbens core and shell following infusion of 60 nmol NMDA. A similar variability in lesion volumes following infusion of different doses of NMDA (60 and 120 nmol) into the lateral hypothalamus has been reported previously (Winn et al, 1990; Clark et al, 1991). A possible explanation could be an irregular distribution of NMDA-receptors within the core-shell or patch-matrix subsystems of the N.Acc., leading to individual variances in lesion volume

subsystems of the N.Acc., leading to individual variances in lesion volume depending on the precise placements of infusion cannulae. However, an irregular distribution of NMDA receptors has not been reported so far. Alternatively, another substance preventing the uptake of NMDA into GABAergic and cholinergic neurones may be present only in particular accumbens regions. It may be possible to minimize individual differences by infusing smaller volumes of excitotoxin into two adjacent sites, thereby reducing the error margin. It may also be necessary to further investigate in a formal comparison the efficiency and accuracy of other excitotoxic substances - such as ibotenate, quinolinate and quisqualate - that have previously been reported to induce N.Acc. lesions (Annett et al, 1989; Churchill et al, 1990; Everitt et al, 1991; Mazzari et al, 1986).

The behavioural consequences of NMDA infusions into the N.Acc. are briefly summarized in Table 10.1. Comparison of behavioural data showed that excitotoxic lesions in the N.Acc. led to enhanced locomotor activity and exploratory behaviour, while leaving intact the locomotor response to *d*-amphetamine, the acquisition of schedule-induced drinking and amphetamine-induced place preference. Differential N.Acc. lesions therefore produced distinct patterns of responding to a number of behavioural paradigms, indicating that it is important to distinguish the functional roles of DA release from terminal fields in the N.Acc. on one hand and of neuronal activity originating within the N.Acc. on the other. In addition, complex behavioural responses such as schedule-induced drinking or place preference may be differentially affected by depletion of DA terminal fields or neuronal loss during different stages of acquisition or display of the response. It has, for example, been shown that prior acquisition of SIP can protect it from the disruptive effects of 6-OHDA lesions (Robbins et al, 1983).

Table 10.1 Summary of behavioural consequences of different excitotoxic lesion volumes in the nucleus accumbens. NC, no response change in comparison to sham-lesioned control groups.

Average lesion volume (% accumbens core, ± SE)			
	146.36% (± 23.2)	105.64% (± 10.6)	59.33% (± 8.05)
Regulatory responses			
Body weights	NC	NC	decrease
Food intake	NC	increase	decrease
Water intake	decrease	NC	NC
Spontaneous locomotion	increase	---	---
Locomotor resp. to d-amphetamine	NC	---	NC
Locomotor resp. to apomorphine	NC	---	---
Stereotyped resp. to d-amphetamine and apomorphine	NC	---	---
Exploration	increase	---	---
Schedule-induced polydipsia	---	---	NC
Conditioned place preference	---	NC	---

Substances other than N.Acc. DA that may be involved in the mediation of the stimulating and rewarding effects of *d*-amphetamine should also be considered in more detail. The olfactory tubercle, for example, innervated by lateral parts of the substantia nigra - VTA complex, has been proposed as an important site involved in mediating short-lasting, stimulatory effects of DA and apomorphine in a familiar environment (Cools, 1986). A further possible substrate by which *d*-amphetamine may act to enhance locomotion and place preference following neuronal degeneration in the N.Acc. are serotonergic pathways originating in the dorsal raphe nucleus. *D*-amphetamine has been shown to affect 5-HT activity (Robbins et al, 1983) and isolation-reared rats with reduced levels of the 5-HT metabolite 5-HIAA in the N.Acc. and enhanced levels of extracellular DA respond more strongly to reward-associated stimuli (Jones et al, 1990). N.Acc. DA release has also been found to be attenuated by infusion of 5-HT into the dorsal raphe nucleus. (Yoshimoto and McBride, 1992). Serotonergic pathways from the dorsal raphe nucleus to the VTA or N.Acc. may therefore be involved in mediating the stimulant and rewarding effects of *d*-amphetamine. Alternatively, supersensitivity of GABAergic receptors in the ventral pallidum - which is strongly implicated in locomotor control (Swerdlow and Koob, 1987b) - following denervation of GABAergic N.Acc. efferents (Churchill et al, 1990) may be responsible for the enhanced locomotor response to *d*-amphetamine observed here. Other likely sites of action for *d*-amphetamine during CPP are limbic structures; amygdaloid-striatal interactions in particular have been shown to mediate the response to reinforcement and reward (Cador et al, 1989; Everitt et al, 1990).

It is important to point out that despite substantial within-group variances, differences in lesion volume and site may result in different

behavioural effects. This assumption would explain the apparent discrepancies in the locomotor responses to *d*-amphetamine and saline observed in the two lesion experiments reported here. Spontaneous locomotion and hyperactivity following administration of *d*-amphetamine were significantly enhanced in rats which had sustained 68.86% (SE = $\pm$ 15.07) damage to N.Acc. neurones on average, while this effect was not observed in rats with smaller average lesions of 33.32% (SE = $\pm$ 4.52). In the light of behavioural differences in groups of rats with differing average damage, individual lesion volumes would be expected to correlate with at least some aspects of behaviour. Few of such correlations, however, were found in the present experiments. This may indicate either that only a small portion of the N.Acc. is responsible for the mediation of the relevant behavioural effect, or, in the case of absence of an effect, that the entire structure is involved and if damage occurs in one region, other parts can compensate for the loss.

Manipulation and measurement of dopaminergic activity in the nucleus accumbens

Microinjection studies showed that enhanced dopaminergic activity in the N.Acc. induced by intra-accumbens administration of DA did not change schedule-induced drinking after SIP onset, although the same dose of DA had previously produced significant behavioural effects (increased locomotion) in a dose-dependent manner. These data were supported by *in vivo* voltammetric recordings that did not indicate changes of DA efflux in the N.Acc. during the acquisition and emission of SIP. However, substantial within-session increases in DA were observed before and after the onset of SIP, suggesting that two different processes may underlie the acquisition of drinking in response to a reinforcement schedule. First, DA

overactivity may develop during a test session, regardless of whether SIP has been acquired; second, a process unrelated to dopaminergic firing may be responsible for the acquisition of excessive drinking. The results obtained in the microinjection and voltammetric experiments would therefore suggest that although dopaminergic activity in the N.Acc. may be necessary to allow the development of SIP, other substrates are involved in mediating the polydipsic response itself (Devenport, 1978; Mittleman et al, 1990; Taghzouti et al, 1985; Winn et al, 1992).

'Gating' hypothesis of nucleus accumbens function

On the basis of its distinct connections with cortical, limbic and midbrain structures, the role of the N.Acc. is best understood in the context of a complex neuronal circuitry. A model accommodating interactions and feedback loops has been proposed by Swerdlow and Koob (1987a) and is outlined in some detail above (see Chapter I). Briefly, according to this model, three feedback loops interact to form a ventral striatal motor system, where excitatory inputs from the limbic cortex and inhibitory GABAergic efferents from the N.Acc. maintain a continuous pattern of firing necessary for the execution of a specific motor program. Inhibitory input to the N.Acc. (stimulated by limbic afferents to the ventral pallidum mediated via the N.Acc.) can disrupt this ongoing process and thereby allow the initiation of a new pattern of activity. Thus, dopaminergic input to the N.Acc. may serve to prevent cortical overexcitation, filtering out irrelevant patterns of activity and facilitating the switching of cognitive, emotional or sensory information. (It is important to note that this integrative process does not provide information about the direction of change, for which additional striatal input is needed.) The 'gating' hypothesis of dopaminergic modulation of cortical

information is in line with work by Mogenson and co-workers demonstrating that dopaminergic projections from the VTA significantly attenuated responses of N.Acc. neurones to stimulation of both hippocampus and amygdala (Yang and Mogenson, 1984; Yim and Mogenson, 1982). It was proposed that dopaminergic activity in the N.Acc. may act as a 'limbic-motor interface' transforming motivation into action by acting as a gating mechanism for signals from limbic to motor systems (Mogenson et al, 1980).

The 'gating' hypothesis of neuronal integration is used to interpret the role of dopaminergic activity in the pathology and treatment of several psychiatric conditions, such as Parkinson's disease, depression, mania and schizophrenia. Underactivity of the forebrain DA circuitry is thought to lead to a fixed pattern of cortical activity and an inability to switch to new information, resulting in the repetitive cognitive and motor impairments typically associated with depression and Parkinson's disease. By contrast, schizophrenia and mania are associated with overactivity of the DA forebrain system, which leads to fast switching between activity patterns and deficits in the filtering of cortical information, resulting in psychotic symptoms (Swerdlow and Koob, 1987a). Impaired performance in tests of the influence of previous consistent experience on the perception of current events, such as sensorimotor gating, latent inhibition and the partial reinforcement extinction effect, has been demonstrated in acute schizophrenic patients, as well as in animal and human subjects following administration of intra-accumbens DA (Swerdlow et al, 1990a/b), *d*-amphetamine or damage to the subiculo-accumbens pathway (Gray et al, 1991). In an extensive literature review, Gray and colleagues (1991) suggest that the main neuronal basis of schizophrenic symptoms may be a combination of dysfunction in the limbic system and DA hyperactivity,

resulting in a disruption of the normal interaction between excitatory glutamatergic input from hippocampal areas and inhibitory DA input from the VTA to GABAergic N.Acc. efferents. In addition, glutamatergic activity in the cortico-striatal-thalamic circuitry may control motor outflow independently of dopaminergic processes, suggesting that the role of DA within the striatal motor system may not be as prevalent as generally assumed (Carlsson and Carlsson, 1990).

The data reported here confirm and extend the proposed model of the N.Acc. motor system. It could be argued that increased spontaneous locomotor activity and exploration following NMDA lesions in the N.Acc. both reflect delayed inhibition to novel environments. However, in the light of a locomotor response to *d*-amphetamine that is enhanced proportional to baseline levels the observed behavioural effects of excitotoxic N.Acc. lesions are more likely to represent a control deficit of general motor output. Thus, in line with the 'gating' hypothesis, removal of neurones in the N.Acc. has led to a disruption in the inhibitory functions of N.Acc. output, resulting in increased levels of activity and an inability to change firing patterns according to directional input from the cortex or other striatal areas. Similarly, the observed reduction in the number of door presses and increase in drinking bout lengths during SIP following infusion of NMDA into the N.Acc. may reflect deficits in the ability to switch appropriately between different activities or to terminate behaviour in response to internal or external cues.

Subsystems of the ventral striatum

The data obtained from the experiments presented here using excitotoxic lesions, microinjections or *in vivo* voltammetry underline the importance of taking into account the diversity of the N.Acc. in terms of

its efferent and afferent connections (Groenewegen and Russchen, 1984; Heimer et al, 1991), its suggested compartmentalization (Gerfen, 1992), and the resulting functional implications. The accumbens core, for example, is innervated primarily by areas of the limbic cortex and amygdala and projects to dorsolateral aspects of the ventral pallidum, substantia nigra, laterodorsal parts of the lateral hypothalamus, as well as to a clearly defined area in the mesencephalic tegmentum. By contrast, the accumbens shell is thought to be innervated by hippocampal efferents and its target neurones are located in ventromedial areas of the ventral pallidum, VTA, rostrocaudal lateral hypothalamus, extended amygdala and in diffuse areas of the mesencephalic tegmentum (Heimer at al, 1991; Kelly and Domesick, 1982; Kelly et al, 1982). In addition, striatal compartmentalization into patches (receiving input from the limbic cortex) and matrix (receiving neocortical information) has been identified in the accumbens core, but not the shell (Voorn et al, 1989), implying further differential connections and distribution of neuropeptides (e.g., enkephalin and substance P) and DA. An extended model of N.Acc. function would therefore have to be based on the integrating, modulatory role of the nucleus and comprise additional information not only about its interaction with related structures, but also about its heterogeneity regarding a variety of neuroanatomical markers, such as patterns of afferent and efferent connections, the distribution of neurotransmitter receptors, immunoreactivity to neuropeptides and second messenger systems.

Directions for further research

Further experiments directed at investigating the functional role of the N.Acc. should take into account the following points that have become particularly significant in the course of the experiments presented here.

First, the diversity of afferent and efferent projections of the N.Acc. needs to be considered in some detail; in particular, there appears to exist a lack in studies tracing accumbens afferents with regard to differential distribution in core and shell areas. Another important consequence would be a detailed examination of the effects of localized lesions whose sites correspond to various input and output pathways. Attempts to identify in more detail changes in receptor regulation in the ventral pallidum following specific lesions of accumbens core and shell areas have been reported by Churchill and colleagues (1990), but more detailed analyses, as well as functional investigations are needed. This clearly is a challenging task due to the fact that it appears to be difficult to induce uniform lesions in the first place. Similarly, localized infusions would still not solve the problem of damaging overlapping afferents or efferents connected to different structures. However, attempts should be made at inducing more localized lesions within the N.Acc. by infusing smaller volumes of toxin at two or more different sites, depending on lesion size. Further, control experiments should be conducted to investigate the possibility of confounding effects of demyelination and breakdown of the blood-brain barrier in response to infusion of excitotoxic substances in the N.Acc..

Second, it not only seems to be inappropriate to regard the N.Acc. as a unitary structure, but it should also be considered within its integrative framework in more detail. Previous research has underlined the fact that the N.Acc. is not a homogeneous structure with a certain number of one-way connections to other areas. Instead, the ventral striatum should be regarded as part of a complex interactive and flexible system. Removal of one set of neurones may not necessarily result in measurable functional deficits since other structures may be able to compensate for the

disruption. It also seems plausible that induced transmitter overactivity can be balanced out to some extent by up- or downregulation of other transmitter substances. The microinjection, voltammetric and lesion studies presented here have shown that neurones within the N.Acc., as well as dopaminergic activity in the nucleus appear to be less involved in the acquisition of schedule-induced behaviours than previously thought. Similarly, since excitotoxic lesions in the N.Acc. did not attenuate the effects of *d*-amphetamine on locomotion and place preference, other sites within the DA circuitry may be involved in mediating its effects. Structures anatomically related to the N.Acc. that ought to be considered in conjunction with N.Acc. functions are the hippocampus, lateral hypothalamus and lateral septum, all of which have been implicated in the development of SIP (Devenport, 1978; Mittleman et al, 1990; Taghzouti et al, 1985; Winn et al, 1992). In addition, the ventral pallidum, substantia nigra and olfactory tubercle are closely associated with the control of locomotion (Cools, 1986; Garcia-Rill, 1986; Mogenson et al, 1989; Swerdlow and Koob, 1987b), and the PPTg, as well as an interaction between the amygdala and the N.Acc. have been suggested to be involved in the mediation of reinforcement and reward (Bechara and van der Kooy, 1989; Cador et al, 1989; Iwamoto, 1990; Yim and Mogenson, 1989). This last aspect becomes particularly interesting considering the close interaction that has been observed between the accumbens shell and parts of the extended amygdala (Alheid and Heimer, 1988).

A number of different techniques have been developed and applied in the experiments described here, and in order to extend the view of N.Acc. function presented above it seems appropriate to use combinations of these techniques (e.g., lesions and microinjections, lesions and

voltammetry). The application of such combination would greatly improve the ability to examine interactions and integrative processes involving two or more related structures.

Finally, the above techniques should be applied to further investigate some of the animal models developed to reflect the cognitive and behavioural characteristics of psychiatric disorders associated with dopaminergic overactivity (Swerdlow and Koob, 1987a) or glutamatergic underactivity (Carlsson and Carlsson, 1990), such as deficits in sensorimotor gating (Swerdlow et al, 1990a/b), latent inhibition and the partial reinforcement extinction effect, all of which are thought to reflect schizophrenic symptomology in humans (Gray et al, 1991).

CONCLUSION

The present work has examined functional aspects of the N.Acc. using comparative lesion techniques, intra-accumbens administration of DA and electrochemical recordings of extracellular DA levels. The data support hypotheses of the N.Acc. as a 'limbic-motor interface' and 'gating' mechanism acting as part of an integrative feedback system allowing the appropriate initiation of new motor programs in response to relevant directional input from cortical areas. It is suggested that these hypotheses should be further considered within the complex framework of the ventral striatal circuitry, taking into account the heterogeneity of the N.Acc. with regard to its core and shell subsystems, patch-matrix compartmentalization, and the corresponding differential connectivity and distribution of neuroactive substances.

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APPENDIX: FIGURE AND TABLE LEGENDS

Fig. 1.1 Mesostriatal (A) and mesolimbocortical (B) dopamine projections. ACC, anterior commissure, AMY, amygdala, CE, entorhinal cortex; cp, caudate putamen, hi, hippocampus; OB, olfactory bulb; ot, olfactory tubercle; nas, nucleus accumbens; PFC, prefrontal cortex; pi, piriform cortex; sl, lateral septum.

Fig. 1.2 Ventral striatal circuitry and its interaction with the dorsal striatal complex (adapted from Bjorklund and Lindvall, 1986). CFR, cuneiform region; CG, central gray; DA, dopamine; EPN, entopeduncular nucleus; GLU, glutamate; PBR, parabrachial nucleus; PPTg, pedunculopontine tegmental nucleus; SC, superior colliculus; SN, substantia nigra; STN, subthalamic nucleus; VTA, ventral tegmental area.

Fig. 1.3 Core and shell areas of the nucleus accumbens. Horizontal stripes: accumbens core; vertical stripes: accumbens shell; a, anterior commissure; cp, caudate putamen,; ot, olfactory tubercle; vp, ventral pallidum. Section (1.20 mm anterior to bregma) drawn from the atlas of Paxinos and Watson (1986).

Fig. 1.4 Differential afferent and efferent connections of nucleus accumbens core and shell. LH, lateral hypothalamus; VP, ventral pallidum.

Fig. 1.5 Nucleus accumbens motor system. Limbic cortico-striato-pallido-thalamic-midbrain circuitry (Swerdlow and Koob, 1987).

Fig. 3.1 Smallest (A) and largest (B) ventral striatal lesions induced by 60 nmol NMDA. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm.

Fig. 3.2 Smallest (B) and largest (B) ventral striatal lesions induced by 90 nmol NMDA. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm.

Fig. 3.3 Photographs of sections through the midbrain and medial forebrain stained for tyrosine hydroxylase (TOH). (A) and (B) show the distribution of TOH-positive cells in the ventral tegmental area (VTA) in sagittal sections after sham lesions (A) and after 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens (B); (C) and (D) show the distribution of TOH-positive neurones in the VTA, substantia nigra and mesostriatal projection sites after sham lesions (C) and after 6-OHDA lesions of the nucleus accumbens (D); dmn, dorsomedial hypothalamic nucleus, ml, medial lamniscus; mt, mammillothalamic tract; snpc, substantia nigra pars compacta; snzr, substantia nigra zona reticulata; vta, ventral tegmental area; 3v, 3rd ventricle; vertical arrows indicate mesostriatal fibre systems. Scale bars = 1 mm.

Fig. 5.1 Smallest (A) and largest (B) lesions of the nucleus accumbens induced by 60 nmol and 90 nmol NMDA. Sections drawn from the atlas of Paxinos and Watson (1986); numbers indicate the distance from bregma in mm; A, nucleus accumbens; C, caudate putamen; t, olfactory tubercle; vp, channel neurones of ventral pallidum.

Fig. 5.2 Percentages of average pre-operative body weights, food and water intake measured over 26 days following NMDA, 6-OHDA or sham lesions of the nucleus accumbens.

Fig. 5.3 Measures of exploratory behaviour in rats with NMDA, 6-OHDA or sham lesions of the nucleus accumbens. Top panel: average number of quadrant crossings per minute on novel and familiar sides of the Carlsson box, $\pm$ SE. Centre panel: average number of rears per minute on novel and familiar sides of Carlsson box, $\pm$ SE. Bottom panel: percentage of time (10 min = 100%) spent on novel side of Carlsson box, $\pm$ SE. $^{*} = P < 0.05$, $^{***} = P < 0.001$, compared to sham-lesioned control rats.

Fig. 5.4 Average of photocell counts (expressed as square roots of total values) during daily 1 hr tests in activity cages over 2 weeks, following NMDA, 6-OHDA or sham lesions of the nucleus accumbens $\pm$ SE. $^{**} = P < 0.01$, compared to sham-lesioned control rats.

Fig. 5.5 Measures of the locomotor response to dopaminergic agonists in rats with NMDA, 6-OHDA or sham lesions of the nucleus accumbens. Average of photocell counts (expressed as square roots of total values) during 1 hr tests in activity cages following systemic administration of apomorphine, *d*-amphetamine or vehicle, $\pm$ SE. $^{*} = P < 0.05$, $^{**} = P < 0.01$, compared to injection of vehicle.