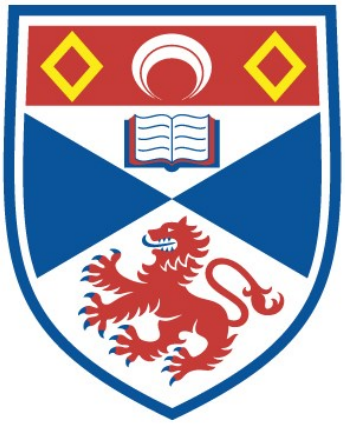
THE ROLE OF THE BASOLATERAL AMYGDALA IN COCAINE SELF-ADMINISTRATION AND COCAINE-SEEKING BEHAVIOUR

Rachel B. Whitelaw

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



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The role of the basolateral amygdala in cocaine self-administration and cocaine-seeking behaviour

by

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B.Sc Hons., St. Andrews

Submitted to the University of St. Andrews in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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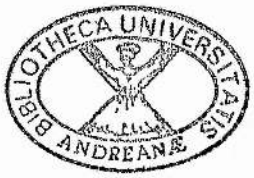

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Abbreviations

ACh	Acetylcholine
aCSF	artificial cerebrospinal fluid
AP5	DL-2-amino-5-phosphono-pentanoic acid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
BLA	basolateral nuclei of the amygdala
BNST	bed nuclues of the stria terminalis
BP(s)	break-point(s)
β-ME	β-mercaptoethanol
CeA	central nucleus of the amygdala
CDP	chlordiazepoxide
CmA	corticomedial nucleus of the amygdala
CPP	conditioned place preference
CNS	central nervous system
CNQX	6-cyano-7-nitroquinoxaline-2, 3-dione
CS(s)	conditioned stimulus/ stimuli
CS-	omission of conditioned stimulus
CS+	reinstatement of conditioned stimulus
CRF	continuous reinforcement
CR(s)	conditioned reinforcer(s)
DA	dopamine
DOPAC	3, 4, dihydroxyphenylacetic acid
EAA	excitatory amino acid
ECD	electrochemical detection
EEDQ	N-ethoycarbonyl-2-ethoxy-1, 2-dihydroquinoline
EEG	electroencephalogram
FI	fixed-interval
FR	fixed-ratio
GABA	γ-amino-butyric-acid
Glu	glutamate
HPLC	high performance liquid chromatography

IP	intra-peritoneal
IV	intravenous
MK-801	dizocilpine
MRI	magnetic resonance imaging
NA	noradrenaline
Nacc	nucleus accumbens
NBM	nucelus basalis magnocellularis
NRF	non-reinforcing
NMDA	N-methyl-D-aspartate
6-OHDA	6-hydroxydopamine
OPA	o-phthaladehyde
PCA	perchloric acid
PDC	L-trans-pyrrolidine-3, 4-dicarboxyl acid
PET	positron emission tomography
PFA	paraformaldehyde
Pfc	prefrontal cortex
PR	progressive-ratio
R	response
S	stimulus
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
Trn	taurine
TTX	tetrodotoxin
US	unconditioned stimulus
VTA	ventral tegmental area

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Abstract

The experiments reported in this thesis have investigated the role of the basolateral amygdala (BLA) in the process by which conditioned stimuli (CS) acquire motivational salience and, as conditioned reinforcers, direct cocaine-seeking behaviour in the rat. Excitotoxic lesions of the BLA did not interfere with the reinforcing effects of cocaine in rats. Intra-peritoneal injections of cocaine produced similar locomotor responses in both lesioned and control animals and both groups also produced equivalent dose-response functions during a within-session dose-response test. Similarly, lesioned and control animals acquired cocaine self-administration under both continuous and progressive-ratio schedules of reinforcement. However, BLA-lesioned animals were (i) severely impaired in the acquisition of second-order schedules of cocaine self-administration; (ii) more sensitive than control animals to reductions in drug dose under a progressive-ratio schedule of cocaine self-administration and (iii) less sensitive than control animals to the omission of a drug-related CS, under a fixed-interval schedule of self-administration. *In vivo* microdialysis showed that lesions of the BLA were associated with an impaired glutamatergic response to intra-nucleus accumbens infusions of cocaine, but that the dopaminergic response of lesioned and control animals of were identical. These findings suggest that drug-seeking behaviour in rats with lesions of the BLA is influenced more by the primary reinforcer and concomitantly less by secondary, conditioned reinforcers. This would indicate that the BLA is significantly involved in the development of cue-elicited drug-seeking behaviour and, by inference, this structure may also play an important role in the development of problem drug-use in humans.

The role of the basolateral amygdala in cocaine self-administration and cocaine-seeking behaviour

Chapter 1: Introduction

Investigation of the aetiology of addiction and of the neural substrates of drug-seeking behaviour have become important areas of neuroscience research (Koob 1992). Numerous influential figures including scientists and physicians endorsed the use of cocaine as a panacea and local anaesthetic during the late 19th and early 20th century and the popular opinion of cocaine as safe and glamorous drug have been contrived and promoted by numerous American media sources, as recently as the early 1980's (Byck 1987; Gawin 1991). Although the hazards of chronic cocaine use were noted by Freud in the 1880s and thoroughly documented in the 1920s, the recreational use of cocaine has periodically swung in and out of fashion for more than 100 years. The escalating demand for, and usage of, psychomotor stimulants such as cocaine and amphetamine has created serious socio-economic problems for many western countries. Despite the absence of firm evidence for the development of true physiological dependence to this class of drug (Wise 1987; Koob and Bloom 1988; Jaffe 1992), there is now a growing body of literature which supports the view that psychological factors are critically involved in the development and maintenance of problem drug use (O'Brien et al. 1992; Grant et al. 1996; Mass et al. 1998).

One approach to the study of drug reinforcement processes is to develop models of drug self-administration in laboratory animals. Cocaine is readily self-administered

by both primates and rodents which have been surgically prepared with chronic intravenous catheters (Goldberg and Tang 1977; Caine et al. 1992). Using this technique it is possible to investigate the neural mechanisms underlying the rewarding properties of a drug and to assess the development of drug-seeking behaviour.

It is now widely agreed that the nucleus accumbens (Nacc) and ventral striatal dopaminergic system are significantly involved in the mediation of the rewarding properties of psychomotor stimulants (Zito et al. 1985; Koob et al. 1987; Koob and Weiss 1990; Cador et al. 1989; Everitt et al. 1989, Robbins et al. 1989; Corrigal and Cohen 1989; McGregor and Roberts 1993; Fontana et al. 1993; Pesold and Treit 1995; Wilson et al. 1994). However, the exact neural substrates and processes by which drug addiction develops are as yet unclear. In addition to physiological alterations which arise following repeated drug use (such as sensitisation and tolerance) and which are exacerbated during periods of drug withdrawal, conditioning is thought to be an essential determinant of drug-seeking behaviour. Robinson and Berridge (1993) have suggested that repeated drug administration enhances the formation of conditioned associations and consequently increases the potential of drug related cues to elicit drug-seeking behaviour, while the perceived rewarding efficacy of the drug are simultaneously reduced through the development of tolerance to the drug's effects. They proposed (Robinson and Berridge 1993) that addiction occurs as a result of this dissociation between the subjective rewarding effects and the sensitised desire for drug. In support of this hypothesis, drug users report enhanced subjective feelings of craving following exposure to drug-related cues such as drug paraphernalia, money, dealers,

drug associated environments or video footage of drug preparation (Gawin 1991; Ehrman 1992; Childress et al. 1996; Grant et al. 1996; Mass et al. 1998). Such cues have also been identified as contributing to the propensity to relapse in human subjects who attempt to give up their drug-taking habit (Childress et al. 1987,1988; Ehrman 1992; Grant et al. 1996; Mass et al. 1998). Understanding the mechanisms by which arbitrary cues gain motivational significance and come to elicit powerful control over drug-seeking behaviour will increase the likelihood of finding both pharmacological and clinical solutions for the current explosion of problem drug use.

Psychomotor Stimulants

Cocaine and amphetamine are reported to produce intense feelings of well being and euphoria in humans and show clear dose related behavioural effects in primates and rats (Woods et al. 1987; O'Dell et al. 1996). Low doses of psychomotor stimulants produce increased locomotor activity in the rat whereas higher doses induce stereotyped behaviours (such as repetitive head movements, sniffing or grooming). Although cocaine has a considerably shorter half-life than amphetamine and also has anaesthetic properties, both of these drugs show high abuse potential in humans and are readily self-administered by many laboratory animals.

Psychomotor stimulants produce their effects by modifying the transmission of central neurotransmitters including excitatory amino acids (EAA's), acetylcholine and the monoamines dopamine (DA), noradrenaline (NA) and serotonin (5-HT). Psychomotor stimulants are distinguished from other stimulants (strychnine,

pentylenetetrazol) by their ability to activate locomotor activity (Wise 1989). As potent catecholamine agonists, both cocaine and amphetamine act to enhance post-synaptic DA-ergic stimulation but, despite producing similar overall effects on extracellular DA levels, they do not share the same neuropharmacological mechanisms. Amphetamine directly stimulates the release of DA, NA and 5-HT pre-synaptically and may act directly on DA receptors, whereas cocaine blocks pre-synaptic DA reuptake by binding to the respective DA, NA and 5-HT transporter molecules (Woods et al. 1987; Cervo and Samanin 1994). This subtle distinction may have implications for the acquisition and development of cocaine abuse (Graybiel et al. 1990). Although enhanced DA stimulation is considered to be the key to psychomotor stimulant reinforcement (Wise 1987), it is likely that additional more complex neuropharmacological processes are involved. Tricyclic antidepressants also produce catecholamine reuptake blockade but, unlike cocaine, fail to act as reinforcers and do not stimulate locomotor activity in rats (Woods et al. 1987).

Conditioned Reinforcement

A motivationally neutral stimulus that is reliably paired with a primary reinforcer, such as water, food or drug, may gain motivational significance and subsequently act as a conditioned reinforcer (CR) (Mackintosh 1974, 1983). As a CR, this previously neutral stimulus may act to maintain specific patterns of behaviour (such as responding for sucrose solution) in the absence of a primary reward (sucrose solution).

Much work has been carried out to investigate the neural mechanisms underlying motivational states and incentive motivational processing (Taylor and Robbins 1984, 1986; Cador et al. 1989; Everitt et al. 1989; Robbins et al.1989; Burns et al. 1991; Everitt and Robbins 1992). Ultimately, by pinpointing the specific neural circuits that mediate these processes, it may be possible to synthesise pharmacological compounds which selectively target and disable the formation of unwanted, conditioned associations.

Many of the experiments listed above involve conditioned reinforcement paradigms, in which rats are trained to associate a neutral stimulus (e.g. a brief light presentation) with a natural reward (such as food, water or sexual activity). Through association, a motivationally neutral stimulus may acquire motivational properties, similar to that of the primary reward, and thus become a conditioned stimulus (CS). When an animal is willing to repeat a novel behaviour (such as lever pressing) to gain exposure to the CS in the absence of the primary reward, the CS can be defined as a conditioned reinforcer (CR) which may influence or direct the animal's behaviour (Mackintosh 1974, 1983).

Recently, it has become clear that control over behaviour by a CR is enhanced by activation of the ventral striatal dopamine system and possibly the interaction of glutamatergic limbic efferents at this site (Burns et al. 1994). Taylor and Robbins (1984) demonstrated that micro-infusions of d-amphetamine into the Nacc of thirsty rats selectively increased responding for a CR which had previously been associated with water. The same investigators later established that 6-hydroxydopamine (6-

OHDA) lesions of the Nacc, but not of the thalamus or the caudate-putamen, attenuated similar selective increases in responding for a CR following micro-injections of d-amphetamine into the Nacc (Taylor and Robbins 1986; Kelley and Delfs 1991). It was concluded from these studies that the ventral, rather than dorsal, dopaminergic innervation of the striatum was critical for the mediation of the reward-related motivational processes. Neural mechanisms underlying CR have since been shown to involve both ventral striatal DA transmission (Taylor and Robbins 1984, 1986) and efferents from the basolateral amygdala (BLA) (Everitt et al. 1989; Cador et al. 1989; Robbins et al. 1989; Burns et al. 1994). Other limbic structures such as the prefrontal cortex and subiculum are also thought to be involved in CR behaviours (Weissenborn et al. 1996; Caine et al. 1996; Burns et al. 1996).

Ventral striatal processes

Much of our understanding of the ascending DA-ergic pathways arose following the introduction of 6-OHDA as a selective DA toxin (Ungerstedt 1971) and the use of neuroanatomical tracing methods (Heimer 1981). By the use of these techniques, ventral striatal DA-ergic projections from the ventral tegmental area (VTA) to the Nacc have been shown to be involved in arousal and locomotor activation and are considered to be the main sites of psychomotor stimulant action (Kelly 1975; Wise 1987, Koob and Bloom 1988).

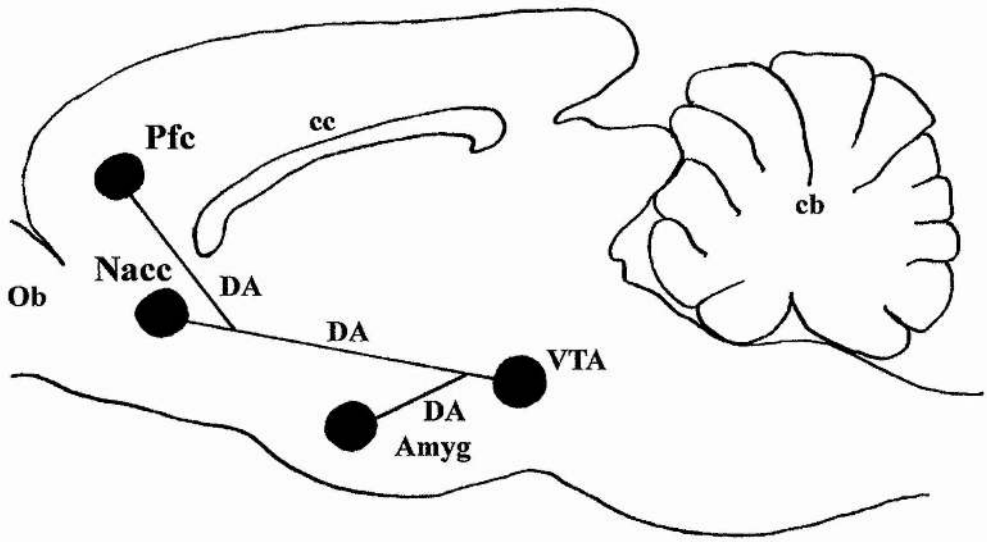


Fig. 1 Schematic representation of the ventral striatal dopaminergic projections in the rat. VTA = ventral tegmental area, Amyg = amygdaloid complex, Nacc = nucleus accumbens, Pfc = prefrontal cortex, cc = corpus callosum, cb = cerebellum, Ob = olfactory bulb, DA = dopamine.

The ventral striatum is innervated primarily by DA-ergic neurons arising in the A10 DA cell group of the VTA but also receives serotonergic innervation from the medial and dorsal raphe and noradrenergic innervation from the locus coeruleus (Fallon 1981). The ascending mesolimbic projection runs from the VTA in the midbrain and terminates primarily in the Nacc, which is situated in the forebrain. Unlike the dorsal striatum, the ventral striatum also receives excitatory inputs from various limbic cortical structures including the hippocampus, pre-limbic cortex and amygdala. On the basis of cytoarchitectural differences, the Nacc can be divided into three main subregions: the core, shell, and rostral pole (Jongen-Rêlo et al. 1994). Limbic efferents have been shown to preferentially project to different Nacc subregions (Heimer et al. 1991). The ventral subiculum, caudal BLA and paraventricular thalamic nuclei project to the Nacc shell, whereas the dorsal subiculum, rostral BLA, parataenial and central medial thalamic nuclei project to the Nacc core and rostral pole. It appears that amygdaloid projections to the Nacc stem almost exclusively from the BLA (Kelley et al. 1982). These factors indicate that the structure and functional significance of the ventral and dorsal striatum are different. Mogenson et al. (1980) proposed that the ventral striatum operated as a 'limbic-motor' interface, integrating limbic inputs and initiating the selection of appropriate behavioural responses via Nacc efferents to the pedunculopontine tegmental nucleus and nigro-striatal motor neurons.

Nacc neurons send out inhibitory GABA-ergic efferent projections to the ventral pallidum which in turn projects to the subthalamic nucleus and substantia nigra reticulata (SNr) and mediodorsal nucleus of the thalamus. Groenewegen (1990)

proposed that at least two populations of ventral striatal efferents project from the Nacc: one to the dorsomedial part of the SNr and another to the VTA, SNr and retrorubral field (cell groups A10, A9, A8 respectively). In this way the ventral striatum appears to influence dorsal striatal DA activation (which arises in A9 DA cell group of the SN), and potentially modulates the transmission of cortical information in the basal ganglia, ultimately influencing the selection of behavioural actions (Nauta and Domesick 1978). A more comprehensive review of this topic is presented by Groenewegen et al. (1991).

The limbic system

As mentioned above, the ventral striatum differs significantly from the dorsal striatum in that it receives cortical afferents from the amygdala, hippocampal formation and the prelimbic cortex (Burns et al. 1996). The limbic system was first named by MacLean (1949), who extended the initial theories of emotion proposed by Papez in 1937. In his original paper, Papez suggested that awareness and expression of emotional states must depend upon mutual communication between viscero-endocrine responses of the hypothalamus and higher cognitive functions of the cortex. He postulated that the cingulate gyrus, hippocampus, hypothalamus and anterior thalamic nuclei were linked together by a neural circuit which mediated the formation and expression of emotional behaviour. MacLean (1949) developed this theory, recognising that other structures such as the amygdala, septal area, Nacc, and specific cortical and thalamic nuclei (which were closely associated with the hypothalamus), were also involved in emotional or 'limbic' processing. Much of this circuitry has been upheld by modern anatomical studies and currently the limbic

system refers to: the hippocampal formation (including the subiculum, entorhinal, perirhinal and parahippocampal cortex), fornix, septum, thalamic nuclei, mammillary bodies, hypothalamus, preoptic area, prefrontal and cingulate cortices, bed nucleus of the stria terminalis and the amygdaloid complex.

The amygdaloid complex

Within the last 30 years understanding of the structure and functional significance of the amygdaloid complex has increased dramatically. Originally considered as a modulator of hypothalamic activity (Heimer 1981), it is now clear that the amygdala is an important component of the limbic system involved in some of the most complex functions of the brain - emotion, motivation, learning and memory (McDonald 1992).

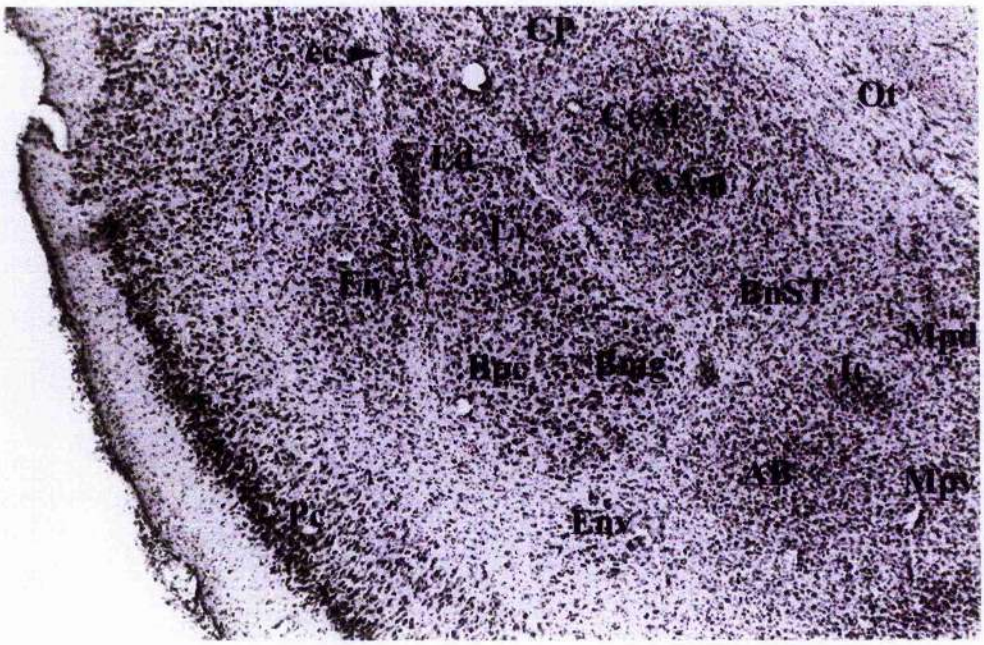
Kluver and Bucy (1939) noted that discrete bilateral lesions of the temporal lobe in monkeys produced an array behavioural characteristics which involved extreme tameness, general loss of motivation and were often associated with inappropriate responding in social situations. Weiskrantz (1956) extended this work in the monkey by investigating the behavioural effects of discrete anteromedial temporal lobe lesions, in an attempt to functionally fractionate the behavioural symptoms associated with general temporal lobe lesions. He reported that the amygdala, which is positioned in the medial aspect of the temporal lobe, may be involved in mediating the rewarding impact of biologically significant stimuli such as primary reinforcers (Weiskrantz 1956). This idea has since been supported by many reports which strongly implicate the amygdala in conditioned reinforcement, stimulus-

reward learning and incentive motivational processing (Jones and Mishkin 1972; McDonough and Manning 1979; Spiegler and Mishkin 1981; Gaffan and Harrison 1987; Cador et al. 1989; Everitt et al. 1989, 1991; Robbins et al. 1989; Gaffan and Murray 1990; Burns et al. 1994, 1996) as well as conditioned fear-motivated behaviour (Davis 1992; Helmstetter 1992; Faneslow and Kim 1994; LeDoux et al. 1990; Killcross et al. 1997; Gewirtz and Davis 1997). Within the limbic system, both the amygdala and hippocampus receive multisensory inputs from visual, auditory, olfactory and somotosensory association areas. Although the hippocampus receives afferent projections via parahippocampal-entorhinal processing, the amygdala receives direct sensory input from the association cortices of the temporal cortex. The cortico-amygdaloid projections are directed primarily to the lateral, basal and basal accessory nuclei of the amygdala (Turner 1981; Witter et al.1989). Functionally, the amygdala is thought to be concerned with the processing of distinct attributes of stimuli (Gaffan and Harrison 1987) whereas the hippocampus is thought to be involved with relationships between multiple stimuli, such as contextual and spatial information (Morris et al. 1982).

Neuroanatomy of the amygdala

"It is no longer appropriate to view the amygdala as a complex poorly understood region of the brain with little systematic organisation. Studies of the internal circuitries show that the amygdala has clear and precise organisation that is tailored to the computational functions it performs." (Pitkänen et al. 1997)

Plate 1



Photomicrograph of the amygdaloid complex taken from a 60µm coronal section approximately -2.8mm from Bregma and stained with Cresyl Violet. Nomenclature from McDonald and Mascagni (1997).

L_d	lateral nucleus, dorsolateral subdivision
L_v	lateral nucleus, ventrolateral subdivision
B_{pc}	basal nucleus, parvicellular division
B_{mg}	basal nucleus, magnocellular division
AB	accessory basal nucleus
En	endopiriform nucleus
En_v	ventral endopiriform nucleus
CeA_l	central nucleus, lateral division
CeA_m	central nucleus, medial division
M_{pv}	medial nucleus posteroventral nucleus
M_{pd}	medial nucleus posterodorsal nucleus
Ic	intercalated mass
Pc	perirhinal cortex
ec	external capsule
CP	caudate putamen
Ot	optic tract
BnST	bed nucleus of the stria terminalis, intra amygdaloid portion

Located in the medial aspect of the temporal lobe, the amygdaloid complex extends rostrally to the level of the suprachiasmatic nucleus and caudally to the level of the mammillary bodies (Rainnie et al. 1991a). The amygdala is reciprocally connected with the hypothalamus, hippocampus, medial and lateral prefrontal cortex (Pfc) and thalamus and it's major efferent projections follow two discrete pathways: i) the stria terminalis, which innervates the bed nucleus of the stria terminalis (BNST), Nacc, Pfc and hypothalamus, and ii) the ventral amygdalofugal pathway, which innervates the hypothalamus, dorso-medial nucleus of the thalamus, and rostral cingulate gyrus (Price et al. 1987; McDonald 1991a, b).

The amygdaloid complex is innervated in a heterogeneous manner, in accordance with the diversity of functions assigned to it. Amygdaloid monoamine innervation enters primarily via the ventral amygdalofugal bundle and the stria terminalis (Fallon 1981). DA-ergic fibers arising in the substantia nigra pars compacta (SNc) and VTA (DA cell groups A9, caudal A8 and A10 respectively) project via the ventral amygdalofugal bundle and to a lesser extent the stria terminalis, to the amygdala via the supraoptic decussation, entering the central and medial nuclei of the amygdala (Fallon and Ciofi 1992). Low to moderate DA innervation is found in the anterior amygdala, basomedial and postero-lateral nucleus, and occasional fibers are found in the cortical medial and lateral nuclei (Fallon 1981). Dopaminergic innervation of the amygdala is unique in that SNc and VTA projections to this structure are relatively specific with very few axon collaterals innervating other forebrain areas, unlike the majority of SNc-VTA collaterals which innervate many cortical, striatal and forebrain regions (Fallon and Loughlin 1982; Fallon and Ciofi

1992) Within the amygdala only central nucleus reciprocates DA projections from the VTA (Wallace et al. 1992).

Noradrenergic innervation of the amygdala is more uniform than DA, emanating from the locus coeruleus and the lateral tegmental cell group (NA cell groups A6 and A1-5 respectively) (Fallon and Coifi 1992). Locus coeruleus fibers project via the ventral amygdalofugal bundle and the stria terminalis showing moderate to dense innervation of the central and basolateral nuclei and sparser innervation of the intercalated masses and anterior nuclei. Larger fibers from the lateral tegmental group also project via the ventral amygdalofugal bundle innervating the basolateral and anterior amygdaloid area (Fallon 1981). Serotonergic (5-HT) innervation of the amygdala arises in the dorsal and median raphe (cell groups B7 and B5 respectively) which project to the amygdala via the ventral amygdalofugal bundle and to a lesser degree the stria terminalis. Relative to DA and NA projections, the amygdala receives modest 5-HT innervation which terminates preferentially in the anterior, cortical, basolateral, central and lateral nuclei (Fallon 1981). The amygdaloid complex also receives relatively heterogeneous cholinergic innervation which arises in the basal forebrain and terminates most densely in the BLA (Ohno et al. 1993).

The amygdaloid complex is made up of more than 10 discrete nuclei, which can be divided into 3 main groupings: basolateral (BLA), corticomedial (CmA) and the central nucleus (CeA). Efferent projections from these groups terminate in diverse brain regions and are also thought to be involved in distinct functional processes. The BLA projects to the ventral striatum (Nacc), prefrontal cortex, and has

reciprocal connections with the thalamus, and hippocampus (via the entorhinal cortex; McDonald and Mascagni 1997). The CmA is directly connected with the accessory olfactory system and the CeA is interconnected with the autonomic control regions of the hypothalamus and brainstem (Price et al. 1987). More recently, anatomical work has highlighted further cytoarchitectural divisions between and within each of the discrete amygdaloid nuclei and it has become clear that the amygdala consists of a highly organised array of inter and intra-divisional connections. The lateral amygdaloid nucleus comprises the dorso-, ventro- and medio-lateral divisions each of which can be further delineated into caudal, mid and rostral components. Connectivity patterns within this nucleus are mainly *inter*-divisional. However, the converse is true of the basal amygdaloid nucleus which can be delineated in a similar manner. The majority of basal amygdaloid connections are *intra*-divisional, with fewer inter-divisional connections, of which a small number are reciprocal. Lateral, basal and accessory basal nuclei all project to the CeA, which in turn projects heavily to the hypothalamus and brainstem nuclei and is a major output nucleus of the complex. Divisional connections within and between these nuclei appear to distribute sensory inputs to other amygdaloid nuclei in parallel, therefore allowing information to be assimilated by different functional systems simultaneously (Pitkänen et al. 1997).

Sensory inputs from cortico-amygdaloid projections are directed primarily to the lateral, basal and accessory basal nuclei (Turner 1981; Witter et al. 1989) and projections from the amygdala to the Nacc stem almost exclusively from the BLA (Kelley et al. 1982). It would appear therefore, that the BLA is in good position to

influence both the motor system, via the ventral striatopallidal system, and the extended amygdala which has outputs to autonomic and somatosensory centres in the hypothalamus and brain stem (Carlsen 1989). Mogenson and Nielsen (1984) proposed that limbic and particularly BLA connections with the ventral striatum (Nacc) may be important for the dynamic integration of sensory information and the subsequent selection of appropriate behavioural actions. In light of putative role of the BLA in tasks involving conditioned reinforcement with natural reinforcers (Cador et al. 1989; Everitt et al. 1989; 1991; Burns et al. 1993) it possible that the BLA is also an important component in the neural mechanisms underlying conditioned associations in drug-seeking behaviour and cue-elicited drug craving.

Theories of Drug Addiction

Most theories concerning the psychological and neurobiological factors underlying drug addiction can be divided into two main categories depending on the incentive motivational valence thought to perpetuate compulsive drug use i.e. whether the pattern of drug-related behaviour is supported by negative or positive reinforcement.

Theories of negative drug reinforcement are based on the suggestion that opioid-like drugs which produce clear physical dependence, tolerance, and symptoms of physiological withdrawal in abstention, sustain drug-seeking behaviour because they *alleviate* physical discomfort and pain (Wise and Bozarth 1987). In a similar way, negative reinforcement theories propose that psychomotor stimulants which do not appear to produce clear physical withdrawal symptoms (Wise 1987; Koob and Bloom 1988; Jaffe 1992) sustain drug-taking behaviour because they *alleviate*

'psychological distress' which develops following extended drug use (Gawin et al. 1984, 1986). In contrast, positive drug reinforcement theories propose that drugs of abuse establish and maintain drug-seeking behaviour because they *produce* positive internal states, often associated with subjective feelings of pleasure (Wise 1987).

It has been known for some time that neural adaptations occur as a result of exposure to drugs of abuse. For instance, intra-Nacc amphetamine micro-injections have been shown to decrease thresholds for intra-cranial electrical self-stimulation (ICSS) in rats (Broekkamp et al. 1975). This suggests that psychomotor stimulants act to sensitise brain reinforcement systems and that optimal levels of ICSS reinforcement may be achieved by lower electrical currents as a result of psychomotor sensitisation. This is a short-lived effect and converse findings have been reported in rats withdrawn from chronic amphetamine or cocaine self-administration which may reflect the development of tolerance to the properties of psychomotor stimulants (Koob and Bloom 1988).

In general, rates of drug self-administration are remarkably consistent in rats given limited daily access to psychomotor stimulants (e.g. 2hr/ day). Yet when permitted free access to these drugs they invariably 'binge' for long periods, often administering lethal overdoses within a matter of days (Bozarth and Wise 1986; Koob and Bloom 1988). This pattern of behaviour is very similar to that observed in human cocaine addicts who typically binge and remain 'high' for several days at a time, before the ensuing 'crash' when their drug supply runs out. A cocaine 'crash' is considered to be an adaptive rebound condition which occurs following

prolonged, powerful activation of positive reinforcement mechanisms and is associated with an affective state similar to depression - sleepiness, dysphoria, anhedonia and general psychological distress (Gawin and Kleber 1986, Wise 1987, Koob and Bloom 1988). Proponents of *negative* reinforcement argue that these symptoms act as potently as opioid related physical withdrawal symptoms to maintain the cycle of drug-seeking behaviour and development of addiction.

There is, however, less conclusive evidence to support the view that negative reinforcement is involved during the initial acquisition of drug-taking behaviour. Both rats and monkeys readily self-administer psychomotor stimulants and opiates without prior drug experience (Jaffe 1992) and many do so repeatedly without producing signs of dependence (Deneau et al. 1969). Therefore, the ability of a drug to induce positive reinforcement appears to make an important contribution to the establishment of drug-taking behaviour; but this finding, in itself, is not an explanation of that effect (Wise and Bozarth 1987). Opiates are known to produce physiological dependence and tolerance with repeated use and chronic cocaine administration may act to de-sensitise positive reinforcement mechanisms (Wise 1987), which lead to the development of tolerance to the drug effects. Positive reinforcement theories fail to justify both the persistence of drug-craving and the propensity for relapse in chronic drug users who commonly report diminished satisfaction and pleasure from their drug-taking experiences (Robinson and Berridge 1993).

Elements of both positive and negative drug reinforcement theories appear attractive but neither one fully accounts for the way in which drug-taking behaviour is acquired and the process by which this behaviour is maintained. Robinson and Berridge (1993) have argued that a comprehensive theory of problem drug use must explain three main aspects of addictive behaviour:

i) drug craving and the development of compulsive drug-taking behaviour
ii) mechanisms underlying the enduring propensity for relapse
iii) the subjective dissociation between 'wanting' and 'liking' drugs of abuse.

In rats, repeated *low dose* administration of psychomotor stimulants or opiates have been shown to induce behavioural sensitisation (increased locomotor activity) in response to subsequent drug administrations and to engender conditioned locomotor activity in the absence of drug, when an animal is re-exposed to the previously drug-paired environment (Shuster et al. 1975; Gold et al. 1988). Behavioural sensitisation to psychomotor stimulants has also been reported to facilitate the acquisition of both cocaine (Horger et al. 1990) and amphetamine (Piazza et al. 1989) self-administration in the rat. In addition, rats exhibit reliable conditioned place preference (CPP) for a distinct drug-paired environment over an equally distinct saline-paired environment (Van Der Kooy 1985). Furthermore, microdialysis studies have shown that cocaine related cues enhance mesolimbic DA release in the rat, when presented in the absence of the drug reinforcer (Fontana et al. 1993).

Robinson and Berridge (1993) argue that a fundamental property of addictive drugs is their ability to induce neural sensitisation to the incentive motivational properties of a drug (several studies have demonstrated significant cross-sensitisation between both opiates and psychomotor stimulants). Thus, discrete cues which are repeatedly paired with the euphoric experiences of early drug-use gain motivational significance and (as CRs) evoke increasingly powerful stimulus control. Compulsive drug-use emerges as an addict's behaviour is progressively controlled by these drug-related CRs at the expense of all other interests. Despite experiencing less and less satisfaction from their continuing drug-use, persistent drug-seeking and drug-taking behaviour is maintained in addicts by these drug-related CRs and the 'high' they symbolise (Robinson and Berridge 1993). Reports that cocaine and opiate addicts experience enhanced craving when exposed to discrete or environmental cues associated with the procurement of drugs (Childress et al. 1986, 1987, 1996; Ehrman 1992; Grant 1996; Mass et al. 1998) lend weight to this theory, although negative drug reinforcement theorist would suggest that drug related cues enhance craving because they elicit withdrawal like symptoms and associated dysphoria (Gawin and Kleber 1986).

In conclusion, it would appear that enhanced sensitivity to drug-related CRs are involved in the development of problem drug use and addiction, irrespective of whether they illicit positive or negative reinforcement. Studies carried out in the early 70's indicated that the expression of behavioural sensitisation to psychomotor stimulants could be enhanced if an animal was exposed to an environment which had been repeatedly paired with the drug (Tilson and Rech 1973). This indicated

that behavioural sensitisation relied not only on the pharmacological properties of a drug but also on a significant experiential component, termed conditioned drug effects. More recently it has been proposed that conditioned drug effects enhance the reinforcing potency and addicitve quality of psychomotor stimulants (Carey and Gui 1998). As the BLA is known to play an important role in the organisation and assembly of direct sensory inputs (Pitkänen et al. 1997; McDonald and Mascagni 1997) and is thought to be involved in the formation of conditioned associations (Cador et al. 1989; Everitt et al. 1989; Robbins et al.1989; Burns et al. 1993) it is also possible that the BLA mediates conditioned drug effects which support the development and maintenance of drug addicition.

Methods of assessing behavioural effects of drugs

The behavioural actions of a drug may be assessed in a number of different animal models such as locomotor activity, conditioned place preference (CPP), and drug discrimination. Briefly, drug-related locomotor activation and the capacity of a drug to establish conditioned locomotor activity can be measured in a computer-controlled locomotor cage in which successive photo-beam interruptions and breaks can be recorded (Burns et al. 1994). Conditioned place preference (CPP, described earlier) is thought to give an indication of the reinforcing value of a drug and is demonstrated by the bias in time a rat spends in or out of a choice chamber, following discrete pairings of drug or saline with one or other of two adjoining chambers (Van Der Kooy 1985). Drug discrimination on the other hand is based on an operant food reinforcement paradigm: animals are initially trained to lever respond for food reinforcement and later the appropriate lever response is made

contingent on the animals internal state. For example, pretreatment with drug x supports left lever reinforcement, whereas pretreatment with drug y supports right lever reinforcement. Test sessions investigate the discriminative stimulus properties of an unknown drug by assessing the bias in lever responding following pretreatment with that drug. A drug can be assumed to possess discriminative stimulus properties similar to one or other of the training drugs depending on the response bias observed (Stolerman 1992). Similar techniques have also been used to evaluate anxiolytic compounds and to assess to what extent behavioural states induced by anxiogenic compounds generalise to natural stressors such as aggressive defeat (Velluci et al. 1988).

All of these methods provide useful information about the central actions of a particular drug and the way in which these properties may generalise to those of other known drugs (drug discrimination). However, to fully understand the neural mechanisms underlying the effects of drugs of abuse it is vital that an animal is able to control and regulate it's own drug consumption. Amygdala DA levels and the firing pattern of Nacc neurons have been reported to increase significantly during self- but not passive administration of cocaine (Wilson et al. 1994; Carelli et al. 1993). These findings may indicate that action-outcome contingency in cocaine self-administration (i.e. lever response-drug reinforcement) also makes a significant contribution to the pharmacological actions of cocaine which may, in turn, influence the development of conditioned associations and therefore cue-elicited cocaine-seeking behaviour. Models of drug self-administration are, therefore, uniquely suited to this purpose and allow for the systematic analysis of drug-taking and drug-

seeking behaviour. For drug self-administration each animal is surgically implanted with a chronic indwelling IV catheter which can be connected via a swivel to an automated pump during self-administration session. A fixed dose of drug is delivered, contingent upon every 'correct' operant response. By pairing each drug delivery with a discrete stimulus (such as a brief light presentation) it is possible to assess the neural mechanisms underlying the rewarding properties of a drug as well as the impact of discrete cues on subsequent drug-seeking behaviour.

Cocaine self-administration was first reported in primates (Deneau et al. 1969) and has since been developed in rats (Dougherty and Pickens 1973; Corrigal and Cohen 1989) dogs (Risner and Jones 1980) and mice (Deroche et al. 1997). Many initial investigations in the primate concentrated on the pharmacokinetic and discriminative stimulus properties of cocaine, although psychological dependence was already considered an important factor in the maintenance of cocaine self-administration (Deneau et al. 1969). There are a number of different schedules by which drug self-administration can be assessed: continuous reinforcement (CRF), fixed-ratio (FR), fixed interval (FI), progressive-ratio (PR) and second-order schedules of drug reinforcement (Pickens and Thompson 1968; Goldberg 1973; Johanson and Shuster 1981; Corrigal and Cohen 1989). Drugs which maintain self-administration typically produce an inverted U-shaped function as drug dose is plotted plotted against operant response rate (Woods et al. 1987). Once a steady rate of self-administration is achieved under a schedule of continuous reinforcement, systematic reductions in drug dose produce reciprocal increases in operant response rates; similarly increments in drug dose result in reductions in overall response rate.

Drug pretreatments or neural manipulations which significantly shift this inverted U-shaped function to the left or right of baseline recordings are taken to represent alterations in the perceived rewarding effect of the self-administered drug (Wilson et al. 1971; Kelleher 1975).

Psychomotor stimulants are known to induce dose-dependent locomotor activation and stereotyped behaviours (Woods et al. 1987) which may physically interfere with operant responding during a self-administration session such that response rates increase or decrease for reasons unrelated to the reinforcing effect of the drug. Continuous reinforcement, fixed-ratio, fixed-interval and progressive-ratio schedules of drug self-administration all measure operant responding which may be confounded by the direct actions of the drug because all responses recorded (with the exception of the first, first-ratio or first-interval) are under the influence of the self-administered drug i.e. drug-driven.

In contrast, second-order schedules of drug reinforcement can be used to evaluate motivational processes unconfounded by the direct effects of a drug reinforcer (Goldberg 1973; Goldberg et al. 1976; Goldberg and Tang 1977). When the specific interest is in assessing cue elicited drug-*seeking* behaviour, and the effect of specific neural manipulations on this behaviour, it is clearly of benefit to arrange an extended period of responding in which behavioural control exerted by a drug-related conditioned reinforcer (CS) can be demonstrated prior to the first drug infusion. Both rats and monkeys have been shown to work under a fixed-ratio schedule of drug self-administration and typically produce 'scalloped' response patterns when

drug delivery is paired with the brief presentation of a CS during FI schedules (Goldberg 1973; Kelleher 1975; Goldberg et al. 1976; Goldberg and Tang 1977; Corrigal and Cohen 1989).

Under a second-order schedule of drug reinforcement, responding for the first drug infusion is maintained by the presentation of a drug-related CS and the response requirement for each CS presentation can be systematically increased over subsequent sessions. Therefore deficits in incentive motivational processing following neural manipulations or drug treatments may be reflected in the pattern of operant responding recorded prior to the first drug infusion, and unconfounded by the direct actions of the drug.

Experimental plan

The objective of the present work was to assess the role of the BLA in an animal model of drug-seeking behaviour. Following the findings of Cador et al. (1989), Everitt et al. (1989, 1991), and Burns et al. (1993, 1994) it has become clear that the BLA is an important component underlying the development of conditioned reinforcement, i.e. the process by which neutral stimuli gain incentive salience and subsequently sustain patterns of behaviour despite the absence of primary reinforcement. Conditioning is also thought to play an important role in the development and maintenance of cocaine abuse (Gawin et al. 1984, 1986; Childress et al. 1987, 1988; Gawin 1991; Ehrman 1992; Grant et al. 1996; Mass et al. 1998) and recently the BLA has also been implicated in cue-elicited re-initiation of responding for a cocaine-related conditioned stimulus (Meil and See 1997). It is

proposed, therefore, that if the BLA is involved in the mediation of stimulus-reward associations which also form the basis of cocaine-seeking behaviour animals with excitotoxic lesions of the BLA will be specifically impaired in the acquisition of second-order schedule of cocaine self-administration in which performance relies on the formation or utilisation of cocaine-related conditioned stimuli (CS). The experiments undertaken aimed to replicate and extend the findings of Everitt et al. (1989) who demonstrated that male rats with excitotoxic lesions of the BLA were impaired, relative to controls, in the maintenance of a second-order schedule of sexual reinforcement.

First, the effects of excitotoxic lesions of the BLA were assessed on the primary reinforcing properties of cocaine, during the acquisition and maintenance of cocaine self-administration and in response to the locomotor stimulant propterties of IP cocaine injections. The effect of BLA lesions on drug-seeking behaviour maintained by the periodic presentation of a cocaine-related CS, in the absence of cocaine, were assessed using a second-order schedule of cocaine self-administration. These findings were then compared with the effects of BLA lesions on drug-seeking behaviour under the influence of cocaine, using a progressive-ratio schedule of cocaine self-administration. The importance of cocaine-paired conditioned stimuli in the maintenance of cocaine-seeking behaviour in both BLA-lesioned and control animals were then assessed under a fixed-interval 15 min schedule of cocaine self-administration, by the omission and reinstatement of a drug-paired CS. The use of a fixed-interval schedule allows for differences in the effects of CS manipulations to be assessed, both under the influence of cocaine (second interval) and prior to the

first drug infusion, drug-free (first interval). Finally, the neurochemical response to either intravenous or intra-nucleus accumbens infusions of cocaine were assessed using *in vivo* microdialysis in both BLA-lesioned and control animals. The results of this experiment provide insight into the neurochemical correlates of excitotoxic lesions of the BLA and, furthermore investigate the ways in which the BLA is involved in mediating the reinforcing properties of cocaine. Thus, this thesis is concerned primarily with understanding the neural mechanisms which may underlie cue-elicited cocaine-seeking behaviour.

Chapter 2: Materials and methods

This chapter contains details of methods and procedures which were common to all experiments. Where modifications were adopted for any specific experiment this is indicated in the appropriate chapter. Details of the nature and quantities of reagents used in the preparation of chemical solutions are given in Appendix 1 and 2, as are construction details of certain items of equipment fabricated in the laboratory.

Animals

Pairs of male Lister Hooded rats (Olac, Bicester, UK), weighing between 300-350g at the start of experiments, were housed in smooth-floored holding cages with sawdust bedding under a 12hr: 12hr reversed light/ dark cycle (lights off at 0900hr). Following the initial acquisition of drug self-administration (seven consecutive days), experiments were carried out from 0900-1830hr for six consecutive days each week. Food was made available at the end of the each day between 1900-2000hr. Each animal received 20g of Purina Lab chow/ day, sufficient to maintain preoperative weights. Water was freely available in the home cage. All experiments were undertaken in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act; Project Licence PPL 80/00684.

Stereotaxic surgery

At the time of surgery, animals weighed between 300-350g. Generally excitotoxic lesions of the basolateral amygdala (BLA) were carried out prior to intravenous (IV) catheterisation, which usually took place at least four days later. No testing of

any kind occurred in the first seven days following either lesion or IV surgery; this was the *minimal* time allowed for post-operative recovery.

Excitotoxic lesions of the BLA were made using 0.09M quinolinic acid (Sigma-Aldrich Company, Dorset, UK). This solution was prepared freshly each day in 0.01M phosphate buffer (see Appendix 1a) adjusted to pH 7.0-7.3 with 0.1M NaOH. The acid was kept on ice and protected from light until needed.

Animals were anaesthetised with Avertin (Sigma-Aldrich Company, Dorset, UK), at a dose of 1ml/ 100g body weight/ IP. Details of the preparation of Avertin are given in Appendix 1(b). Once anaesthetised, the rat's head was shaved and swabbed with 70% ethanol before being placed into the stereotaxic frame (Stoelting, Wood Dale, Il., USA). Following the preparation of the skull surface, two infusions of 0.3µl quinolinic acid were made into each hemisphere using a 1µl syringe (SGE, Milton Keynes, UK). The co-ordinates, from Bregma, were: AP: –2.3, –3.0; L: ±4.6; V: –7.3 from the dural surface; incisor bar: –3.3, (Swanson 1992). After each infusion, the syringe was left in position for 2 min at each site to allow complete diffusion of the toxin. The syringe was then flushed repeatedly with double distilled water prior to refilling with quinolinic acid, to ensure that it did not block with blood or other material. Once all infusions had been made the syringe was removed and the initial incision sutured using 5/0 sterile silk (Mersilk; Ethicon Ltd., Edinburgh, UK). Sham-operated controls underwent identical surgical procedures but phosphate buffer vehicle was infused instead of the excitotoxin.

Construction of the intravenous catheter

The component parts of the IV catheter are illustrated in Fig. 2a, b; reference codes and suppliers of the various constituents are listed in Appendix 2(a). The catheters were made from two sizes of silicon tubing (Osteotec Ltd., UK) and guide cannulae supplied by Semat Technical Ltd., St. Albans, Herts, UK. The smaller tubing was cut to lengths of approximately 16 cm and the wider tubing to lengths of approximately 4 cm. Using chloroform to expand the wider tubing, the smaller tubing was slipped inside, until both pieces of tubing were flush at one end. This end was then slipped onto the guide cannula which consisted of a 20 mm stainless steel tube running through, and bonded to, a screw-threaded plastic collar; 5 mm of the guide protruded above the collar and 10 mm below. Before attaching the silicon tubing, the longer end of the guide was curved at right angles to the collar with pliers. Within minutes of being slipped onto the guide cannula the tubing dried and shrank to the original size, creating a tight seal. Next, the end of the catheter which would eventually lie in the right ventricle of the heart was trimmed at right angles with a scalpel blade. Exactly 3.7 cm was then measured from this end and a small 'bobble' of silicon rubber glue (RS Supplies, UK) was used to mark the point. Once the silicon glue had dried the threaded plastic collar was placed into a mould which was used to form the 'pedestal' of the catheter. Dental cement (Associated Dental Products, Swindon, UK) was carefully poured into each mould and, while the cement was still wet, a 3 x 3 cm square of polypropylene marlex mesh (Small Parts Inc. Miami, Florida, USA) was placed on the top of the

Figure 2a Component parts of IV catheter.

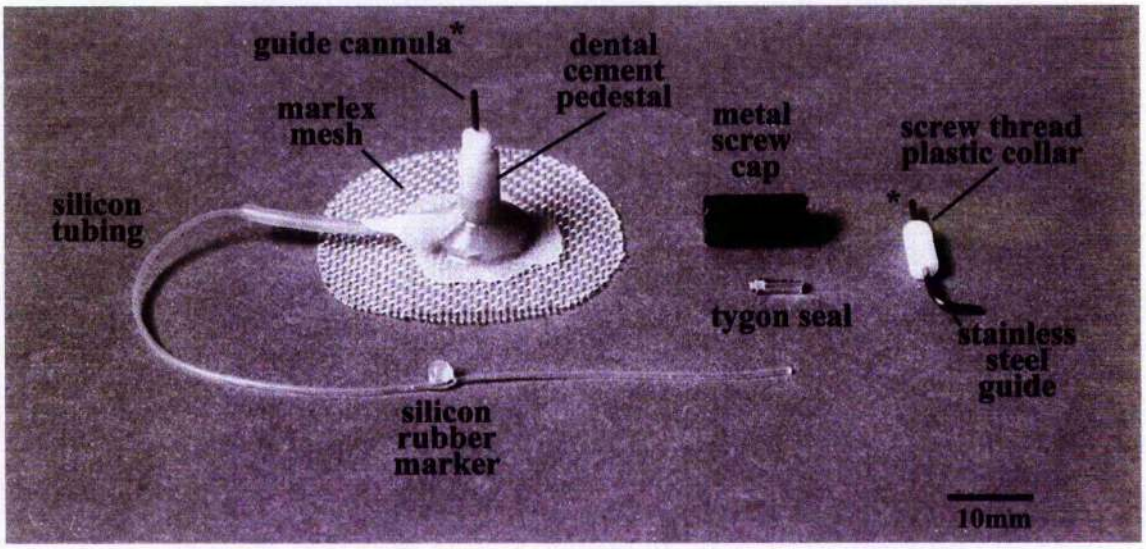
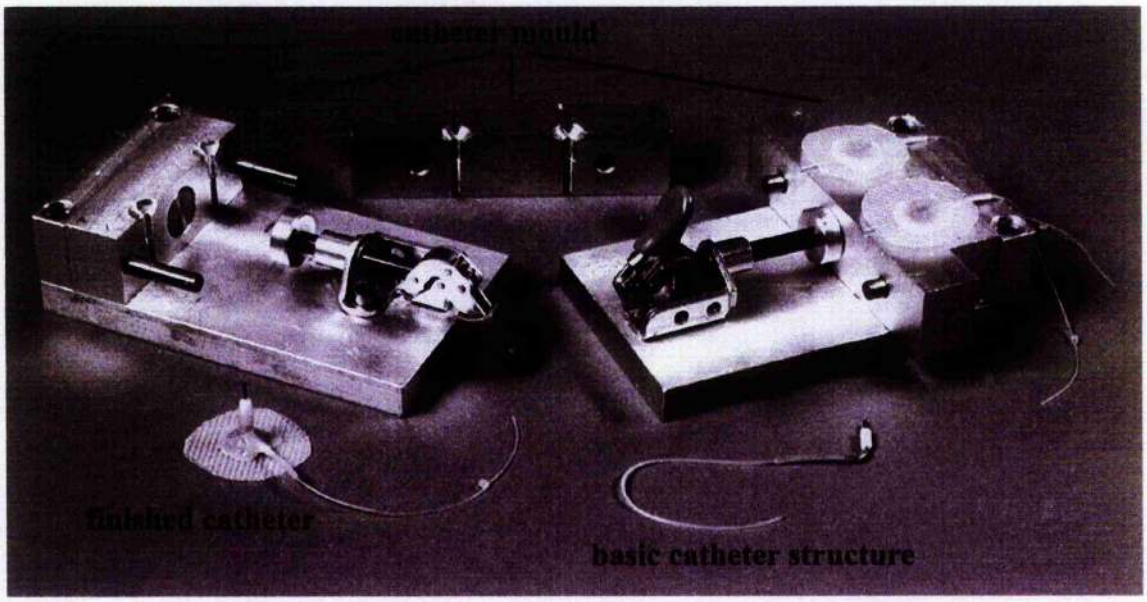


Figure 2b Construction of catheter.



mould to form the base for the pedestal. This was left to dry for several hours or over-night. The catheters were then removed from the mould and the mesh square was trimmed to remove square edges.

Intravenous catheterisation

Following lesion surgery, animals were allowed to recover with free food for four days before undergoing IV catheterisation surgery. On day five, the animals were anaesthetised using Avertin (1ml/ 100g body weight/ IP) and implanted with chronic indwelling intravenous catheters. Prior to surgery, all catheters were soaked in 70% alcohol for at least 30 mins and left to dry thoroughly, wrapped in surgical paper towel. Each rat was shaved between the shoulder blades and down the back in an area approximately 40mm wide by 60mm long; on the ventral side, the area of neck over the right jugular pulse was also shaved. Both areas were then swabbed with distilled water, followed by iodine surgical scrub (Pevidine; BK Veterinary Products Ltd., Bury St. Edmunds, UK). The surgical scrub was then removed with 70% ethanol and the areas finally painted with strong veterinary iodine solution (J.M. Loveridge Plc., Southhampton, UK) and allowed to dry. A 50 mm incision was made caudally from between the shoulder blades. Using small round-tipped scissors the skin was then separated from the underlying muscle layer on all sides of the incision to form a small pocket under the skin. The rat was then turned belly-up and the right jugular vein located; a 15 mm incision was made carefully over the site of the jugular pulse and, using round-tipped scissors, the muscle fibres surrounding the vein were teased apart until the vein was clearly visible and a section, approximately 10 mm long, was freed from all surrounding

fascia. A small channel was then forged under the skin with round-tipped scissors from the incision at the neck, over the shoulder to the incision on the back. Minimal tissue damage or bleeding occurred as a result of this procedure. Once a clean channel had been formed a pair of Spencer-Wells forceps was inserted at the jugular incision and the catheter drawn under the skin from the back incision to the opening at the jugular vein.

A 1ml syringe with a bevelled 23G needle was then attached to the catheter via a length of tygon tubing (Altec PVC tubing, Alton, UK) and sterile saline was flushed through, ensuring the catheter itself was not blocked and also preventing the exposed vein from drying out. A pair of straight forceps was then slipped under the vein and drawn back slightly towards the head, to exert some tension on the vein. Two adapted needles (23G and 21G) which had been filed into canoe shapes were used as guides to help insert the catheter into the vein. The smaller needle was inserted into the taut vein and this was then used to guide the second, larger needle. The first needle was then removed and the catheter was carefully eased into the vein, by picking-up the vein, held fast over the larger needle, with a pair of watch-maker's forceps.

The catheter was inserted 3.7 cm into the vein, to the silicone rubber marker so that the tip of the catheter lay in the right ventricle of the heart. The flow of the catheter was then checked by drawing back on the flusher syringe; if there was any difficulty in drawing blood, adjustments were made to the positioning of the catheter until this was rectified. A series of knots was then tied with sterile 5/0 silk

(Mersilk; Ethicon Ltd., Edinburgh, UK) to secure the catheter in the vein. Catheter flow was monitored continuously throughout this procedure as it was relatively easy to constrict the tubing while tying the knots. Finally the knots were fixed with two drops of 'Superglu' (Bostik Ltd, Leicester, UK). Special care was taken to prevent the glue spreading over the skin and fur of the animal as this was a potential irritant. Once the glue had dried, the incision was irrigated with 0.1ml broad spectrum antibiotic solution (Gentamicin; Life Technologies Ltd., Paisley, UK) and sutured with 5/0 sterile silk. The rat was then turned on it's ventral surface and the pedestal of the catheter was slipped into the pocket under the skin on the back. Care was taken to ensure that the catheter tubing lay as smoothly as possible under the skin. The catheter was then flushed with 0.1ml antibiotic solution (Timentin; Beecham Research, Welwyn, UK; see Appendix 1(c) for composition and preparation) and sealed with a tygon seal. To construct a tygon seal 3 mm acrylic fishing line were pushed into a 7 mm length of tygon tubing and cut flush to make a 'plug'. On the pedestal the tygon seal was then protected by a metal screw cap (RS Supplies, UK) to prevent it being removed or chewed. (Details of the seal and screw cap are given in Appendix 2(b). The dorsal incision was then irrigated with 0.1ml gentamicin solution and sutured with 3/0 sterile silk (Mersilk; Eithicon, Edinburgh, UK). Finally, both incisions were painted with strong veterinary iodine solution and left to dry. The IV catheters were flushed daily with the Timentin solution for five days post-operatively and free access to food was allowed for the first three days. Thereafter, the catheters were flushed daily with 0.1ml heparin solution, to minimise the risk of the catheter blocking (30 units/ ml 0.9% sterile saline; CP Pharmaceuticals Ltd., Wrexham, UK).

Dialysis probe construction

All dialysis probes were made in the laboratory and were of the conventional concentric design as illustrated in Fig. 3. Firstly, 24 G stainless steel cannula (Cooper's Needle Works, Birmingham, UK) were cut in 25 mm lengths and filed at both ends. A 10 mm length of Hospal dialysis tubing (Hospal Industrie, 69330 Meyzieu, France) was then inserted approximately 7 mm into one end of the stainless steel cannula and glued in place with epoxy resin (Araldite; Ciba-Geigy Plastics, Duxford, UK). The membrane was handled with forceps at all times to prevent contamination of the dialysis surface and special care was taken to ensure that the glue did not cover more than 0.2 mm of the membrane. After drying for 1hr, a 10 mm length of rubber sealing tube (Elkay, Shrewsbury, MA, USA) was pushed over the opposite end of the stainless steel cannula. The outer wall of the sealing tube was then pierced with a 30G needle and a 90 mm length of polyamide coated silica glass tubing (SGE; Milton Keyenes, UK) was fed through the needle and down the entire length of the stainless steel cannula into the Hospal membrane. Once the silica tubing was in place the needle was removed and the free end of the silica tubing was trimmed to 30 mm length to form the dialysate outlet. Finally, the Hospal membrane was trimmed to exactly 2.5 mm from the end of the cannula. Epoxy resin was used to carefully plug the end of the membrane (0.5 mm) and excess glue was wiped away from the dialysis surface, leaving a total surface length of 2 mm, suitable for dialysis of the nucleus accumbens (Nacc).

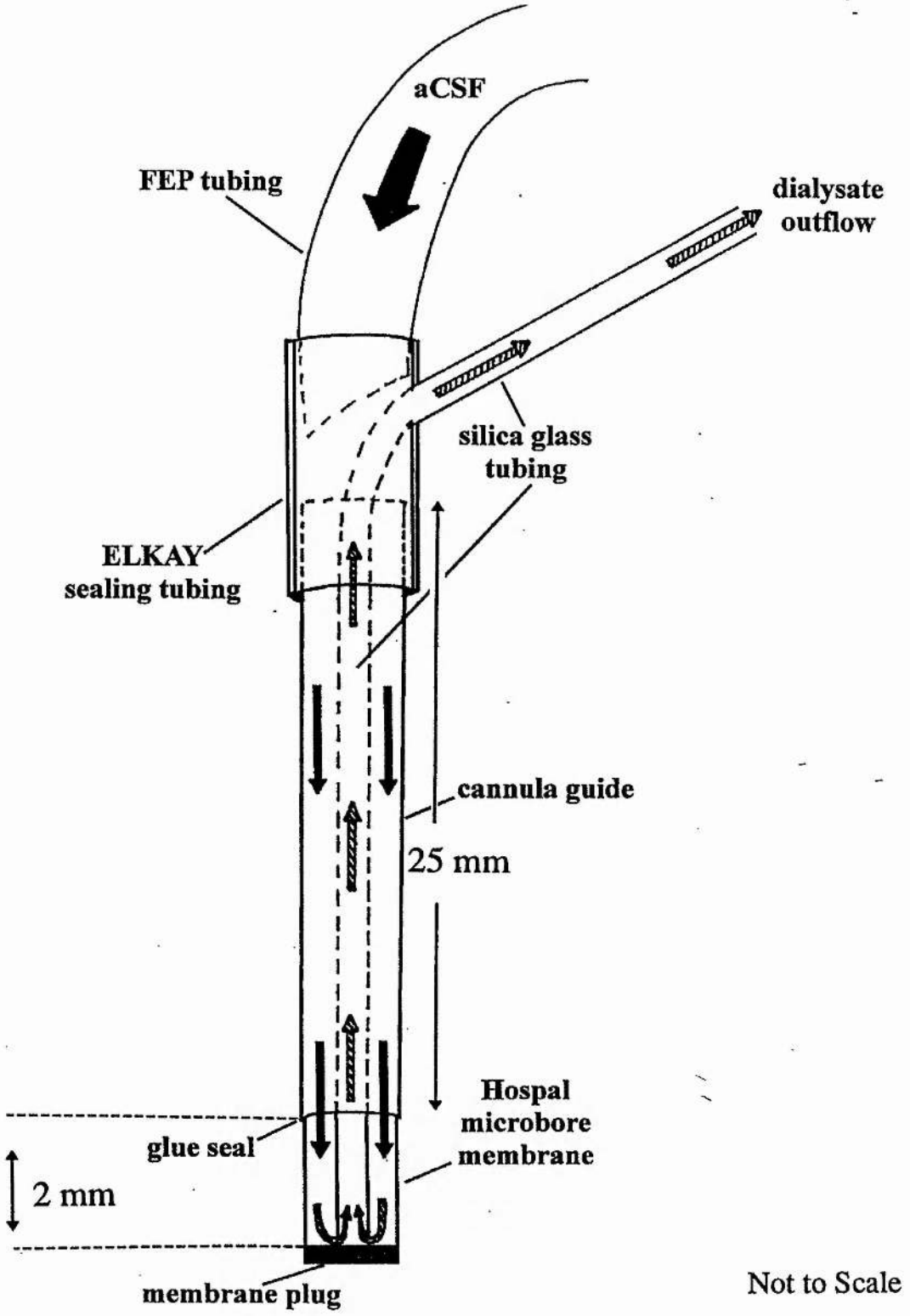


Fig. 3 Schematic representation of a microdialysis probe. Artificial cerebrospinal fluid (aCSF) flow rats = 1µl/ min. Dialysate indicated by striped arrows, dialysate samples collected once every 10 min.

Once the probe was completely dry (at least 1hr) the silica tubing was eased further into the Hospal membrane until it almost touched the end plug. This increased the efficiency of the dialysis process by ensuring that the artificial cerebrospinal fluid (aCSF) had maximal contact with the dialysis surface. The silica tubing was then glued in position at the outlet end and the probes were again left to dry until needed. Details of the component parts used in the construction of the probes and the respective suppliers are given in Appendix 2(c). During dialysis, aCSF entered the probe through the Elkay tubing, flowed down the stainless steel cannula, dialysed across the 2 mm Hospal membrane and the dialysate was then driven up through the silica glass tubing and collected in a sample vial.

Perfusion and histological assessment

At the end of each experiment animals were sacrificed under deep pentobarbital sodium BP anaesthesia (Euthatal, 1.5 ml; Rhone Merieux Ltd., Harlow Essex, UK) and perfused transcardially with 0.9% saline solution for 3 min followed by 4% paraformaldehyde (PFA) for 6 min (Merck, Darmstadt, Germany). The brains were then removed, post-fixed in 4% PFA for 2hr and then left overnight in 20% phosphate buffered sucrose solution, to dehydrate the tissue. Once the brains had 'sunk' they were blocked and placed individually on a freezing microtome platform (Anglia Scientific, Norwich, UK); coronal sections of the BLA were cut at 60μm and mounted on microscope slides (Scientific Laboratory Supplies Ltd., Wilford, UK), pretreated with a gelatine solution dip (BDH Laboratory Supplies, Poole, UK). Details of the preparation of PFA and gelatine solutions are given in Appendix 1(d) and (e).

Nacc probe placement was assessed following the dialysis study (see Chapter 6 p138). The brains were bisected between the Nacc and the BLA. The Nacc was cut horizontally so that the depth and placement of the dialysis probe could be established whereas the BLA was cut coronally in the normal manner. The slides were left to dry fully for 2-3 days before staining with Cresyl Fast Violet nissl cell body stain (Raymond A Lamb, London, UK).

Cresyl Violet staining

Slides were loaded into carriages which hold 15 slides at a time. Each carriage was taken through a series of solutions to dehydrate the sections and prepare them for staining. The sections were first placed in absolute alcohol for 3 min, followed by 95% alcohol for 3 min and finally 70% alcohol for 3 min. They were then briefly washed in ultra pure water before being placed in the stain solution for 2-4 min. The process was then repeated with the order of solutions reversed. The slides were briefly washed again in ultra pure water, followed by 70% and 95% alcohol in which the sections were left for the differentiation of the stain. This was determined by eye, until the desired intensity of stain was achieved (between 2-3 min). The slides were again briefly washed in absolute alcohol before being placed in a histological clearing agent (Histoclear; National Diagnostics, Atlanta, Georgia, USA) for at least 3 min. Each slide was individually removed from the carriage and several drops of a mounting agent (DePeX; BDH Laboratory Supplies, Poole, UK) were dropped onto the sections. A coverslip (Scientific Laboratory Supplies Ltd.,Wilford, Notts. UK) was then carefully placed on top and pressed down slowly from one end to exclude all air bubbles. The slides were then laid out to dry for

several days before excess glue was peeled off and each slide was polished, ready for microscopic assessment.

Histological assessment

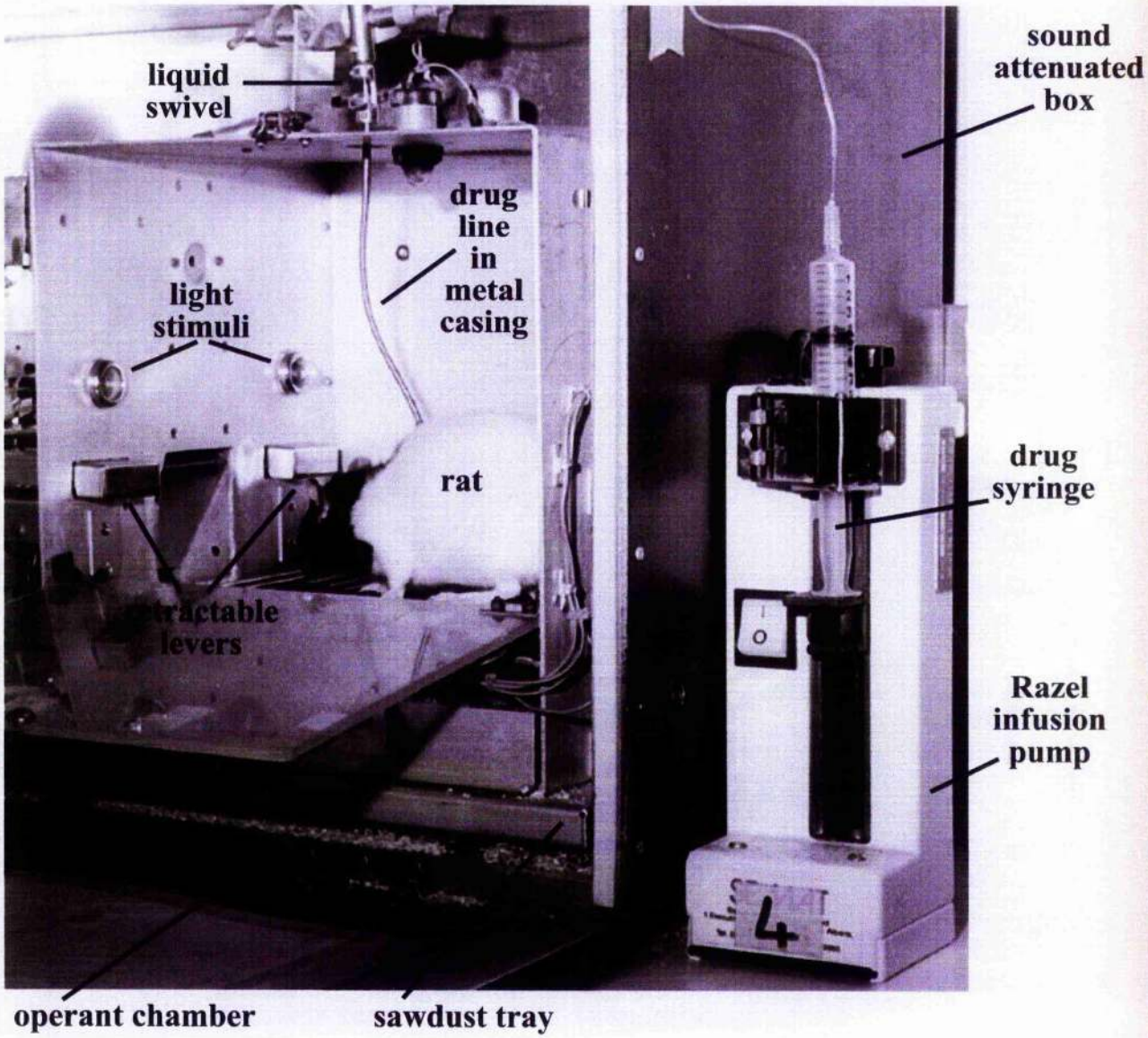
Lesions were assessed according to the pattern of neuronal loss. The areas of neuronal loss were mapped individually onto schematic representations of coronal slices of the brain (Swanson 1992). Assignment of the presence of bilateral or unilateral BLA lesions or damage to other structures such as the central amygdaloid nucleus, perirhinal or entorhinal cortex, was done by an observer 'blind' to the experimental conditions. Nucleus accumbens probe placements were assessed in the same manner by schematic representation of the position and depth of the dialysis probe. Photomicrographs of representative lesions of the BLA are shown in Chapter 3 p53-54.

Self-administration apparatus

Experiments were carried out using six operant chambers, four of which were Gerbrands (Gerbrands Corporation, Arlington, Mass, USA) and two of which were Campden (Campden Instruments Ltd., London, UK) (Fig. 4). The dimensions were 26 x 28 x 28 cm, and 24 x 26 x 26 cm respectively. Each chamber was housed in a sound-attenuated box with an infusion pump outside. External noise was further masked by ventilating fans mounted on the side of each box. The apparatus was controlled and data was collected by an Acorn Archimedes microcomputer (Acorn Computers Ltd., Cambridge, UK), running the control language Arachnid (an extention of BASIC; Fray 1980).

Figure 4

Self-administration Chamber



The total number and temporal pattern of responses was recorded for each lever. Each chamber contained two retractable levers 4.8 cm wide, positioned equidistantly on one wall, 17.5 cm apart and 9 cm from the grid floor. The chamber was illuminated by a red-enamelled 2.5W, 24V house light, positioned on the top of the chamber. A circular opaque disc 2.5 cm in diameter, positioned 10 cm above each lever could also be illuminated by a 2.5W, 24V light bulb as a stimulus light. Each box was equipped with a Razel infusion pump (Semat Technical Ltd., St. Albans, Herts, UK) which was operated via computer controlled software. Intravenous infusions of cocaine were delivered via tygon tubing (Altec PVC tubing, Alton, UK) through a single channel liquid-swivel (Stoelting Wood Dale, Ill., USA) with connector attachments (Lomir Biomedical Inc., Quebec, Canada). Within the chamber the tygon tubing was protected by a metal spring casing (Mackays, Cambs, UK). Three rapid presses on either lever initiated the session, signalled by illumination of the house light. The initiating presses automatically registered this lever as the *drug* lever; responses on the other lever had no programmed consequence and this was designated the non-reinforced lever, providing an index of basal levels of activity only. Subsequent depression of the drug lever caused the house light to extinguish and the drug stimulus light to be illuminated. Both levers then simultaneously retracted and the infusion pump was activated for 4s, delivering 0.1ml intravenous infusion of cocaine solution. After a further period of 16s, the levers were again extended into the chamber, the stimulus light went out (total duration 20s) and the house light was illuminated. Further depression of the drug lever repeated this sequence of events and resulted in further infusions of cocaine.

Locomotor Activity

Locomotor activity was measured using 16 fixed wire activity cages (25 x 40 x 18 cm), each fitted with two parallel infra-red photo beams, 2 cm from the cage floor and 20 cm apart on the long axis of the cage. Successive or continuous beam interruptions were processed by a BBC Master computer (Acorn Computers, Cambridge, UK) situated in an adjacent room. Activity counts were recorded cumulatively in 10 min time bins.

Microdialysis

Microdialysis 10μl syringes (SGE UK, Milton Keynes, UK) were used in a Harvard micropump (Harvard Apparatus, Southnatick, Mass., USA). FEP microbore tubing (Biotech Inst., Kimpton, UK) which has exceptionally small dead volume (1.2μl/ 100 mm), was used for the flow lines. A tubing kit (Biotech Inst., Kimpton, UK) was used to connect the FEP tubing to syringes and to a 3-way liquid-switch (Biotech Inst., Kimpton, UK). Details of these components are given in Appendix 2(d).

HPLC Analysis

Dopamine (DA) was determined in brain dialysates by high performance liquid chromatography and electrochemical detection (HPLC-ECD). Dialysates were injected via a 9125 Rheodyne 5μl loop valve (Rheodyne; Cotati, CA, USA) onto the HPLC system (BAS LC-4C; BAS, West Lafayette, IN, USA). Monoamines were separated at ambient room temperatures (22°C) on a microbore C18 column (Sep Stik 50DS 100 x1mm) and detected by oxidation (+750 mV). The mobile

phase (pH 2.7) consisted of sodium dihydrogen orthophosphate (2.3 g/l), trisodium citrate (8.82 g/l), triethylamine (1000 µl/l), sodium octysulphonic acid (500 mg/l) ethylenediaminetetraacetic acid (10 mg/l), methanol (22.5% v/v) and was delivered at 50 µl/ min. The absolute detection limit of dopamine was approximately 1 fmol (1×10^{-15}).

Dialysate levels of taurine and glutamate were determined by conventional HPLC and fluorescence detection (254 nm) at room temperature (22°C). Prior to injection, samples were derivitised with an equal volume of β-mercaptoethanol/ o-phthaladehyde (β-ME/ OPA) (Fisher Scientific; Loughborough, UK) working reagent (6µl) for exactly 2 min. Each sample was then injected via a 7125 Rheodyne valve (Rheodyne; Cotati, CA, USA) in a loop volume of 10µl onto a Hypersil analytic column (3ODS: 80 x 4.6mm). The β-ME/ OPA stock solution was prepared weekly (27 mg OPA dissolved in 1ml methanol, 9ml of 0.2M potassium tetraborate (pH10) and 5µl β-ME) and stored at 4°C, protected from light. OPA working reagent was prepared each day by diluting the OPA stock solution with 0.2M potassium tetraborate (pH10) buffer (1:3) and stored on ice.

Drugs

Cocaine hydrochloride (McFarlan-Smith, Edinburgh, UK) was dissolved in sterile 0.9% saline (Animalcare; Dunnington, UK). All doses of cocaine were calculated as the salt. Quinolinic acid (Sigma-Aldrich Company Ltd, Dorset, UK) 0.09M was dissolved in sterile phosphate buffered saline and the pH was adjusted to between 7.0-7.3, with 0.1 M NaOH. For dialysis, artificial cerebrospinal fluid (aCSF) was made up daily from the stock solution (see Appendix 1(f) for composition).

Dialysis cocaine solutions were also made up daily from 1nM stock solution and dissolved in aCSF. The potassium solution (see Chapter 6 p153) was made up by replacing 60nm of the sodium chloride solution with potassium chloride; thus the potassium solution contained 87nm NaCl and 60nm KCl. Chlordiazepoxide ($C_{16}H_{14}CIN_30.HCl$; Sigma Chemicals Co St. Louis, MO, USA) was made up daily from the salt, dissolved in ultra-pure water and protected from light until needed.

Statistical analyses

All statistical analyses excepting the Fisher Exact Probability test were carried out using CLR ANOVA (Clear Lake Research, USA) for Apple Mac computers, or SPSS version 6.0 for Windows (SPSS Inc. Chicago Il, USA) for IBM compatible computers. Simple Effect and Newman-Keuls statistical tests were used post-hoc, where applicable.

Chapter 3: Acquisition of intravenous cocaine self-administration

Introduction

Earlier studies of conditioned reinforcement have demonstrated that intra-nucleus accumbens (intra-Nacc) microinjections of amphetamine, dose-dependently enhance lever responding for the presentation of a conditioned stimulus (CS) which had previously been paired with the delivery of a natural reward during training. Therefore, the capacity of a previously neutral stimulus to exert behavioural control is potentiated by the action of amphetamine in the Nacc (Taylor and Robbins 1984, 1986). Later studies demonstrated that conditioned reinforcement was blocked by excitotoxic lesions of the basolateral amygdala (BLA) (Cador et al. 1989; Robbins et al. 1989; Everitt et al. 1989; 1991) and it was concluded that BLA efferent projections convey important associational information to the ventral striatum (Taylor and Robbins 1986; Robbins et al. 1989; Wolterink et al. 1993; Burns et al. 1993; 1996) and that these projections underly the process of conditioned reinforcement. Several reports have implicated conditioning as an important component in the development and persistence of drug-taking and drug-seeking behaviour, particularly with respect to cocaine abuse (Grant 1996; O'Brien and McLellan 1996; Gawin 1991; Childress et al. 1988, 1987; Gawin and Kleber 1986, 1984). There is little substantial evidence that cocaine produces physiological dependence, yet it is considered a highly addictive drug (Volkow et al. 1996) and it is possible that cocaine alos acts to potentiate conditioned associations in a manner similar to that described in the work of Taylor and Robbins (1984) above. As the BLA is known to be involved in the process of conditioned reinforcement the experiments

reported in this chapter aimed to investigate the role of the BLA in the acquisition and maintenance of cocaine self-administration in the rat, to determine whether the BLA is also involved in drug-seeking behaviour.

First, three separate groups of animals were assessed in their acquisition of cocaine self-administration at three different doses of cocaine (either 0.5, 0.25 or 0.083 mg cocaine/ infusions). Approximately half the animals in each group were surgically prepared with excitotoxic lesions of the BLA while the remaining animals were given sham-lesions of the BLA with phosphate buffer replacing the toxin (exact group numbers are reported in the results section of each experiment). Comparing the rate with which lesioned and control animals acquired cocaine self-administration at each dose would indicate whether or not excitotoxic lesions of the BLA interfered with the primary reinforcing properties of cocaine. An alteration in the reinforcing efficacy of cocaine would be evident as a shift to the left or right in the acquisition dose-response function produced by lesioned animals relative to controls.

Second, in a separate group of unlesioned animals, the role of the conditioned light stimulus (CS) paired with each drug infusion was investigated. During the acquisition of cocaine self-administration each infusion of cocaine administered was accompanied by the brief illumination of a light stimulus, positioned above the designated drug lever (detailed in Chapter 2 p40). In this experiment, both groups were allowed to self-administer cocaine for 2hr each day, but only half of the animals were presented with the brief light stimulus with each infusion of cocaine

(normal CS-drug condition) while the remaining animals were never presented with the light stimulus (No-CS condition). Differences in the rate with which each group acquired cocaine self-administration could be attributed to the presence or absence of the CS light and therefore, would indicate whether or not the CS light was an important component in the acquisition of cocaine self-administration.

In the third experiment, the role of the BLA on the maintenance of cocaine self-administration was assessed. Half of the animals from the normal CS-drug condition in Expt. 2 were given excitotoxic lesions of the BLA while the remaining animals were prepared with sham-lesions. Following a period of recovery, both groups were allowed to re-acquire cocaine self-administration. Differences in the rate at which each group re-acquired cocaine self-administration could therefore be attributed to a loss of BLA neurons and indicative of BLA involvement in the maintenance of cocaine self-administration.

Finally, in the fourth experiment, locomotor stimulant properties of intra-peritoneal (IP) cocaine injections were assessed in BLA-lesioned and sham-operated control animals. Brown and Fibiger (1993) reported that excitotoxic lesions of the amygdala which encompassed both the basolateral and central nuclei did not affect the locomotor stimulant properties of a single IP injection of cocaine. The present experiment assessed the effect of discrete BLA lesions on the locomotor stimulant properties of three doses of IP cocaine, in animals with no prior drug experience (i.e. drug-naive). As the BLA is considered to be involved in the mediation of

discrete conditioned associations, it was predicted that both lesioned and control animals should respond similarly to non-contingent injections of cocaine.

Methods

All procedures are outlined in Chapter 2 (p28-44). In each experiment, rats were assigned to an operant chamber and specific drug and non-reinforced levers which were counterbalanced within each group. These parameters remained constant for each rat throughout each experiment. With the exception of the No-CS group in Expt. 2, all animals experienced the following sequence of events during the acquisition of cocaine self-administration. First, depression of the assigned drug lever resulted in the house light being extinguished followed by a 1s delay before the presentation of a 20s light stimulus (CS). Second, each infusion of cocaine (0.1ml) was administered intravenously over the first 4s of each CS presentation. Access to the drug was restricted to 2hr/ day for 7 consecutive days. The number of infusions administered and responses made on the non-reinforced lever were recorded and compared between the groups. In an attempt to maximise potential drug-related, conditioned stimuli effects, the animals in the present experiments were never administered non-contingent, 'priming' infusions of cocaine.

Statistical analyses

The drug and non-reinforced lever responding in Expt. 1 were analysed individually in two, three-way analyses of variance. Each test had two between subject factors: Group (BLA-lesioned and control) and Dose (0.5, 0.25, 0.083 mg cocaine/ infusion) and one within subject factor Day (1, 2, 3, 4, 5, 6, 7). Statistically significant main

effects were analysed further with separate two-way analyses of variance at each dose of cocaine, with between subject factor of Group (BLA-lesioned and control) and the within subject factor Day (1, 2, 3, etc.). Individual means of statistically significant main effects were then compared using the Newman-Keuls post-hoc test. Experiment 2 was assessed initially using a three-way analysis of variance with one between subject factor: Group (BLA-lesioned and control) and two within subject factors Lever (drug reinforced and non-reinforced) and Day (1, 2, 3, etc.). Following this, the data were analysed further using contingency tables for the proportion of animals administering more than 15 infusions/ session and for those administering less than five infusions/ session; these proportions were analysed non-parametrically using the Fisher Exact Probability test. In Experiment 3, the last four days of pre-lesion self-administration data were compared with the first four days post-lesion acquisition. Between subject factors were Group (BLA-lesioned and control) and within subject factors were Lesion (pre-surgery and post-surgery) and Day (1, 2, 3, 4). Locomotor data in Expt. 4 were collected over successive 10 min intervals (bins) and subjected to an analysis of variance with two-factors, Group (BLA-lesioned and control) and Time (12 x 10 min bins).

Experiment 1: The effect of BLA lesions on the acquisition of cocaine self-administration

Seventy rats were surgically prepared with intravenous catheters, 48 of these were also given excitotoxic lesions of the BLA while the remaining 22 rats underwent identical sham lesions, with phosphate buffer replacing the toxin (for details of surgical procedure see Chapter 2 p28 and p32). All rats were allowed to recover for at least seven days before being divided into three groups of lesioned and control animals, to assess the effect of excitotoxic lesions of the BLA on the acquisition of three different doses of cocaine self-administration - 0.5, 0.25 and 0.083 mg cocaine/ infusion. The groups were: Group A - 0.5 mg cocaine/ 16 BLA lesions eight sham-operated controls; Group B - 0.25 mg cocaine/ 16 BLA lesions and eight sham-operated controls; Group C - 0.083 mg cocaine/ 16 BLA lesions and six sham-operated controls.

Results

i) Histological assessment

Excitotoxic lesions of the BLA were assessed by microscopic examination following Cresyl Violet staining as described in Chapter 2 p38. Only animals which sustained bilateral lesions of the major antero-posterior extent of the BLA were included in each experiment. Any animals showing bilateral damage of the CeA, cortical, medial nuclei or caudate or that had sustained only unilateral BLA lesions, were discounted from the final behavioural analyses.

Of the 48 animals given excitotoxic lesions of the BLA, 11 were excluded on histological grounds and four were discounted because of catheter blockade or leakage. No sham-operated control animals were discounted on the grounds of histological assessment, but two out of 22 control animals were discounted because of catheter blockade or leakage. Very few BLA-lesioned animals sustained damage of the CeA (4 animals), this nucleus appeared to be relatively insensitive to 0.09M quinolinic acid and in the majority of animals the toxin diffused laterally away from the CeA producing unilateral and occasionally bilateral damage to the piriform and perirhinal cortex. Five animals were discounted because of insufficient bilateral cell loss in the BLA. Two lesioned animals were discounted because they sustained large lesions which extended along the path of the injection cannula into the caudate putamen and entorhinal cortex, unilaterally. A schematic of the largest and smallest BLA lesions observed throughout the whole experimental programme is illustrated in Fig. 5. Representative photomicrographs of the BLA in lesioned and control brains are shown in Plates 2 and 3. The final group sizes used for statistical analyses were: Group A: 0.5mg cocaine - nine BLA lesions, six sham-operated controls; Group B: 0.25mg cocaine - 12 BLA lesions, eight sham-operated controls; Group C: 0.083mg cocaine - 12 BLA lesions, six sham-operated controls.

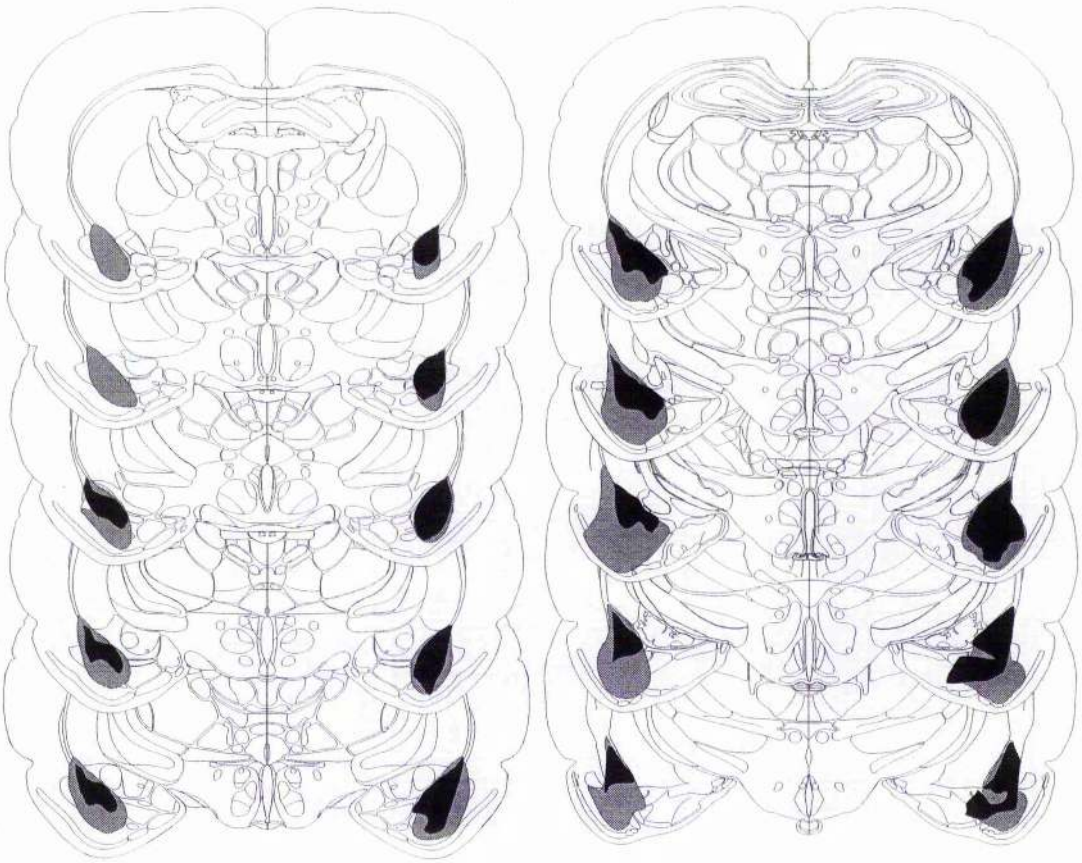


Fig. 5 Representative illustration of the largest (stippled shading) and smallest (solid shading) BLA-lesion included in the final behavioural analyses. In the majority of cases lesions encompassed ~ 90% of the antero-posterior extent of the lateral and basal magnocellular nuclei, with variable damage to acccessory basal and basomedial nuclei. Adapted from Paxinos and Watson (1992) showing sections –2.12 to –3.3 from Bregma.

Plate 2 Photomicrographs of excitotoxic lesions of the basal and lateral amygdala made with quinolinic acid (0.09M). Sections A and B are photomicrographs from a sham-operated control subject, which received infusions of phosphate buffer intra-amygdala. Sections C and D are photomicrographs of quinolinic acid lesions of the BLA. Gliosis and extracellular deposits associated with the path of the cannulae are seen in all four sections. Note that in C and D the lateral (L_d and L_v) and basal (B_{pc} and B_{mg}) nuclei have been completely destroyed and as a result the central nucleus (CeA) lies adjacent to the external capsule (compare A with C and B with D). In C, there is obvious damage to the region of the piriform cortex (Pc) which is seen in some animals sustaining larger lesions. In D, the damage to the piriform cortex is minimal.

Plate 2

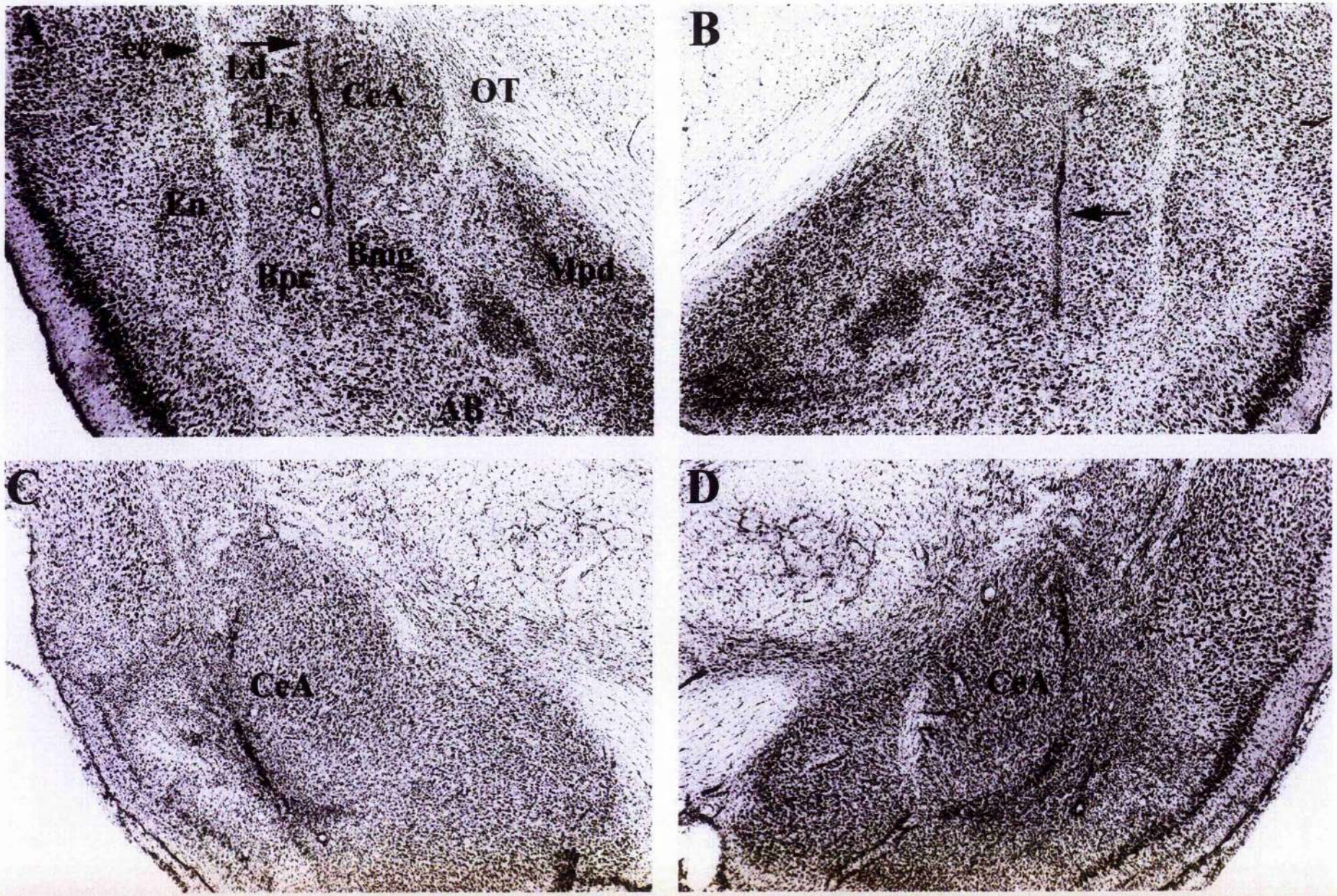
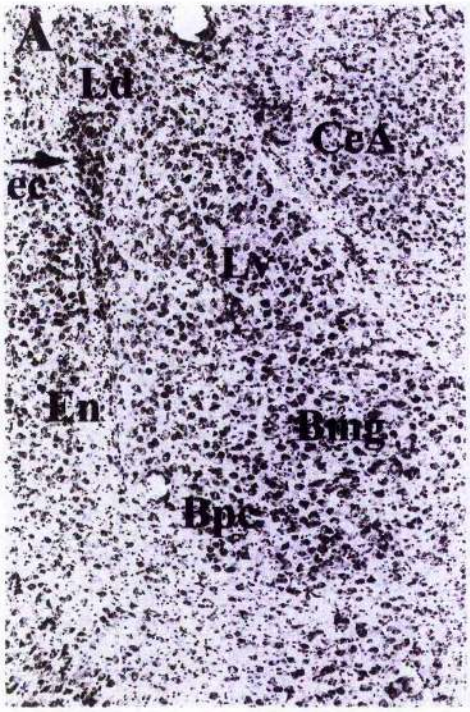
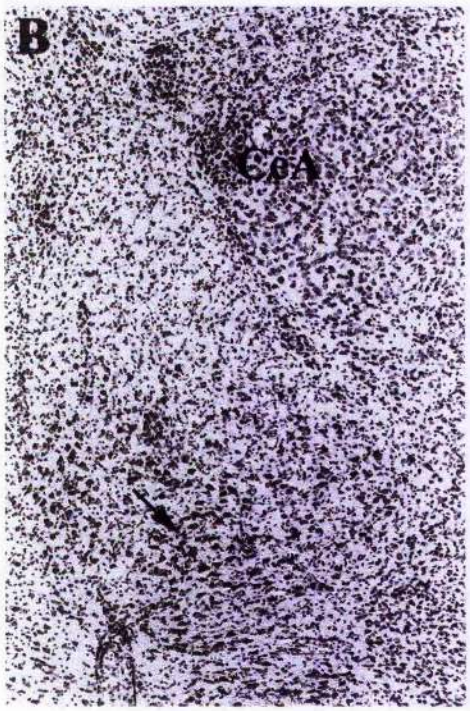
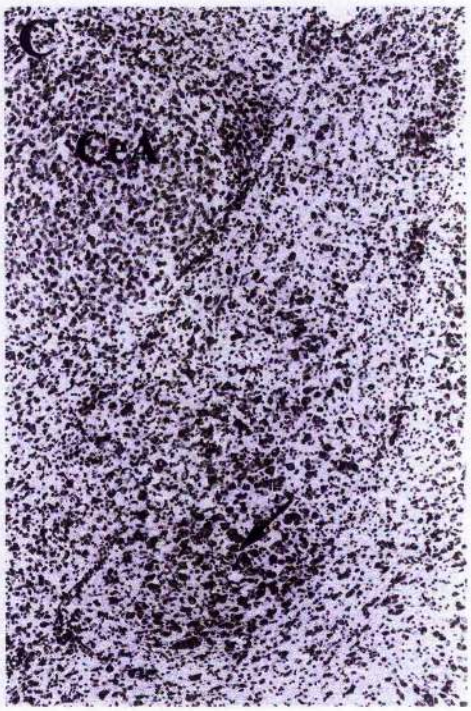


Plate 3







Photomicrograph of excitotoxic lesions of the basolateral parts of the amygdala. Section A is a magnified photomicrograph of Plate 1, and shows a sham-operated control animal. Sections B and C show photomicrographs of quinolinic acid lesions of the BLA (0.09M). These lesions are smaller and more discrete than those in Plate 2C and 2D, and the shape of the BLA remains intact. Contrast the 'fat' healthy neurones in A with the ruptured cells in B and C. In C and D, the arrows represent slight cell sparing (<10%).

ii) Behavioural analyses

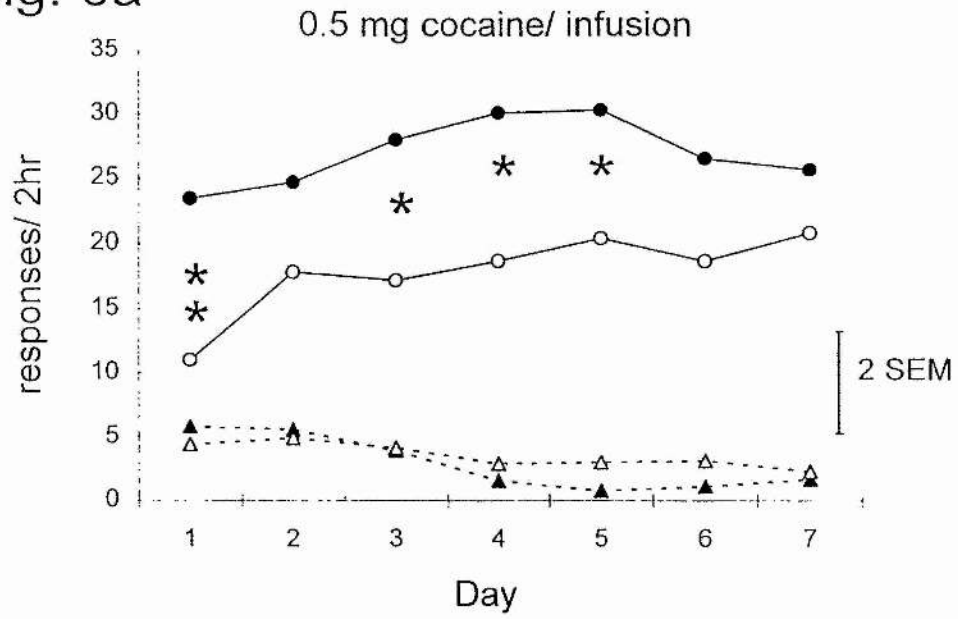
Drug and non-reinforced lever responses for lesioned and control animals at each dose are shown in Fig. 6a, b and c. Overall, both groups responded more on the drug reinforced lever than on the non-reinforced lever, and both groups increased the number of infusions administered over successive days. A three-way analysis of variance of drug lever responding in BLA-lesioned and control animals at all doses showed there was a highly significant main effect of Dose [$F(2,46)=11.66$, $p<0.001$] and Day [$F(6,276)=5.69$, $p<0.001$], but no main effect of Group [$F(1,46)=0.36$, p=NS]. However, there was a significant Group x Dose interaction [$F(2,46)=3.19$, $p<0.05$] which reflected the fact that BLA lesioned animals responded more than control animals for the highest dose of cocaine but responded less than controls for the lowest dose of cocaine. No Group x Day, Dose x Day or Group x Dose x Day interactions were significant.

A three-way analysis of variance of non-reinforced lever responding in BLA-lesioned and sham-operated control animals at all doses showed there was also a significant main effect of Dose [$F(2,46)=6.63$, $p<0.005$] and Day [$F(6,276)=6.70$, $p<0.001$], but no main effect of Group [$F(1,46)=1.11$, p=NS]. However, there were significant Group x Day [$F(6,276)=2.52$, $p<0.05$] and Dose x Day interactions [$F(12,276)=3.71$, $p<0.001$]. This reflected that fact that lesioned and control animals' non-reinforced lever responding was different during the acquisition of each cocaine dose. Both groups responded at a similar low level during the acquisition of the highest dose of cocaine (0.5mg/ infusion), control animals responded considerably more than lesioned animals during the first days of

acquisition at the middle cocaine dose (0.25mg/ infusion) and both groups resopnded within a similar range at the lowest dose of cocaine (0.083mg/ infusion) but the pattern of respondng differed over days. Group x Dose and Group x Dose x Day interactions were not significant.

Drug and non-reinforced lever responding were analysed further in separate two-way analyses of variance with factors Group (BLA-lesioned and control) and Day (1-7) at each dose of cocaine. Drug at lever responding at 0.5 mg cocaine/ infusion showed there was a significant effect of Group [$F(1,13)=6.87$, $p<0.05$] and Day [$F(6,78)=2.68$, $p<0.05$] but no Group x Day interaction [$F(6,78)=0.72$, p=NS]. BLA-lesioned rats self-administered between seven and 12 infusions more than control animals on the first six acquisition days, and a subsequent one-way analysis of variance showed that the groups differed significantly in their drug self-administration on the first, third, fourth, and fifth days ($p<0.05$). Control animals clearly differentiated between the levers by the second acquisition day and self-administered progressively more drug on each subsequent day, until both groups self-administered equivalent amounts of cocaine on the sixth and seventh days of acquisition. Responding on the non-reinforced lever at this dose also showed a significant effect of Day [$F(6,78)=5.8$, $p<0.001$] but no effect of Group [$F(1,13)=0.01$, p=NS] or Group x Day interaction [$F(6,78)=0.57$, p=NS] as both groups gradually decreased non-reinforced lever responding over days.

Fig. 6a



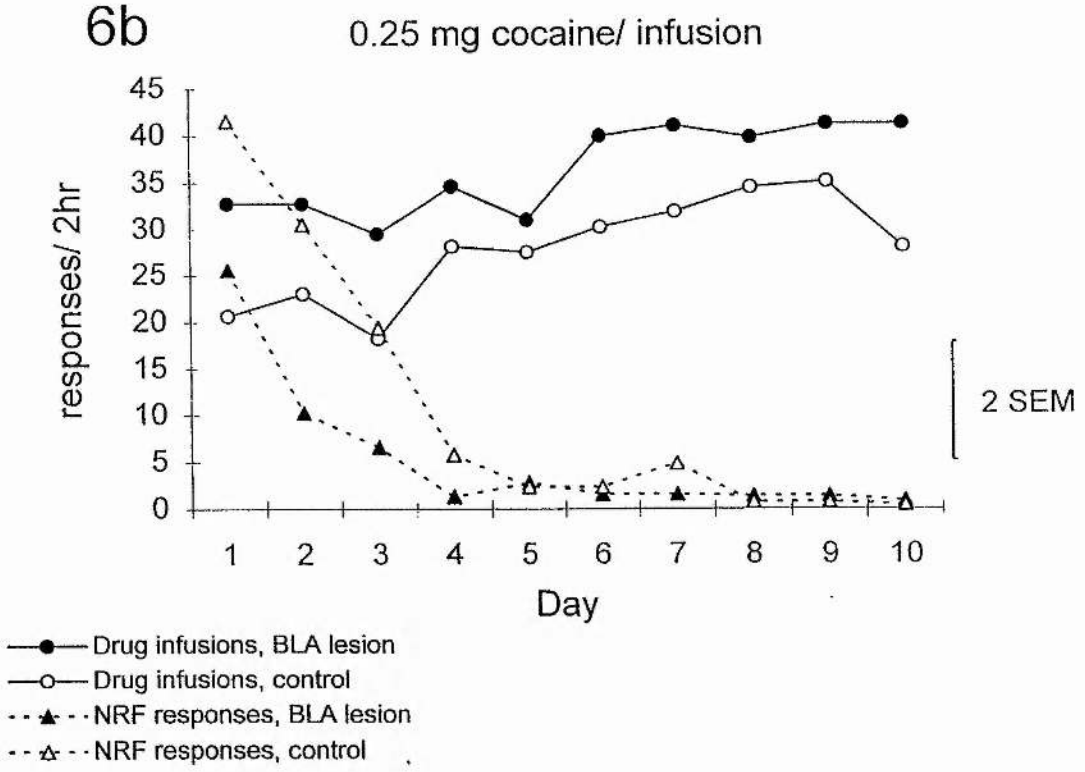


Fig. 6 a, b, c Mean daily drug and non-reinforced (NRF) lever responses for BLA-lesioned and sham operated control animals over the first 7-10 days of acquisition of cocaine self-administration. Doses tested were: 0.5mg/ infusion (Fig. 6a), 0.25mg/ infusion (Fig. 6b) and 0.083mg/ infusion (Fig 6c, over page). Note that the number of infusions administered per session is greater for lower doses of cocaine. The error bars represent standard error of the mean. * = $p \leq 0.05$, ** = $p \leq 0.01$

Fig. 6c

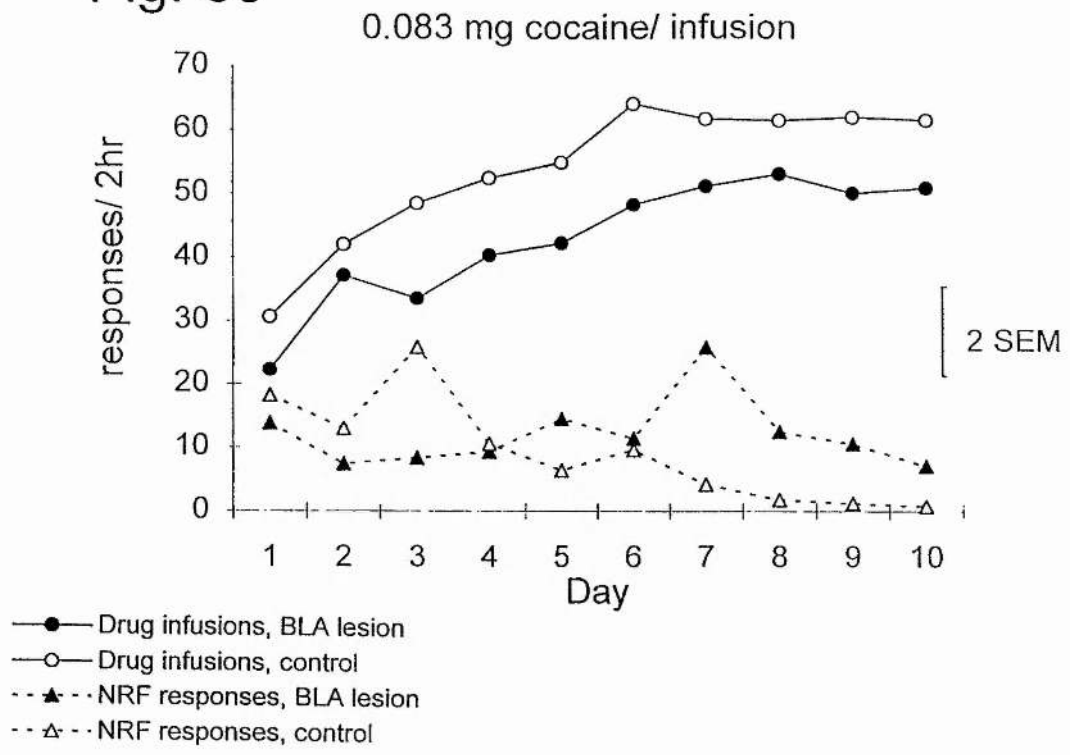


Fig. 6c Mean daily drug and non-reinforced (NRF) lever responses for BLA-lesioned and sham operated control animals over the first 10 days of acquisition of self-administration at 0.083 mg cocaine/ infusion (see previous page for higher doses).

Analysis of drug lever responding at 0.25 mg cocaine/ infusion showed a significant main effect of Day [$F(6,108)=3.44$, $p<0.005$] but no effect of Group [$F(1,18)=2.01$, p=NS] or Group x Day interaction [$F(6,18)=0.37$, p=NS]. However, control animals appeared to take four sessions to differentiate between the levers, while BLA-lesioned animals responded significantly more on the drug lever by the second acquisition session. Non-reinforced lever responding at this dose also showed a significant main effect of Day [$F(6,108)=9.72$, $p<0.001$] but again, no effect of Group [$F(1,18)=3.14$, p=NS] or Group x Day interaction [$F(6,108)=1.19$, p=NS].

Analysis of drug lever responding at 0.083 mg cocaine/ infusion also showed a significant effect of Day [$F(6,90)=2.72$, $p<0.05$] but no effect of Group [$F(1,15)=1.97$, p=NS] or Group x Day interaction [$F(6,90)=0.09$, p=NS]. By the second day of acquisition, both groups responded more on the drug lever than on the non-reinforced lever. Non-reinforced lever responding at this dose showed no effect of Group [$F(1,15)=0.01$, p=NS] or Day [$F(6,90)=0.66$, p=NS] but a significant Group x Day interaction [$F(6,90)=2.51$, $p<0.05$]. A subsequent one-way analysis of variance showed that the groups differed significantly on the third day of acquisition ($p<0.05$) when control animals responded significantly more than BLA-lesioned animals on the non-reinforced lever.

Discussion

The results of Expt. 1 indicate that both BLA-lesioned and sham-operated control animals acquired cocaine self-administration at each dose of cocaine but that the rate of self-administration was dependent on the dose of cocaine. Lower doses of cocaine produced higher rates self-administration, although both lesioned and control groups appeared to require a similar number of sessions to stabilise their self-administration regardless of cocaine dose. Relative to controls, BLA-lesioned animals self-administered significantly more drug over the first five days of acquisition at the highest dose of cocaine (0.5 mg cocaine/ infusion). However, there was no significant difference between the groups' acquisition of the two lower doses (0.25, 0.83 mg cocaine/ infusion) which suggests that the accelerated rate of acquisition observed at the high dose was not a result of a reduction in the reinforcing efficacy of cocaine. If lesions of the BLA impaired the reinforcing efficacy of cocaine, enhanced self-administration at the highest dose should have been accompanied by a reduction in the rate of acquisition at the lowest dose of cocaine, therefore producing an overall shift to the right in the acquisition dose-response function.

Differences between the groups at the highest dose of cocaine declined with repeated drug exposure over successive days, primarily as a result of the selective increase in drug intake by control animals. By the sixth and seventh acquisition days, both groups self-administered comparable amounts of cocaine at the high dose. Rather than interfering with the primary reinforcing effects of cocaine, this may indicate that lesions of the BLA selectively mitigate an aversive component of cocaine reinforcement, associated with the initial self-administration of high doses. Several

reports have suggested that high doses of cocaine may elicit an aversive component of cocaine reinforcement (McGregor et al. 1994; Ettenberg et al. 1993; Glick et al. 1993) and the BLA has been implicated in the actions of anxiolytic compounds such as benzodiazepines, which are thought to produce their effects by enhancing GABA-ergic inhibition (Tallman and Gallagher 1985; Vellucci et al. 1988; Davis 1992, 1994).

Gamma-amino-butyric acid (GABA) is an important inhibitory neurotransmitter in the brain. The majority of GABAergic synapses mediate neural inhibition by enhancing the conductance of chloride ions which act to hyperpolarise postsynaptic cells. The GABA receptor complex is made up of 5 subunits with specific binding sites for GABA, benzodiazepine, barbiturate, steroid and picrotoxin, as well as an integral chloride ion channel. Chloride dependent effects are mediated primarily by $GABA_A$ receptors and these have been found in high concentration within the BLA (Niehoff and Kuhar 1983).

Vellucci et al. (1988) investigated the effects of intra-amygdala benzodiazepine administration and reported that infusions of the benzodiazepine midazolam or muscimol, dose-dependently antagonised the introceptive discrimination of systemic pretreatment of two anxiogenic compounds (FG-7142 and pentylenetetrazol, respectively). Furthermore, Davis (1992) reported that during an operant conflict test, intra-BLA benzodiazepine infusions increased the time taken for rats to switch-off an electric shock, following the brief presentation of a conditioned light stimulus, predicting shock. Davis (1994) went on to show that these effects could also be reversed by systemic administration of bicuculine, a $GABA_A$ antagonist. Together

these studies indicate that the BLA is involved in mediating the effects of anxiolytic drugs.

The activity of BLA neurons is modulated by GABAergic projections from the thalamus. Benzodiazepines appear to facilitate the binding of GABA to $GABA_A$ receptors which act to increase the inhibitory power of GABA-transmission (Tallman and Gallagher 1985). In the present experiment, BLA lesioned animals showed enhanced acquisition of a high dose of cocaine self-administration, relative to controls. Therefore, excitotoxic lesions of the BLA that disrupt excitatory projections from the BLA to the Nacc and ventral striatum may also produce effects similar to benzodiazepines, in some way diminishing aversive elements of cocaine reinforcement, which are particularly likely during the acquisition of higher doses.

Experiment 2: The role of the CS during acquisition of cocaine self-administration

Twenty animals were prepared with IV catheters and allowed to acquire cocaine self-administration for 2 hr/ day for 10 days (0.25mg cocaine/ infusion). Twelve of these animals were exposed to the normal drug-CS light pairings (described in the methods p48) and these animals were identified as the CS-group. For the remaining eight animals, the houselight in each chamber was permanently illuminated during each self-administration session and the animals never experienced drug-CS light pairings; this group was identified as the No-CS group. The acquisition of cocaine self-administration was then compared between CS and No-CS groups. Neither group underwent any stereotaxic surgery.

Fig. 7

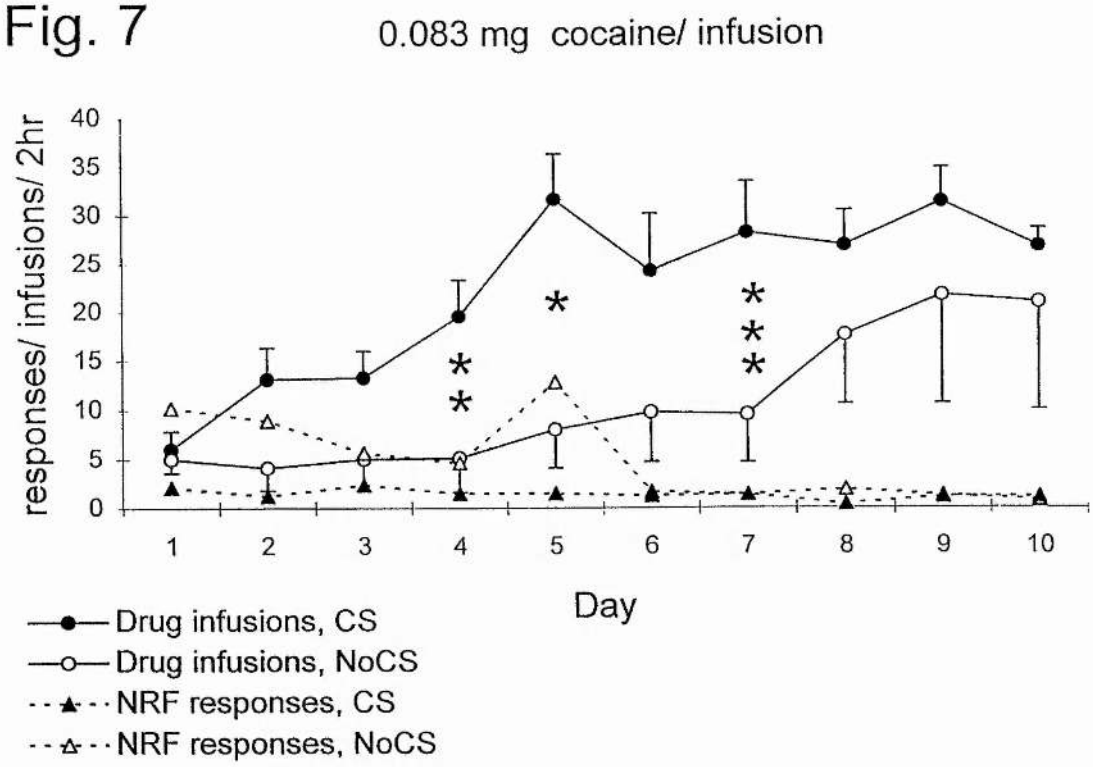


Fig. 7 Mean daily drug and non-reinforced (NRF) lever responses during the 10 day acquisition of cocaine self-administration (0.25mg/ infusion). Each infusion of cocaine was paired with the presentation of a 20s CS light stimulus for rats in the CS group, while the CS light stimulus was never presented to rats in the NoCS group. The error bars represent standard error of the mean.

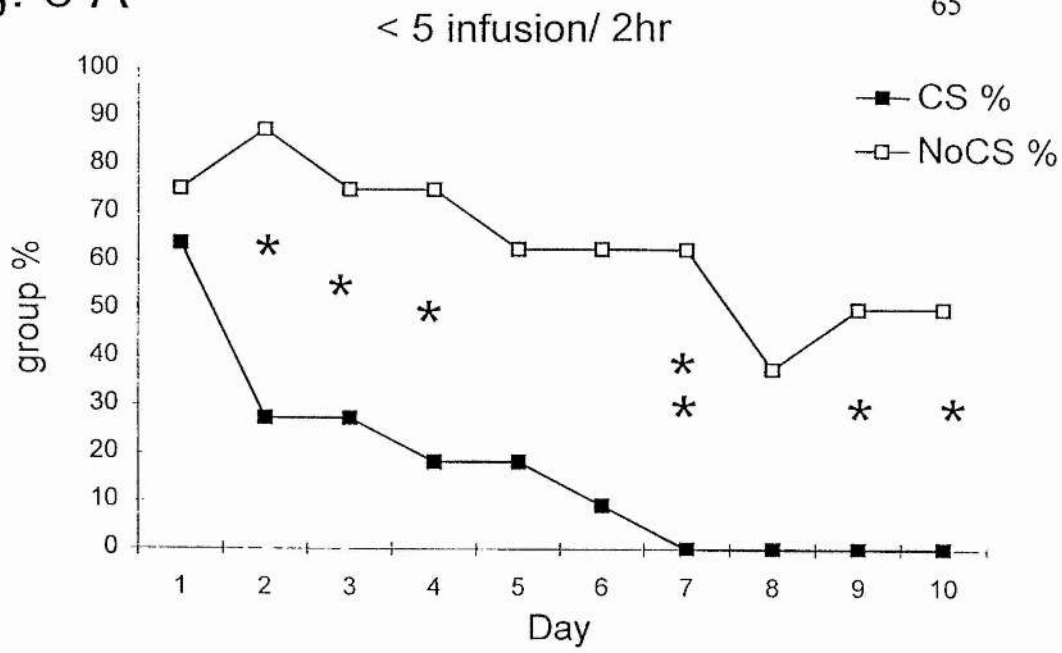
* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.005$

Results

One animal was lost from the CS group due to a catheter related infection and the numbers used in the statistical analyses were therefore, 11 for CS and eight for No-CS condition. Fig. 7 shows drug and non-reinforced lever responses for both CS and No-CS groups. A three-way analysis of variance showed that the CS group acquired cocaine self-administration at a significantly faster rate than the No-CS group and this was reflected as a significant main effect of Group [$F(1,17)=10.17$, $p<0.005$] and Lever [$F(1,17)=32.87$, $p<0.001$].

Both groups gradually increased responding on the drug reinforced lever and decreased responding on the non-reinforced lever over successive days, but there was no significant main effect of Day [$F(9,153)=1.31$, p=NS]. Similarly, there were no significant Group x Lever [$F(1,17)=2.89$, p=NS] or Group x Day interactions [$F(9,153)=1.60$, p=NS]. Post-hoc analyses with Student-Newman-Keuls test showed that the groups differed significantly in their drug lever responses on the fourth ($p\leq0.05$), fifth ($p\leq0.01$) and seventh ($p\leq0.005$) days of acquisition, whereas non-reinforced lever responses differed significantly only on the first day of testing ($p\leq0.05$). The proportion of animals in each group self-administering less than five, or more than 15 infusions/ 2hr on each of 10 successive days were compared between the two groups using the Fisher Exact probability test. These findings are shown in Figs 8a and 8b. For six out of the 10 days of study, the proportion of each group self-administering five or fewer infusions per session was significantly higher for the No-CS group than for the CS group ($p<0.05$-0.01; Fig. 8a). By the 10th day of acquisition, four out of eight No-CS animals continued to administer less than five infusions per session

Fig. 8 A



B

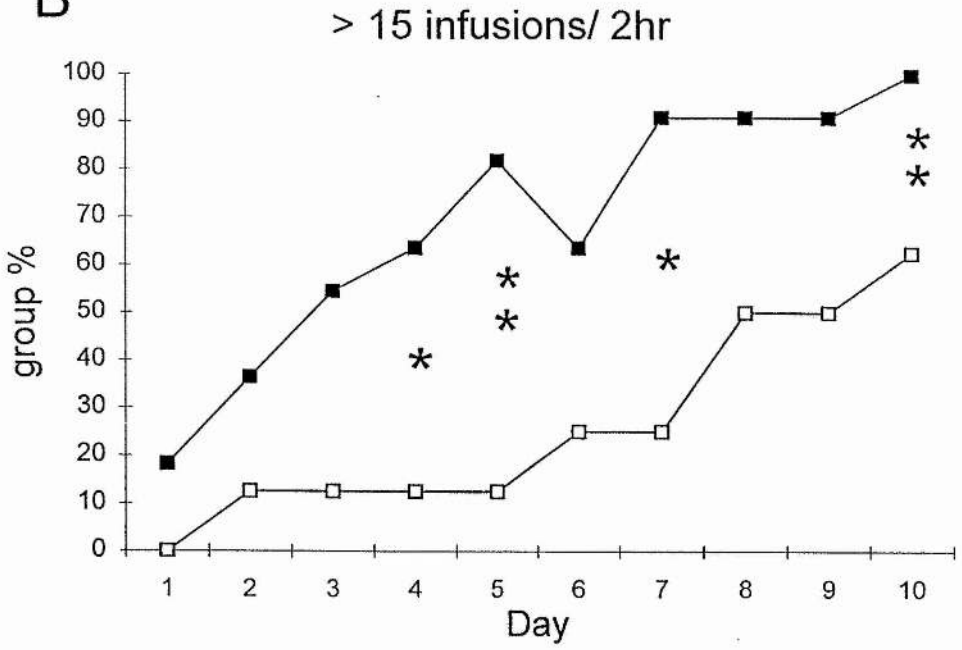


Fig. 8a, b The proportion of rats self-administering less than five (Fig. 8a) or more than 15 (Fig 8b) infusions of cocaine (0.25 mg/ infusion) during each 2hr session over the first 10 days of acquisition. Each infusion of cocaine was paired with the presentation of a 20s CS light stimulus for rats in the CS group, while the CS light stimulus was never presented to rats in the NoCS group. * = $p \leq 0.05$, ** = $p \leq 0.01$

whereas all 11 animals in the CS group self-administered more than five infusions per session. Over the same period, the proportion of each group self-administering 15 or more infusions per session was significantly higher in the CS group than in the NoCS group on first, fourth, fifth, seventh and 10th days ($p \leq 0.05$ - 0.005; Fig. 8b).

Discussion

The results of this experiment indicate that the presence of a light stimulus (CS) paired with each drug infusion facilitates the acquisition of cocaine self-administration in control animals. This may have occurred because the CS light initially acts as a discriminative stimulus, therefore aiding the recognition and learning of the correct response-reinforcement contingency. Animals in the No-CS group may have found it more difficult to make the association between the correct lever response and the ensuing drug reinforcement, which may have also altered the reinforcing properties of cocaine and as a result, retarded the acquisition of cocaine self-administration.

As mentioned above, both amygdala dopamine levels and the firing pattern of Nacc neurons have been reported to increase significantly during self- but not passive administration of cocaine (Wilson et al. 1994; Carelli et al. 1993), suggesting that response-reinforcement contingency may be an important factor in the acquisition of cocaine self-administration. More recently, Hemby et al. (1997) also investigated the effects of self- and passive-administration of cocaine on Nacc DA levels using a yoked-littermate design. One animal in a litter was trained to actively self-administer cocaine while another littermate passively received identical cocaine

infusions yoked to those self-administered by the first rat. *In vivo* microdialysis revealed that during self-administration sessions, extracellular Nacc DA and cocaine levels were elevated in both yoked and self-administering animals. However, during the first hour of each session Nacc DA levels were significantly higher in self-administering animals relative to their yoked counterparts, despite equivalent Nacc cocaine levels recorded in both groups during this period. On a second test day, animals previously trained to self-administer cocaine were, instead, yoked to their own self-administration pattern from an earlier session. Interestingly, when these animals were passively administered cocaine they also showed reduced Nacc DA release relative to earlier self-administration sessions.

The findings of the experiments described above support the contention that response-reinforcement contingency is an important factor in cocaine reinforcement during both the acquisition and maintenance of cocaine self-administration. But the authors also suggest that reductions in extracellular Nacc DA release may arise because passively administered, non-contingent infusions of cocaine might act as a 'startling' stimulus. Similar reductions in Nacc DA release have been reported following acoustic startle (Humby et al. 1996) and more recently excitotoxic lesions of the BLA have been shown to reduce prepulse inhibition of acoustic startle and fear potentiated startle in rats (Wan and Swerdlow 1997). In the present experiment, omission of CS-drug pairings in the No-CS condition may have produced effects similar to non-contingent, yoked cocaine administration (Hemby et al. 1997) because it was more difficult for No-CS animals to identify an action-outcome origin of the cocaine-induced state. Taken together, these findings imply that not only are the reinforcing effects of cocaine enhanced by response contingent

self-administration, but that pairing a salient light CS with each infusion also facilitates the acquisition of cocaine self-administration in control animals. Furthermore, the self-administration findings in Expt. 1 indicated that lesions of the BLA may diminish an aversive component of cocaine reinforcement associated with higher doses of cocaine. If non-contingent infusions of cocaine also produce an aversive 'startle' response when administered intravenously, it is possible that lesions of the BLA might also alleviate these effects.

Experiment 3: The effect of BLA lesions on the maintenance of cocaine self-administration

This experiment investigated the effect of excitotoxic lesions of the BLA on the maintenance of cocaine self-administration. The 11 non-lesioned animals from the CS group in Expt. 2, that acquired cocaine self-administration (0.25mg/ infusion) under the normal CS-drug contingency were randomly divided into two groups, following the initial 10 day acquisition period. Six of these animals received bilateral lesions of the BLA and the remaining five were given identical sham lesions. Once the animals had recovered for seven days, they were again allowed to re-acquire cocaine self-administration (0.25mg/ infusion) for a further seven days.

Results

All six BLA-lesioned animals were included in this study although the catheters of several animals from each group blocked shortly after the post-lesion recovery period. As all animals remained patent for at least the first four re-acquisition days, statistical analysis was carried out on the last four days of the initial acquisition period and the first four days of the post-lesion, re-acquisition period.

Fig. 9 A

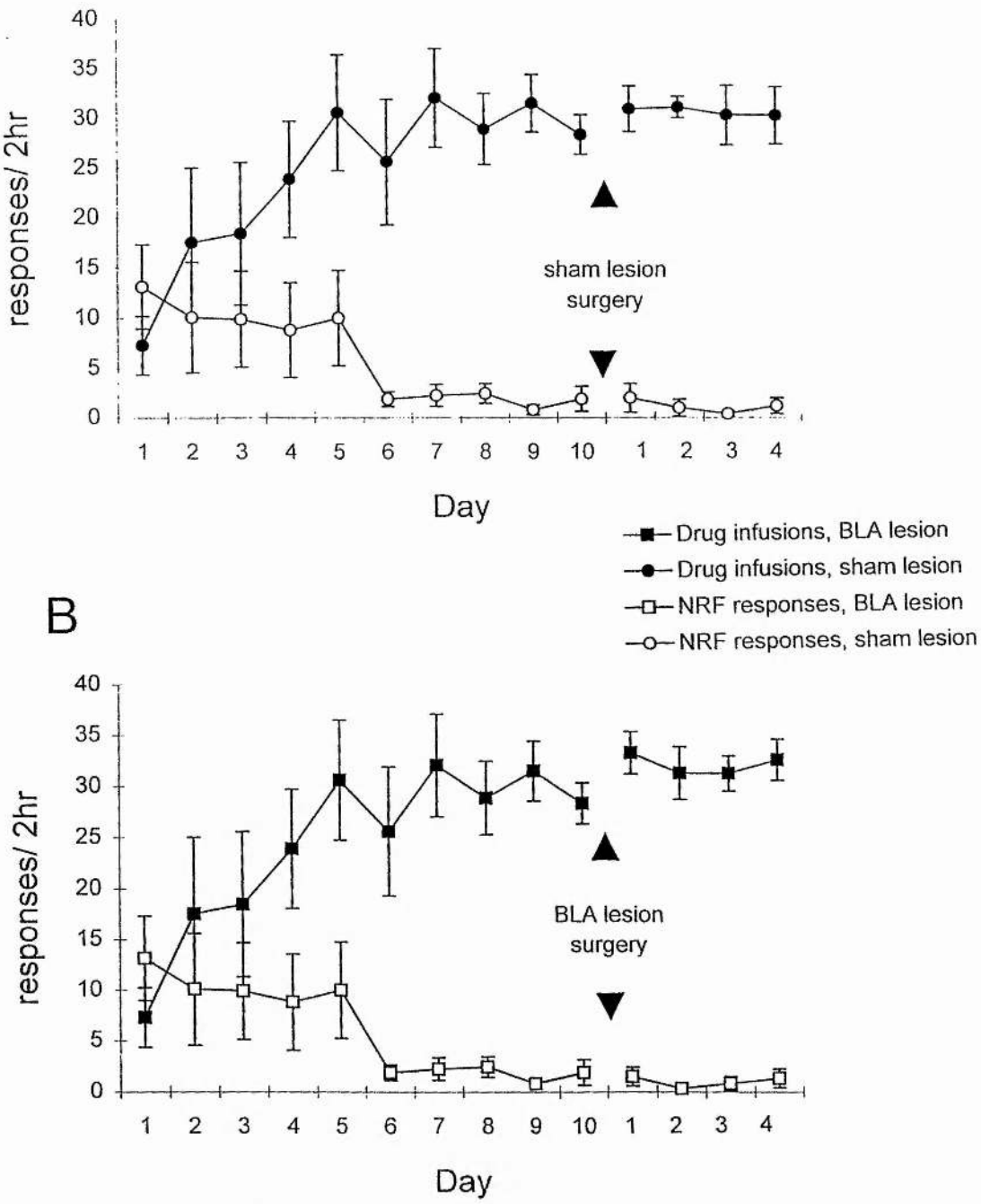


Fig. 9 a, b The effect of excitotoxic lesions of the BLA made after the initial acquisition of cocaine self-administration. Following 10 days self-administration (0.25mg cocaine/ infusion), rats from the CS group in Expt. 1 were randomly allocated for BLA lesion or sham lesion surgery. On recovery, both groups were allowed to re-acquire cocaine self-administration (0.25 mg/infusion). Days 1-10 show the self-administration of the unlesioned rats prior to surgery. Days 1-4 indicate the pattern of self-administration seen following sham lesions of the BLA (Fig. 9a) and excitotoxic lesions of the BLA (Fig. 9b).

Each animal was compared to its own performance prior to lesion surgery. Fig. 9a, b shows the pattern of self-administration before and after BLA and sham lesion surgery. A two-way analysis of variance of drug-lever responding showed that lesions of the BLA produced no effect on the maintenance of cocaine self-administration. There was no significant effect of Group [$F(1, 9)=0.01$, p=NS] or Day [$F(7, 63)=0.46$, p=NS] comparing pre and post-lesion self-administration.

Discussion

The results of this experiment indicate that lesions of the BLA made following the initial acquisition of cocaine self-administration do not affect the rate of subsequent cocaine self-administration. In retrospect this may not be unexpected because lesions of the BLA made prior to the acquistion of cocaine self-adminitration at this dose (0.25mg cocaine/ infusion; Expt. 1) increased self-administration between 10-12 infusions per session over the first three acquisition days, relative to controls, but over seven days this effect was not statistically significant. However, several other studies have also reported that lesions of the BLA made following the initial acquisition of cocaine self-administration did not interfere with subsequent self-administration rates. For instance, Meil and See (1997) reported that lesions of the BLA attenuated cue-elicited responding for cocaine, but that BLA lesions made following the initial training period had no effect on cocaine maintained responding. Similar findings have also been observed by Caine and Koob (unpublished observations) and McGregor et al. (1994) who reported that dopaminergic lesions of the amygdala failed to produce specific deficits in responding under a progressive-ratio schedule of cocaine self-administration. Furthermore, studies investigating the development of

sensitisation to psychomotor stimulants have reported that animals with lesions of the BLA exhibited intensified stereotypy in response to acute amphetamine administration, but failed to develop a sensitised locomotor response (Wolf et al. 1995). Yet, lesions of the BLA made two weeks after a series of cocaine injections did not affect the expression of behavioural sensitisation to a subsequent cocaine challenge (Pierce et al. 1998). Taken together, these findings are also consistent with reports from learning and memory studies which suggest that the BLA is specifically involved in the acquisition, but not the retention of strategies used during a three-panel, four-choice runway task (Ohno et al. 1992, 1993). In conclusion, it would appear that the BLA is not involved in the primary reinforcing effects of cocaine or the maintenance of cocaine self-administration, but that the BLA is involved in the mediation of conditioned association and the selection of appropriate behavioural responses in dynamic situations.

Experiment 4: Locomotor response to intra-peritoneal cocaine injections

In an attempt to elucidate the neural mechanisms underlying differences in the acquisition of cocaine self-administration drug-naive BLA-lesioned and sham-operated control rats were assessed in their locomotor response to three doses of cocaine administered intra-peritoneal (IP). These animals were not surgically prepared with IV catheters and had no previous self-administration experience. Ten BLA-lesioned and six sham-operated control animals were randomly assigned to individual photocell cages. Two consecutive 2hr daily sessions were sufficient to habituate the animals to the novel environment, and preliminary saline injections were administered IP to each animal in the home cage on the day prior to the start of the test, to habituate the animals to the injection procedure. In the

subsequent seven-day test period, a saline injection was administered on day one and drug injections were administered on days two, four, and six; days three, five and seven were drug-free. Each animal received a single IP injection of cocaine (10, 20 or 30 mg/kg, in ascending order) on successive drug test days. All test sessions lasted for 2hr with the IP saline or cocaine injection administered 30 min into the session, so that the development of any conditioned locomotor effects could be observed uncontaminated by the direct stimulant action of cocaine. Locomotor activity cages and computer equipment are described in Chapter 2 p42.

Results

Following histological assessment, two BLA-lesioned animals were discounted because of they had sustained unilateral lesions of the BLA and showed considerable cell sparing; thus the number of animals included in the analysis consisted of eight lesions and six sham-operated control animals. A two-way analysis of variance showed there were no significant differences in spontaneous locomotor activity between BLA-lesioned and sham-operated controls during the two habituation sessions. Fig. 10a, b and c show the locomotor stimulatory effects at each dose of cocaine in both lesioned and sham animals. There were no significant difference between the groups in their locomotor responses to IP cocaine at any dose (10mg/kg: [$F(1,12)=1.22$, NS], 20mg/kg: [$F(1,12)=0.2$, NS], 30mg/kg: [$F(1,12)=0.01$, NS]). Although there was a significant main effect of Time at each dose [$F(11,32)=16.66$, 15.21, and 10.49, $p<0.01$] at 10, 20, 30 mg/kg cocaine, respectively, Group x Time interactions were not significant at any dose.

Fig. 10 A

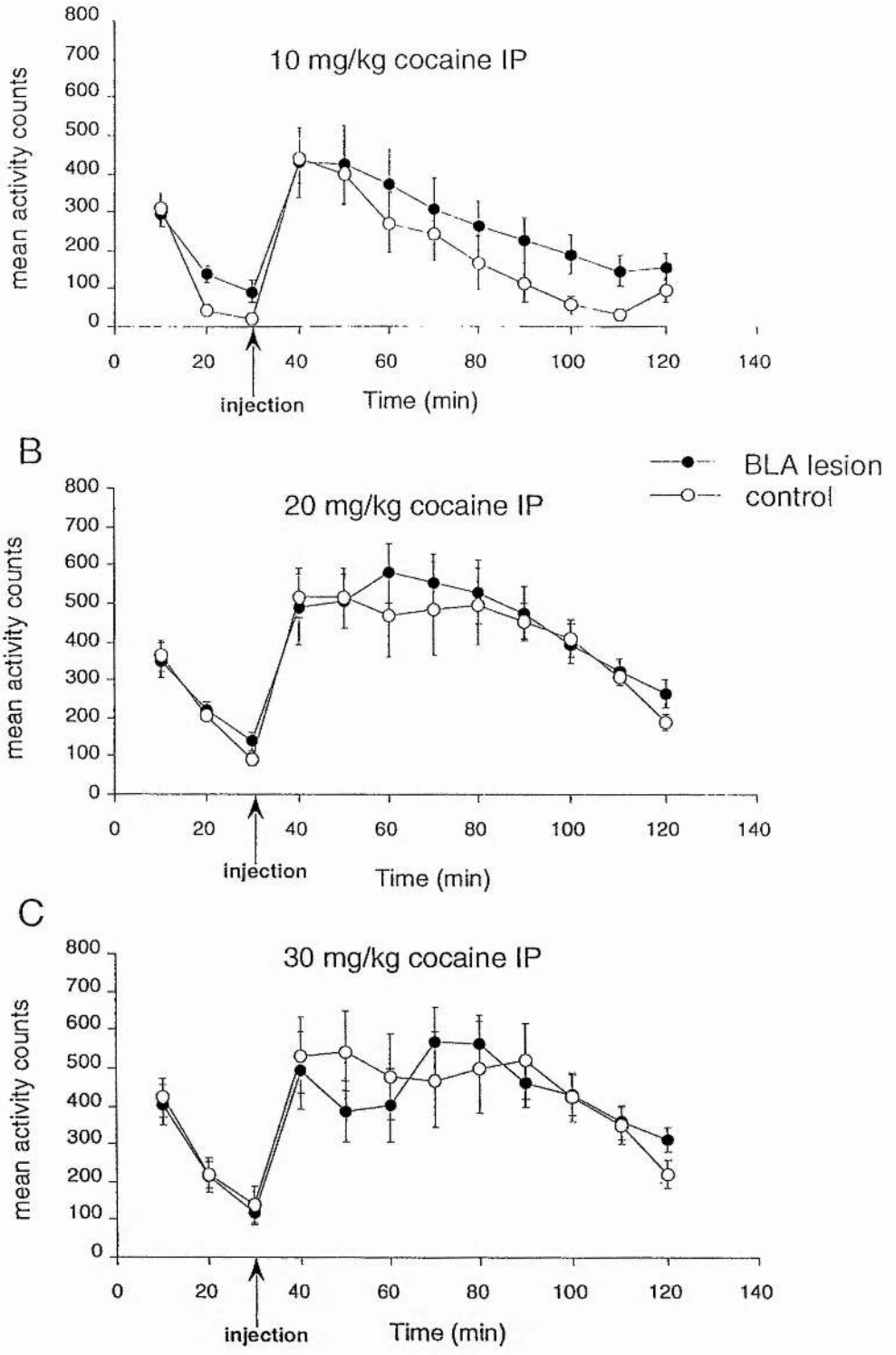


Fig. 10 Locomotor response to IP cocaine in drug naive BLA-lesioned and sham-operated control rats. Cocaine doses were administerd in ascending order on separate days: 10mg/ kg (Fig 10a), 20mg/ kg (Fig. 10b) and 30mg/ kg (Fig. 10c). Cocaine injections were administered 30 min into each locomotor test session.

Discussion

Consistent with Expts. 1 and 3, the results of this experiment indicate that the BLA is not involved in mediating the stimulant effects of cocaine, as neither spontaneous or cocaine-induced locomotor activity were affected by excitotoxic lesions of the BLA. The present results also indicate that lesions of the BLA did not impair general conditioned locomotor activity, elicited by repeated IP injections of cocaine. During the first 10 min of each drug test session, in the absence of drug, both groups increased their locomotor activity by an average of 50 counts from the first to the second test session and again from the second to the third test session. Although this finding may not appear consistent with BLA involvement in the formation of conditioned associations, there are several possible reasons for this lack of effect.

First, the findings in Expt. 2 imply that response-reinforcement contingency is an important factor in the rate with which unlesioned animals acquire cocaine self-administration and other reports have also indicated that such effects may alter the pharmacokinetic action of cocaine (Carelli et al. 1993; Wilson 1994; Hemby et al. 1997) and perhaps, as a result, the interoceptive cocaine cue (Hemby et al. 1997). It is also possible that the neural mechanisms underlying response-contingent behaviour rely more on the amygdala (Wilson et al. 1994) than those mechanisms underlying locomotor responses to an acute systemic injection of cocaine, over which the animal has no control. However, in the present locomotor test, all animals were habituated to the locomotor cages prior to drug testing and each drug injection was administered IP 30 min into each 2hr test session. It would seem

likely therefore that potential cocaine-induced conditioned activity was diminished by extensive pre-exposure to the locomotor cages in a drug-free state (Badiani et al. 1995).

The dose and route of cocaine administration have also been reported to differentially influence the contribution of contextual factors to conditioned drug effects (Post et al. 1992) and as a result, these effects may be optimal at different time spans following IV or IP administration (O'Dell et al. 1996). Specifically, Post et al. (1992) proposed that repeated low dose administration of cocaine produces context-dependent behavioural sensitisation, whereas the repeated administration of higher doses produces context-independent sensitisation to the behavioural effects of cocaine. O'Dell et al. (1996) reported that IV infusions of cocaine produced reliable conditioned activity following 20 min but not 40 min exposure to the test environment, whereas IP injections produced robust context-independent sensitisation of head-bobbing stereotypy, which was not as prevalent following IV injections. This might suggest that the rapid action of IV cocaine is more likely to support the formation of specific conditioned associations whereas the protracted action of IP cocaine is more likely to produce context-independent sensitisation to the drugs effects which may not involve the BLA. It is possible therefore, that the non-contingent manner in which cocaine was administered in the present locomotor study may have concealed latent behavioural effects associated with lesions of the BLA.

Earlier reports have also shown a lack of effect of BLA lesions on the locomotor response elicited by intra-Nacc amphetamine infusions and in response to IP cocaine injections (Cador et al. 1989; Burns et al. 1993; Brown and Fibiger 1993). Interestingly, however, Brown and Fibiger (1993) reported that although their non-specific amygdala lesions (including both the basal and central nuclei) did not interfere with the locomotor stimulant properties of IP cocaine, or cocaine-induced conditioned activity these lesions completely blocked cocaine conditioned place preference. Lesions of the BLA have also been reported to block conditioned place preference for sucrose paired context (Everitt et al. 1991) but recently the validity of procedures used to assess cocaine-induced conditioned activity and conditioned place preference have been called into question. By employing a video recorder to monitor both stimulant and conditioned psychomotor effects in rats, Martin-Iverson and Fawcett (1996) reported that the repertoire of behaviours observed in animals exhibiting conditioned activity differed significantly from those behaviours observed in response to injections of drug. As a result, these authors concluded that automated locomotor test cages produce misleading evidence regarding the stimulant and conditioned effects of psychomotor stimulants. Furthermore, it is interesting to note that Wolf et al. (1995) observed intensified stereotypy in BLA-lesioned animals relative to sham-operated controls, in response to an acute injection of amphetamine but the results of the present locomotor study indicated that both BLA-lesioned and control animals responded similarly to IP injections of cocaine. Taken together, it is possible that these findings also provide support for the conclusions of Martin-Iverson and Fawcett (1996).

Nevertheless, there is substantial evidence to suggest that repeated intermittent cocaine administration increases the magnitude of behavioural responses elicited by cocaine (Kalivas and Stewart 1991; Post et al. 1992) and that these effects are greater when repeated drug injections are made in the same environmental context as a subsequent test injections (Pierce and Kalivas 1997). Thus, sensitisation to the behavioural effects elicited by psychomotor stimulants comprise both conditioned and unconditioned effects. In an attempt to assess the relationship between these factors in repeated cocaine use, Carey and Gui (1998) administered five daily cocaine injections (10mg/ kg, IP) in either a specific context (paired) or the home cage (unpaired), to separate groups of rats. Following the repeated cocaine treatment, paired animals showed clear conditioned locomotor activation in response to a saline injection given in the drug specific context. This conditioned behavioural response was then successfully eliminated in half of these paired animals (extinction group) by repeatedly exposing them to the specific drug context, in the absence of cocaine. Finally, the behavioural response of each group (paired, unpaired and extinction) was assessed in response to a test injection of cocaine in the drug specific context. These authors reported that both paired and extinction animals exhibited a faster onset of behavioural activity than unpaired or saline treated control rats and it was concluded that conditioned drug effects did not contribute to the observed behavioural sensitisation, possibly because the unconditioned cocaine response occluded conditioned cocaine effects (Carey and Gui 1998). These findings might also provide a potential explanation for the lack of effects observed in animals with lesions of the BLA in the present locomotor study, in that the locomotor stimulant properties of cocaine may have masked

possible deficits produced by lesions of the BLA although, differences between the experimental procedures may make these studies difficult to compare directly. For instance, a total of 10mg/ kg more cocaine was administered in the present locomotor study and it is likely that the strength of conditioned effects were greater in the Carey and Gui study (1998), simply because the duration of exposure to the drug-related context was markedly shorter (20 min versus 2 hr) and this is likely to produce more robust conditioned activity (O'Dell et al. 1996). Interestingly, Carey and Gui (1998) also point out that the sensitised behavioural response in their study was context-specific and therefore must have involved some degree of associational processing, that persisted even after extinction training. In conclusion these authors proposed that context specificity is mediated by a compound stimulus complex comprising of both exteroceptive and interoceptive cocaine drug cues and that together these cues facilitate the onset of cocaine's effects, thereby enhancing the addictive potency of cocaine. Clearly it would be of interest to assess the locomotor stimulant effects of cocaine during contingent self-administration in both BLA-lesioned and sham-operated control animals with and without prior self-administration experience.

Conclusions

The experiments reported in this chapter have established: i) that lesions of the BLA do not interfere with the primary reinforcing properties of cocaine; ii) that pairing a brief light stimulus with each drug infusion enhances the rate with which non-lesioned animals acquire cocaine self-administration; iii) that lesions of the BLA are not involved in the maintenance of cocaine self-administration, once the behaviour is established and iv) that lesions of the BLA do not influence locomotor activating properties of IP cocaine injections.

Chapter 4: Acquisition of a second-order schedule of cocaine self-administration

Introduction

Responding for IV infusions of cocaine can be maintained under a number of different schedules of reinforcement including fixed-interval, fixed-ratio and progressive-ratio (Pickens and Thompson 1968; Goldberg 1973; Johnson and Schuster 1981; Woods et al. 1987; Corrigall and Coen 1989; McGregor and Roberts 1993), and with an appropriate dose of drug, these schedules can yield reliable and consistent patterns of responding. Psychomotor stimulants such as cocaine and amphetamine dose-dependently modify locomotor activity in the rat; low doses produce continuous running and rearing activity, whereas higher doses induce repetitive stereotyped behaviours such as sniffing, licking and movements of the head (Woods et al. 1987). As cocaine self-administration is in itself a motor response, an animal's rate of responding for cocaine during a self-administration session may increase or decrease depending on the blood plasma concentration of drug at a given time. Therefore, rates of self-administration may vary for reasons unrelated to an animal's motivation or desire for the drug.

In an attempt to minimise toxicity and reduce the likelihood of an animal indiscriminately self-administering a lethal over-dose of drug, many researchers establish a brief 'time-out' period following each self-administered drug infusion. This is achieved either by briefly deactivating the drug lever, such that further

responses produce no effect, or by completely retracting the drug lever from the operant chamber, thereby denying access for a short time.

Another method of limiting an animal's drug intake is to establish a fixed-interval (FI), progressive ratio (PR) or second-order schedule of drug self-administration. These schedules reliably extend the period of time between successive drug infusions, although each operates in a different way. An FI schedule delivers reinforcement contingent upon an animal's operant response only after a set-time has elapsed, irrespective of the overall number of lever responses made i.e. during an FI 15 schedule the maximum number of infusions an animal could earn in 1hr would be four, one every 15 mins. Progressive-ratio and second-order schedules differ from FI schedules in that reinforcement is dependent upon an animal's rate of drug lever responding. During a PR schedule the response requirement for successive infusions increases systematically, either as an arithmetic or logarithmic ratio. Each session begins on a low ratio but an animal may have to emit several hundred responses for each infusion by the 17th or 18th infusion of a session. In contrast, a second-order schedule relies on an animal completing an extended response requirement prior to receiving the first infusion of each session. This period of responding is maintained by the periodic presentation of a brief stimulus light, previously paired with each drug infusion during the initial acquisition of self-administration. The strength of this conditioned stimulus (CS) to support operant responding in the absence of primary reward largely determines how well an animal will perform under a second-order schedule of reinforcement and the extent to which the response requirement for each infusion can be increased.

A second-order schedule derives its name form the process of conditioning upon which it depends. Animals initially acquire cocaine self-administration under a schedule of continuous reinforcement (CRF), in which each response on the drug lever results in an IV infusion of drug and the simultaneous presentation of a discrete light stimulus. Animal learning theory describes these events in terms of conditioned and unconditioned stimuli. An IV infusion of drug acts as an unconditioned stimulus (US) in that it evokes an unconditioned physiological response upon administration. Coincident presentation of a discrete light stimulus may act as a conditioned stimulus (CS), through contingent presentation and consequent association with each drug administration. In the absence of this contingent relationship, the brief light stimulus will not acquire motivational significance and as a result, will not support the acquisition of a new behavioural response, which in the absence of the primary reinforcer is a critical determinant of conditioned reinforcement (Mackintosh 1974, 1983). Therefore, first-order conditioning can be denoted as:

R	$\Rightarrow$	US	+	CS
(operant response)		(drug infusion)		(light stimulus)

Second-order conditioning relies upon either the transfer of incentive motivational properties of the initial conditioned stimulus (CS_1) to a second stimulus (CS_2) or by the acquisition of a behavioural response, supported solely by presentation of CS_1. Therefore, second-order conditioning can be denoted as either:

(a) i) $R \Rightarrow CS_1 + CS_2$

ii) $R \Rightarrow CS_2 \Rightarrow US$

or (b) $R \Rightarrow n[CS_1] \Rightarrow US$

Where n represents the number of times CS_1 is presented prior to the delivery of the US. Equation (b) also represents a second-order schedule of reinforcement, which can be made progressively more demanding by increasing the response requirement for each CS_1 presentation. For instance, if n is increased over sessions from 1 to 10, the US is ultimately delivered only after CS_1 has been presented 10 times. Following this, the response requirement for each presentation of CS_1 may also be increased over sessions, e.g. from 1-8 lever responses. In this example, once each step has been completed, an animal would require 10 presentations of CS_1 for the delivery of the US and each CS_1 presentation would require 8 correct lever responses. Thus the delivery of each US, would require 80 responses on the correct lever.

Conditioning is thought to play an important role in the development and maintenance of cocaine addiction in humans, and more recently the use of functional neuro-imaging techniques have been used to identify brain structures that may mediate cue-induced drug craving (Childress et al. 1987, 1988, 1996; Grant et al. 1996; Mass et al. 1998) and the actions of psychomotor stimulants on neural activity (Volkow et al. 1996). Using positron emission tomography (PET)

Grant et al. (1996) reported that exposing subjects with a history of cocaine abuse to cocaine-related paraphernalia, elicited a significant increase in glucose metabolism within the dorsolateral prefrontal cortex, amygdala and cerebellum, relative to control subjects with no previous drug experience. Importantly, select regional increases in glucose metabolism in cocaine abusers were also associated with subjective reports of enhanced cocaine-craving, but neither increased glucose metabolism or craving were elicited in these subjects when exposed to motivationally neutral arts and crafts materials. In a similar study using PET Childress et al. (1996) also reported significant increases in blood flow in the amygdala, anterior cingulate cortex and temporal poles in cocaine abusers watching video footage of the preparation and usage of cocaine. More recently, however, Mass et al. (1998) reported the use of magnetic resonance imaging (MRI) to assess blood oxygenation levels as an indication of regional brain activity elicited by cocaine related stimuli. In this study, only the anterior cingulate and dorsolateral prefrontal cortex were reliably activated, but Mass et al. (1998) propose that reasons for these limited effects are most likely attributed to the brevity of the test (10 min compared to 30 min in the Grant et al. 1996 and Childress et al. 1996) and relatively short duration of stimuli exposure - 150s segments of cocaine-related images interchanged with neutral images for a total of 10 minutes, relative to 30 min of cocaine related stimuli (Childress et al. 1996; Grant et al. 1996). Furthermore, in the Grant et al. study (1996) the anticipation of cocaine use at the end of each test session may have enhanced the craving elicited by cocaine stimuli. Taken together these findings strongly support the opinion that specific brain regions, including the amygdala, are selectively activated by cocaine related stimuli

in human cocaine users. Lesions of the basal and lateral nuclei of the amygdala have been shown to disrupt tasks which rely on the formation and utilisation of conditioned associations (Cador et al. 1989; Everitt et al. 1989). Therefore, it is possible that these amygdaloid nuclei also play an important part in the mediation of conditioned associations which underlie cue-elicited cocaine-seeking behaviour.

A second-order schedule of cocaine self-administration in which performance relies heavily on the incentive motivational capacity of a drug related CS, in the absence of primary reinforcement, may provide a model of drug-seeking behaviour. Cador et al. (1989) demonstrated that lesions of the BLA significantly impaired the acquisiton of a new response, supported by a water associated conditioned reinforcer, while Everitt et al. (1989) demonstrated that lesions of the basolateral amygdala (BLA) markedly impaired the performance of male rats working under a second-order schedule of sexual reinforcement, despite the fact that sexual behaviour *per se* was unaffected. From these experiments Everitt et al. (1989) concluded that the BLA played a critical role in the formation of conditioned associations and in consequence, lesioned animals failed to register the significance of reward related CSs; hence they were severely impaired in both the acquisition of a new behavioural response with conditioned reinforcement and in performance under a second-order schedule of sexual reinforcement. Everitt et al. (1991) went on to demonstrate that bilateral lesions of the BLA also disrupted conditioned place preference to a sucrose-paired environment, and that similar attenuation was achieved by combining a unilateral lesion of the BLA with a contralateral lesion of the ventral striatum, therefore demonstrating that projections from the BLA to the

ventral striatum form part of a neural system mediating conditioned associations. In subsequent experiments (Burns et al. 1994) concluded that projections arising in the BLA and terminating in the Nacc and ventral striatum utilise glutamate as a primary neurotransmitter, and that glutamatergic/ dopaminergic interactions within the ventral striatum and Nacc mediate the formation of conditioned associations. This would imply that a significant mechanism for the reinforcing qualities of psychomotor stimulants may be their ability to enhance dopaminergic transmission in the ventral striatum and therefore amplify the impact of drug-related conditioned stimuli (Phillips and Fibiger 1990; Robinson and Berridge 1993). Furthermore, these findings suggest that investigation of the effects of excitotoxic lesions of the BLA on cocaine-seeking behaviour may make an important contribution to addiction research because cue-elicited drug-seeking behaviour is thought to be a fundamental component of compulsive drug use (Childress et al, 1987, 1988; Ehrman et al. 1992).

The experiments reported in this chapter aimed to investigate the effects of lesions of the BLA on the acquisition of a second-order schedule of cocaine self-administration as a novel method of assessing drug-seeking behaviour. It was hypothesised that cocaine self-administration under a second-order schedule would be sensitive to excitotoxic lesions of the BLA, as this structure has been strongly implicated in processes underlying conditioned reinforcement using natural rewards (Cador et al. 1989; Everitt et al. 1989; 1991) and may also constitute a major component in the neural mechanisms underlying cue-elicited cocaine-seeking behaviour.

Secondly, it was considered important to assess the effect of BLA lesions on a within-session dose response test in animals which had acquired and reached stable rates of cocaine self-administration (0.5mg /infusion). Experiment 1 indicated that BLA-lesioned animals showed an accelerated rate of cocaine self-administration relative to sham-operated controls at the high dose of cocaine, although both groups self-administered equivalent doses of cocaine by the sixth and seventh self-administration days. As this dose was chosen to assess the acquisition of a second-order schedule of cocaine self-administration a comprehensive dose response test was carried out to determin whether differences in the acquisition of BLA-lesioned and sham-operated control animals were due to alterations in the rewarding effect of cocaine in BLA-lesioned animals.

Experiment 5: Second-order schedule of self-administration

During the initial acquisition of cocaine self-administration under a schedule of continuous reinforcement (CRF) each response on the drug lever resulted in an IV infusion of cocaine (0.1ml over 4s) and the simultaneous presentation of a 20s light stimulus (CS), positioned directly above the active drug lever. This CRF schedule can be denoted as a second-order schedule type FR1(FR1:S). The information within the brackets denotes the fixed-ratio (FR) of lever responses to CS presentations (represented by 'S'). The numerical suffix denotes the number of times that unit of behaviour (lever response/ CS ratio) must be repeated to gain reinforcement. In this example, both the bracketed component and numerical suffix are represented by one, therefore this schedule denotes continuous

reinforcement (CRF). A more demanding schedule is indicated by the second-order schedule FR10(FR8:S); in this case, the information within the brackets indicates a fixed-ratio of eight lever responses for each CS presentation, and the numerical suffix denotes the number of times this unit of behaviour must be repeated ten times to gain reinforcement i.e. rats must make 80 responses, resulting in the presentation of ten CSs, before the first infusion of cocaine.

Methods

Thirty-four BLA-lesioned animals and 22 sham-operated controls began this experiment. Once stable rates of responding had been achieved under CRF, responding became progressively more demanding over sessions, under the increasing requirements of the second order schedule. In the first stage, each rat was required to respond on the drug lever under a second-order schedule FR10(FR1:S) i.e. each lever response produced a CS presentation and 10 CS presentations were required to produce the first IV infusion of cocaine. Subsequently, the second-order schedule were made more stringent by systematically increasing the ratio of responses required for each CS presentation in successive stages: FR10(FR2:S), FR10(FR4:S) and FR10(FR8:S). Once the first infusion of each session was obtained under a second-order schedule, nine further infusions were allowed under CRF. Therefore, each animal self-administered a maximum of ten infusions/ day. The time taken for each animal to complete the second-order response requirement and therefore attain the first infusion of each session was recorded on each self-administration day. Each animal also progressed at its own rate through the successive second-order schedules. To succeed at any

stage, and therefore progress to the next stage, each rat was required to attain its first daily infusion in under five min, and within five consecutive daily sessions. Those rats that did not reach this criterion were adjudged to have failed, but received further opportunities on subsequent days to achieve criterion. Rats that were successful moved directly to the next stage of the schedule, on their next test day.

Results

(i) Acquisition of cocaine self-administration

Nine lesioned animals were discarded from the final analyses, eight because of incomplete BLA lesions, following histological examination and one animal died post-IV surgery because of an infection. Of these animals, five were discarded because the basal and lateral nuclei of the amygdala were only partially lesioned, either bilaterally or unilaterally. One of these five also showed damage to the lateral central nucleus and partial cell loss in the ventral caudate putamen and entorhinal cortex unilaterally. Three animals showed good lesions but the placement of these lesions were inaccurate. For two of these rats the antero-posterior co-ordinates were incorrect and the lesions did not encompass the entire antero-posterior extent of the basal and lateral amygdaloid nuclei. For the third animal the lateral co-ordinates were incorrect and lesion damage extended into the medial amygdaloid nucleus and ventral central nucleus unilaterally and spared the major extent of basal amygdala contralaterally, primarily damaging the lateral nucleus, endopiriform nucleus and pirifrom cortex. The statistical analysis for the

initial acquisition of cocaine self-administration at a dose of 0.5 mg/ infusions, included 25 BLA-lesioned animals and 22 sham operated controls.

Fig. 11 shows the mean drug and non-reinforced lever responses for BLA-lesioned and sham-operated control animals over the first four days of CRF acquisition. BLA-lesioned animals responded significantly more on the drug lever than did the sham-operated controls. A three-way analysis of variance revealed significant Group x Day [$F(3,135)=5.23$, $p=0.002$] and Group x Lever [$F(1,45)=13.21$, $p<0.001$] interactions. Further post-hoc analyses showed significant differences between sham-operated and BLA-lesioned animals in responding on the drug lever [$F(1,73)=29.46$, $p<0.001$], but there was no significant difference between groups in non-reinforced lever responding [$F(1,73)=3.0$, p=NS]. Drug-lever responding was significantly higher BLA-lesioned animal's than in sham-operated controls over the first four days of acquisition ($p<0.05$ on each day), but both groups self-administered equivalent amounts of cocaine by the fifth self-administration day.

Separate analysis of the BLA-lesioned and sham-operated control groups showed significant differences between the patterns of drug and non-reinforced lever responding. Sham-operated control animals showed a gradual increase in drug lever responding over the first three days and a concomitant reduction in non-reinforced lever responding in the first two days, which gave rise to a significant Day x Lever interaction [$F(3,66)=6.9$, $p<0.001$] for this group. In contrast BLA-lesioned animals, maintained a high rate of drug lever responding relative to non-reinforced lever responding

Fig. 11

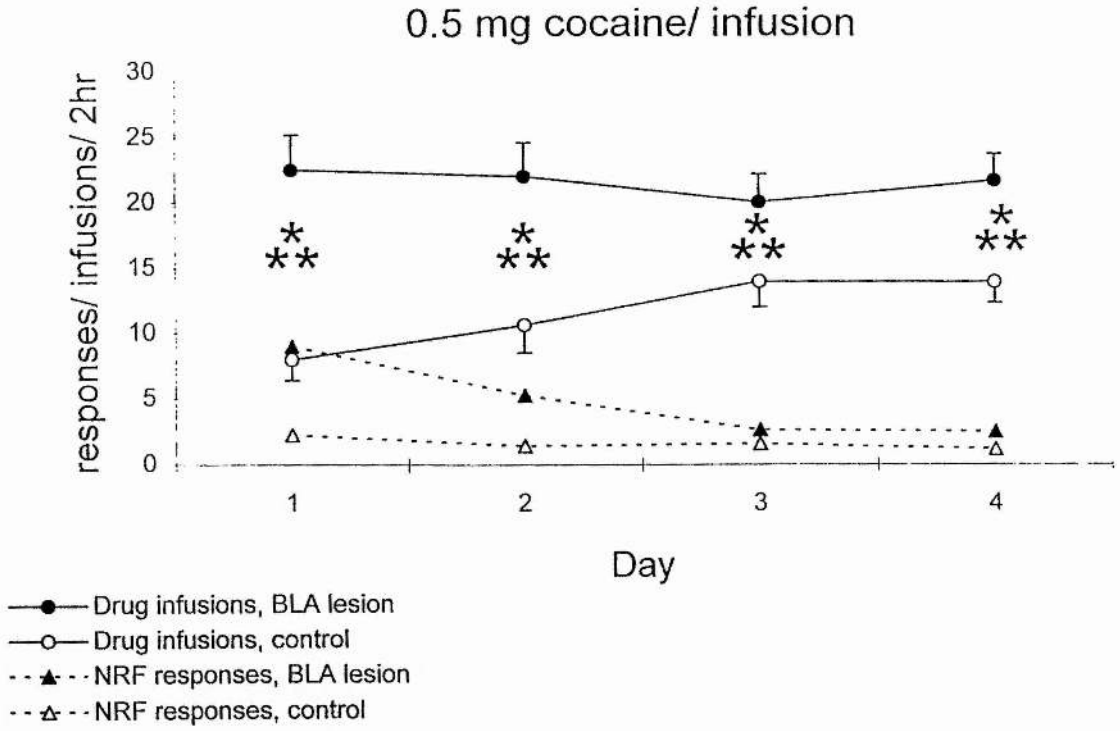


Fig. 11 Mean drug and non-reinforced (NRF) lever responses in BLA-lesioned and sham-operated controls over the first 4 days of cocaine self-administration under a schedule of continuous reinforcement (0.5 mg cocaine/ infusion).

*** = $p \leq 0.005$

from day one and throughout the acquisition period, reflected as significant effects of Lever [$F(1,23)=135.17$, $p<0.001$] and Day [$(3,69)=2.94$, $p<0.05$] for the group but there was no significant Lever x Day interaction ($F<1$).

(ii) Second-order schedules of cocaine self-administration

Following the acquisition of IV cocaine self-administration, nine lesioned animals and ten sham-operated control animals were discarded due to catheter failure. Therefore, the final numbers entering the second-order phase of the experiment were 16 BLA-lesioned animals and 12 sham-operated controls. Fig. 12 shows the proportion of BLA-lesioned and sham-operated control animals reaching criterion for the acquisition at each stage of the second order schedule of cocaine self-administration. Control animals acquired progressively demanding second-order schedules more quickly than BLA-lesioned animals and consequently required fewer repetitions at each stage during the acquisition. In comparison, BLA-lesioned animals required more attempts to reach criterion at each stage and as a result more lesioned animals were also lost because of catheter failure. The size of the lesioned group was necessarily reduced at successive stages, this effect can be seen in the upper panel of Fig. 12.

Fisher Exact probability estimations carried out at each second-order stage, showed a significant difference between the groups in their acquisition of the first ($p<0.01$) and second ($p<0.05$) stages of the second order schedule. Due to diminishing group size, further analyses at higher levels of the schedule were not possible. The lower panel in Fig. 12 shows the proportion of rats reaching criterion each day

Fig. 12 Acquisition of a second-order schedule of cocaine self-administration in BLA-lesioned and sham-operated control animals. The upper 3 panels show the performance of both groups on each day, at each stage of the second-order schedule, whereas the lower panel summarises these results. To reach criterion and pass to the next stage of the of the schedule each rat had to administer the first cocaine infusion of their session within five min, and within five consecutive sessions at each stage. Animals failing to reach criterion did not pass to the next stage but continued to be tested daily until the criterion was achieved.

Fig. 12

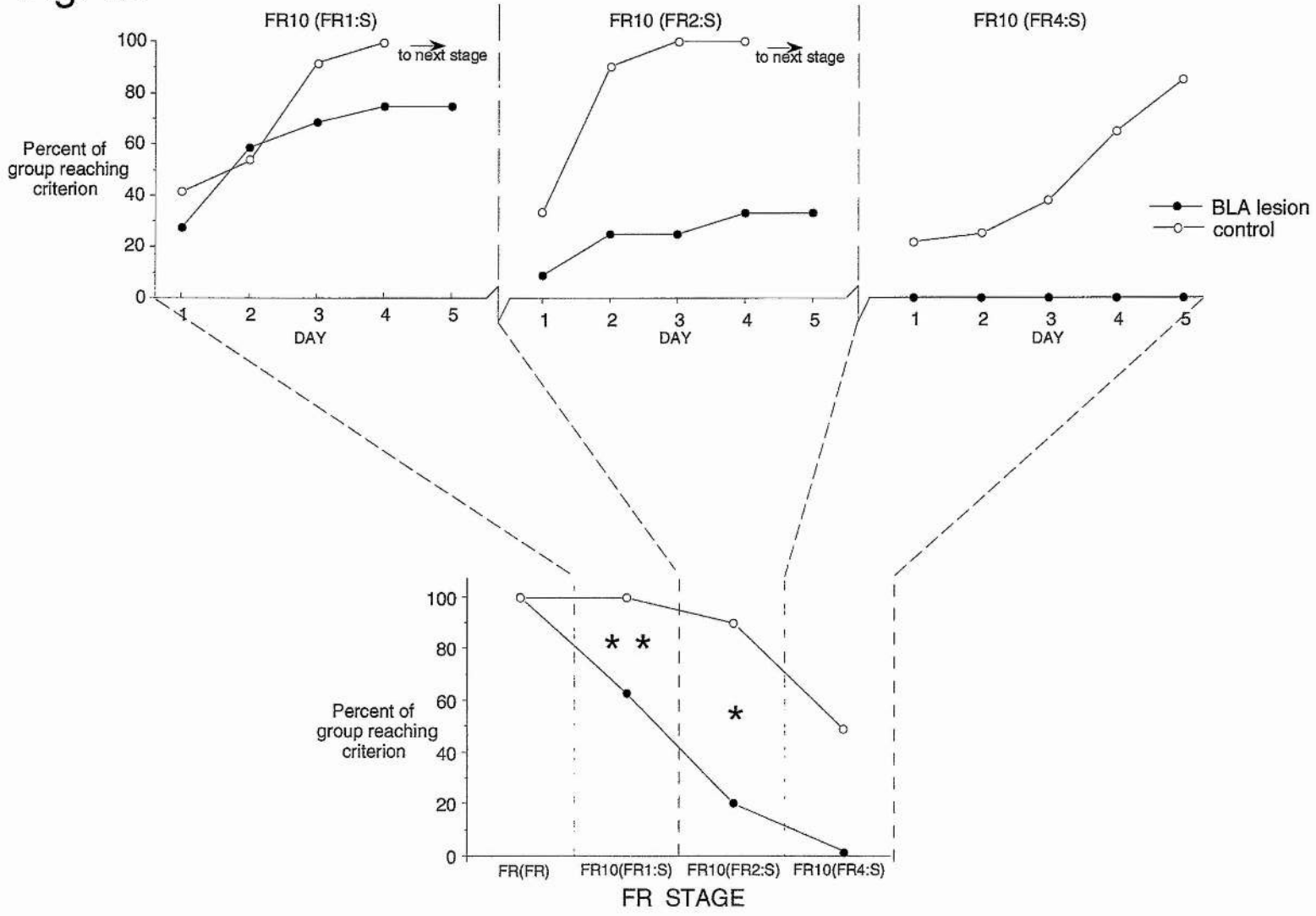

FR10 (FR1:S)
FR10 (FR2:S)
FR10 (FR4:S)
to next stage
to next stage
Percent of group reaching criterion
100
80
60
40
20
0
1
2
3
4
5
DAY
BLA lesion
control
* *
*
FR(FR)
FR10(FR1:S)
FR10(FR2:S)
FR10(FR4:S)
FR STAGE

Fig. 13

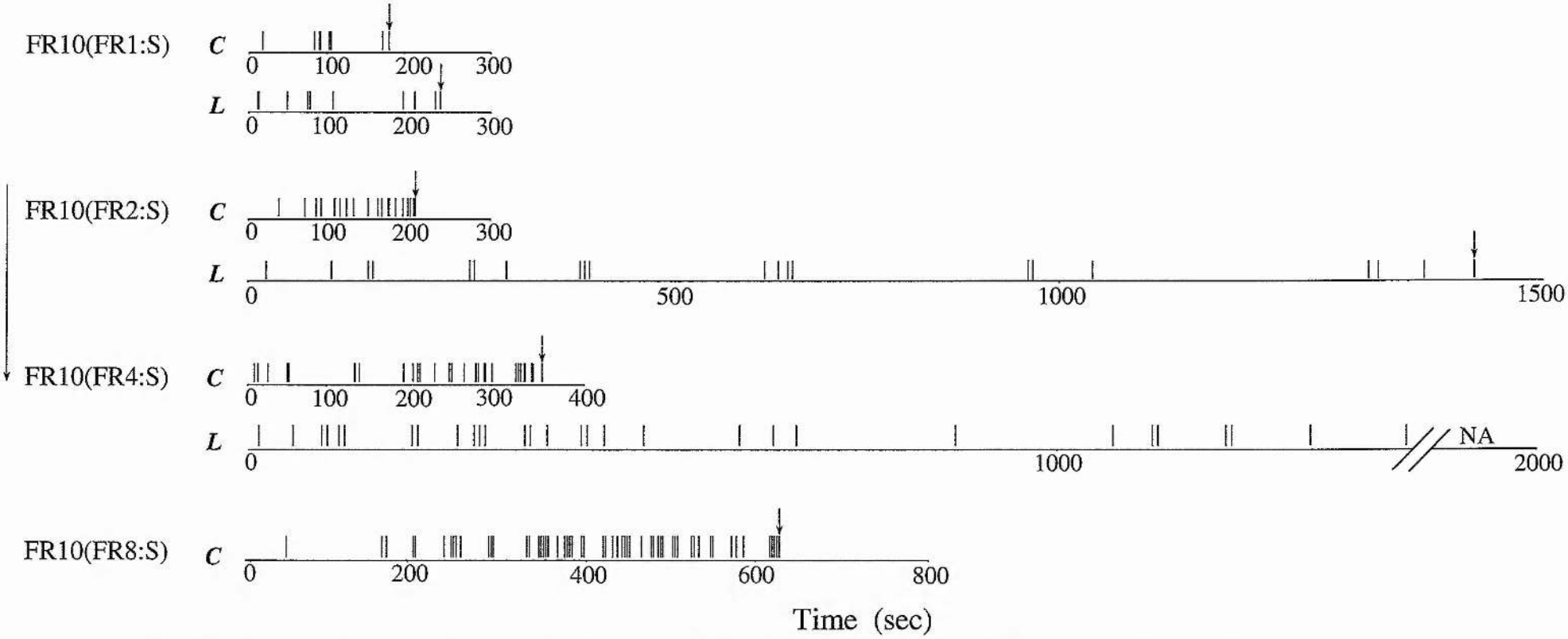


Fig. 13 Representative event records of responding in BLA-lesioned and sham-operated control rats (represented by L and C respectively) at sucessive stages in the second-order schedule of cocaine self-administration. Arrows indicate the administration of a cocaine infusion, and NS indicates that the drug infusion was not achieved. CS presentations are not indicated because the resolution of print is such that several lever presses may appear superimposed on each other. However, in each case, ten CSs were presented for each drug infusion earned.

during the acquisition of each successive stage of the second-order schedule. Fig.13 depicts the event records of responding in BLA-lesioned and sham-operated control subjects at each stage of the second-order schedule; the increased time taken by rats with BLA lesions to complete the response requirement at each stage is illustrated for a typical subject.

Discussion

BLA-lesioned animals showed an elevated rate of cocaine self-administration during the first four days of acquisition on CRF and as a result experienced more CS-drug pairings than sham operated controls (thus replicating the earlier findings in Expt. 1 at 0.5 mg cocaine/ infusion). Yet, these lesioned animals were impaired from the first stage in the acquisition of the second-order schedule FR10 (FR1:S). Fig. 13 depicts the event record for both BLA-lesioned and sham-operated control animals at successive stages of the second-order schedule. The sham-operated control animal responded more quickly than the lesioned animals and also show a pattern of rapid burst-like responding dependent upon the contingent presentation of the CS. In comparison, responding of the lesioned animal appears irregular and unconnected to presentation of the drug-related conditioned stimulus. This would suggest that behaviour of control animals is strongly linked with, and directed by, the presentation of the drug-related conditioned stimulus whereas lesioned animals show no sign of being influenced by the CS.

The use of IV cocaine administration to support operant responding was first reported in the primate by Deneau et al. (1969) while Dougherty and Pickens

(1973) later established a fixed-interval schedule of cocaine self-administration in the rat. Second-order schedules of drug self-administration were also established in monkeys initially (Goldberg 1973; Goldberg et al. 1976; Goldberg and Tang 1977; Katz 1979) and then later in the rat (Corrigal and Coen 1989). These original self-administration studies were concerned with detailing the effects of unit dose alterations and assessing the influence of reinforcement schedules on the pattern of responding maintained by drugs of abuse. In contrast, the present study employed a second-order schedule of cocaine self-administration to investigate the neural mechanisms underlying cue-elicited drug-seeking behaviour, in which performance was dependent upon the formation and utilisation of cocaine-related conditioned associations. In the present experiment, infusions of cocaine were contingent upon the completion of a fixed-ratio of responses, which were themselves reinforced under a unit fixed-ratio of responding for the periodic presentation of a cocaine-related CS. The results of this experiment have shown that rats with lesions of the BLA were severely impaired in their acquisition of a second-order schedule of cocaine self-administration, but were not impaired in the acquisition of self-administration *per se,* under a schedule of continuous reinforcement. These findings lend support to the growing body of literature which implicates the BLA in the process of conditioned reinforcement and in addition, demonstrates that the BLA is an important component in the neural mechanisms underlying cue-elicited drug-seeking behaviour.

Further support for BLA involvement in cue-elicited cocaine-seeking behaviour are found in a recent report by Meil and See (1997). In their study, excitotoxic lesions

of the BLA abolished the ability of a drug-related cue to elicit responding for cocaine self-administration. Animals first acquired cocaine self-administration and were then subject to a period of extinction training in which drug-maintained responding was successfully eliminated, as both cocaine and the drug-related stimuli were omitted for 20 repeated sessions. Following extinction training, in the absence of cocaine, presentation of the drug-related conditioned stimulus significantly increased responding on the lever previously associated with cocaine in control animals but made no impact on the behaviour of animals with lesions of the BLA. Similar to the conclusions of the present work, Meil and See (1997) proposed that BLA-lesioned animals failed to make the association between cocaine reinforcement and the contingent presentation of the drug-related stimulus. As a result, these lesioned animals were unresponsive to the reinstatement of the drug-related conditioned stimuli. Interestingly, Meil and See (1997) also reported that lesions of the BLA made after seven days of cocaine self-administration did not affect subsequent self-administration rates. This finding is also consistent with the conclusions of Expt. 3 (Chapter 3) and suggests that the BLA is not involved in the maintenance of self-administration once the behaviour is established. Therefore, there is now a body of literature which strongly suggests that the BLA is not involved in the primary reinforcing effects of cocaine but is part of a neural system through which stimuli acquire motivational salience and thereby control over instrumental behaviour, including cocaine-seeking behaviour.

Experiment 6: Within-session dose response function

A within-session dose response test involves the systematic reduction of the self-administered drug dose, within one self-administration session. Post-hoc correlations for each animal can then be made between drug dose and subsequent rates of self-administration and non-reinforced lever responding. Drugs of abuse typically produce an inverted-U shaped function when drug dose is plotted against self-administration rate (Woods et al. 1987). Once an animal has acquired self-administration, reductions in the training drug dose give rise to increases in self-administration rate, possibly to maintain optimal plasma drug levels. Similarly, increases in the self-administered drug dose produce initial reductions in self-administration rates, possibly to limit toxicity. If a neural manipulation causes this function to shift significantly, either to the left or right of the appropriate control group's pattern of responding, it can be concluded that the reinforcing efficacy of the self-administered drug has also been altered significantly by the neural manipulation.

Methods

The nine BLA lesioned and 6 sham operated control animals used in Expt. 1 and which had acquired cocaine self-administration at 0.5 mg/infusion, were used in this experiment. In order to reduce the duration of the dose-response test, 8 test doses were allocated to two separate dose response test sessions, A and B, which were conducted on separate days with an intervening baseline day. The order in which the tests were carried out was also balanced across groups, either dose response A followed by B or dose response B followed by A, therefore each animal was tested twice. Each within-session dose response test began with 30 min IV cocaine self-

administration at the training dose (0.5 mg/ infusion), followed by four separate test doses self-administered for 45 min each; dose response test A used doses of 0.5, 0.25, 0.063, 0.016 and 0.004 mg cocaine/ infusion, while dose response test B used doses of 0.5, 0.125, 0.031, 0.0076 and 0.00 (saline) mg cocaine/ infusion. Care was taken to ensure the length of tubing from the pump to the connecting swivel was as short as possible and equal for all boxes, to guard against mixing of doses as the syringes were changed within-session. However, as a further precaution against such problems, data from the first 15 min of each 45 min dose period were excluded from the final analyses. This procedure has been utilised successfully in earlier work (Phillips et al. 1994 a, b). Responding was recorded for both non-reinforced and drug levers throughout.

Results

During the experiment two lesioned animals had to be discarded because of catheter failure. Therefore, the final statistical analysis included seven BLA-lesioned animals and six sham-operated controls. Fig. 14 illustrates mean drug and non-reinforced lever responses at all doses tested for both BLA-lesioned and sham-operated control animals. BLA-lesioned animals showed increased levels of cocaine self-administration across all doses tested when compared with sham-operated controls. However, this elevation in responding was mirrored by the control group and there was no overall lateral shift in the dose response function between the lesioned and control groups. A three-way analysis of variance revealed significant main effects of Group [$F(1,11)=6.24$, $p<0.05$], Dose [$F(9,99)=26.92$, $p<0.001$] and Lever [$F(1,11)=219.72$, $p<0.001$].

Fig. 14

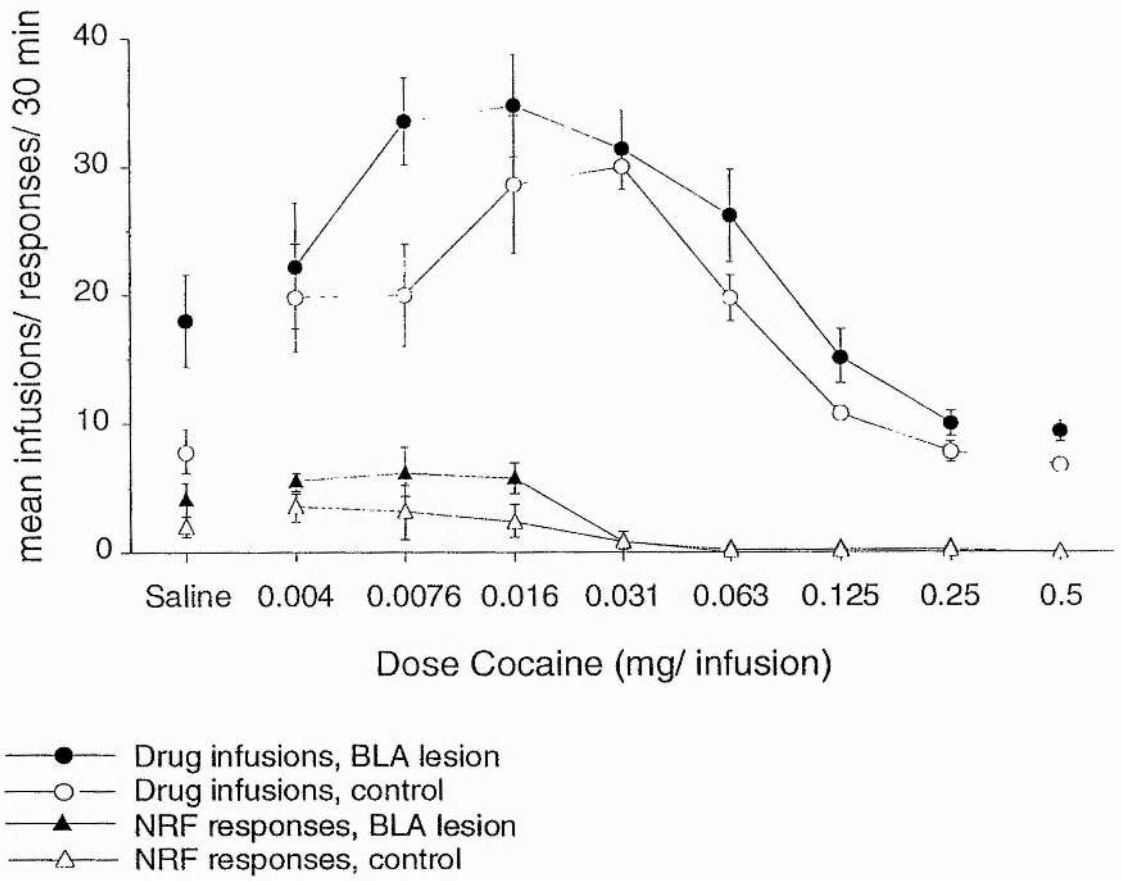


Fig. 14 Mean drug and non-reinforced (NRF) lever responses during a within-session dose response test in BLA-lesioned an sham-operated control animals. The doses were administered in descending order from 0.5 mg cocaine/ infusion. With the exception of the highest dose which was available for the first 30 min of each test session, access to every other dose was restricted to 45 min/ dose.

Both Lever x Dose and Lever x Group interaction were also significant [$F(9,99)=22.95$, $p<0.001$] and [$F(1,11)=4.88$, $p<0.05$] respectively. Although, Group x Dose [$F(9,99)=1.44$, p=NS] and Group x Dose x Lever interactions [$F(9,99)=0.80$, p=NS] were both non-significant. Further two-way analysis of variance of drug lever responding only, using between subject factor of Group (BLA, control) and within-subject factor of Dose (0.5, 0.25, 0.125 etc.) indicated that there was no significant difference between the groups' rate of drug self-administration at any dose [$F(9,99)=1.13$, p=NS].

Discussion

The results of this experiment show that once cocaine self-administration is established at 0.5 mg cocaine/ infusion, both lesioned and control animals respond similarly to reductions in the unit dose of self-administered cocaine. The dose response function of BLA lesioned animals did not differ significantly from control animals, despite the fact that lesioned animals self-administered significantly more drug than controls during the first five days of cocaine self-administration (Expt. 1, Fig. 6a). As a drug dose is reduced below a detectable level of reinforcement, animals often increase non-reinforced lever responding, presumably as an alternative drug-seeking strategy. Both BLA-lesioned and sham-operated animals started to increase their non-reinforced lever responses at the same dose (0.031 mg cocaine/ infusion), although lesioned animals did not show complementary reductions in drug lever responding until a dose four-fold lower than sham operated controls (0.004 as opposed to 0.016 mg cocaine/ infusion). Lesions of the BLA did not alter the reinforcing properties nor the psychomotor stimulant properties of

cocaine (as demonstrated in Expt. 1 and 4; Chapter 3), but they may produce deficits in an animal's flexibility to respond to alterations in the reinforcing outcome of specific patterns of behaviour. Animals with lesions of the BLA have been shown to persist in fixed patterns of behaviour during a run-way task, irrespective of alterations in the magnitude of reinforcement presented (Kemble and Beckman 1970), and to show repetitious 'frustrative' responding following repeated non-reinforced CR (Henke and Maxwell 1973). McDonough and Manning (1979) proposed that neither 'response perseveration' or 'frustrative emotionality' fully explained the pattern of behaviour seen in animals with lesions of the BLA and suggested instead that these animals were *less* under the control of conditioned reinforcers. This suggestion is in keeping with the postulated importance of the BLA in conditioned reinforcement paradigms (Cador et al. 1989; Everitt at al. 1989; Robbins et al. 1989; Everitt and Robbins 1992; Burns et al. 1993).

More recently, Hatfield et al. (1996) have reported the specific involvement of different amygdaloid nuclei in second-order conditioning and reinforcer devaluation, maintained by food reinforcement. Discrete lesions of the BLA but not the central amygdaloid nucleus (CeA) impaired the acquisition of a second-order schedule of food reinforcement in which animals were initially trained to associate the presentation of food with a discrete light stimulus. Following the initial training period the acquired motivational strength of the light stimulus was assessed by its ability to act as a reinforcer for second-order conditioning of a tone stimulus, when light-tone pairings were presented in the absence of food. BLA-

lesioned animals were also insensitive to reinforcer devaluation following conditioned reinforcement training, but like controls, animals with lesions of the CeA were able to adjust their conditioned responding to alterations in reinforcer value. The present findings with cocaine self-administration are very similar to those of Hatfield et al. (1996) in that lesions of the BLA severely impaired the acquisition of a second-order schedule of cocaine self-administration and that animals with lesions of the BLA continued to self-administer low doses of cocaine (ultimately saline), which did not support self-administration in sham-operated controls during the dose-response test. Taken together, the present results, those of Hatfield et al. (1996) and those of Meil and See (1997) provide strong evidence for the specific involvement of the BLA in conditioned associations and the adaptation of ongoing behaviour in response to environmental contingencies.

Conclusions

The experiments in this chapter have established the use of a second-order schedule of cocaine self-administration as a method of assessing drug-seeking behaviour in the rat, un-contaminated by the direct stimulant properties of cocaine. Excitotoxic lesions of the BLA selectively impaired the acquisition of a second-order schedule of cocaine self-administration, and this effect could not be attributed to alterations in the reinforcing effects of cocaine in lesioned animals. These results suggest that the BLA is specifically involved in the formation of conditioned associations which underlie cue-elicited cocaine-seeking behaviour. Both control and lesioned animals responded similarly during a within-session dose response test, although lesioned

animals continued to respond for very low doses of cocaine, it is likely that this reflects insensitivity to reinforcer devaluation rather than a lateral shift in the dose-response function.

Chapter 5: Acquisition of a progressive-ratio schedule of cocaine self-administration

Introduction

The findings of Chapter 4 demonstrated that lesions of the BLA significantly retarded the acquisition of a second-order schedule of cocaine self-administration. Despite experiencing more CS-drug pairings than control animals during the first four days of cocaine self-administration, contingent presentation of the discrete drug-associated stimulus did not maintain responding in BLA-lesioned animals - an important prerequisite for the successful acquisition of a second-order schedule of cocaine self-administration. The second-order experiment described in Chapter 4 identified specific neural substrates which may differentially mediate psychological aspects of cocaine-seeking behaviour, unaffected by the direct pharmacological actions of self-administered cocaine. Many studies have investigated the impact of neural manipulations, antagonist/ agonist pretreatments on drug self-administration under a number of different schedules of reinforcement, but these schedules invariably measure behaviour under the influence of the self-administered drug.

A schedule of this type which has been used extensively is the progressive-ratio (PR) schedule of reinforcement (Griffiths et al. 1979; Deminiere et al. 1988; Roberts et al. 1989; Loh and Roberts 1990; Spear and Katz 1991; McGregor and Roberts 1993; McGregor et al. 1994; Woolverton 1995; Ranaldi and Roberts 1996). Under a PR schedule the response requirement necessary for successive drug

infusions increases systematically throughout each self-administration session. However, as psychomotor stimulants produce dose-dependent rate altering effects on locomotor activation (Woods et al. 1987), the behaviour measured under a PR schedule is at least to some extent under the control of the self-administered drug and the effects of neural manipulations or drug pre-treatments on responding may be confounded by these direct drug effects. None the less, variations of the PR schedule have been used extensively to investigate the rewarding properties of many different kinds of reinforcers. A major benefit of this kind of schedule is that it provides a reliable index of the motivation an animal has to work for reinforcement which can be used to compare the relative reinforcing power of different reinforcers. This index is termed the break-point (BP) and refers to the point at which the response requirement necessary to produce the next reinforcer outweighs the potential reward available, and the animal quits responding altogether.

Progressive-ratio schedules were first reported by Hodos (1961) who assessed the effect of variations in reward value and deprivation state of rats trained to respond for sweetened condensed milk. It was found that increases in BP correlated with increases in both the concentration of the reward solution and an animal's state of deprivation (hunger). This was later replicated using intra-cranial self-stimulation, which also showed that increasing duration (Hodos 1965) or intensity of electrical stimulation (Keesey and Goldstein 1968) correlated with greater BPs. Several studies have since used BP to establish the relative reinforcing effects of different drugs of abuse (Yanagita 1973; Griffiths et al. 1975, 1978; Spear and Katz 1991;

Woolverton 1995) as well as providing a means of investigating the impact of pharmacological compounds on the reinforcing effects of a drug, as opposed to the psychological factors involved in drug-seeking behaviour (Roberts et al. 1989; Loh and Roberts 1990; McGregor and Roberts 1993; McGregor et al. 1994; Ranaldi and Roberts 1996).

During a PR schedule of drug self-administration, the overall number of infusions earned by an animal in any session can be used as an index of the incentive motivational value of the drug in question. Each session begins on a low ratio of responses for the drug infusion and this can be increased according to either an exponential (Roberts and Richardson 1992) or arithmetic ratio (Griffiths et al. 1979). For example, under an arithmetic ratio, the response requirement for the first infusion of a session might be one lever press, and this requirement may double for each infusion thereafter so that the response requirement would increase in the following manner - 1, 2, 4, 8, 16, 32, 64, 128 .... etc., for the first 8 infusions. Once a set period of non-reinforcement elapses (e.g. 1hr) because an animal fails to fulfil the necessary response requirement for the next infusion in the series, the schedule automatically terminates and the last infusion earned is taken as the BP.

McGregor and Roberts (1993), investigated the effects of intra-cranial infusions of the D_1 dopamine receptor antagonist SCH 23390 infused either intra- amygdala or Nacc, shortly before cocaine self-administration sessions under both continuous (CRF) and PR schedules of reinforcement. It was reported that under a CRF schedule, SCH 23390 intra-amygdala had a greater rate increasing effect on cocaine

self-administration than identical infusions intra-Nacc. However, under a PR schedule, SCH 23390 intra-Nacc produced greater reductions in BP than identical intra-amygdala infusions. Increases in the rate of self-administration have primarily been regarded as indicative of reductions in the rewarding efficacy of a drug (Yokel and Wise 1976) and the authors concluded that the apparent dissociation of effects between PR and CRF schedules may measure different aspects of a drug's rewarding properties. It was suggested that performance under a PR schedule may rely more on intact DA function within the Nacc, as lesions of this structure have been shown to disrupt cocaine self-administration (Roberts et al. 1980) and that the Nacc may play an important part in the translation of motivation to action (Mogenson et al. 1980; Cador et al. 1991). On the other hand, amygdaloid DA afferents may make a significant contribution to the interoceptive stimulus properties of cocaine which would be more important under a CRF schedule (McGregor and Roberts 1993).

McGregor et al. (1994) went on to assess the impact of 6-OHDA lesions of the amygdala on performance under PR schedules of cocaine reinforcement. Amygdaloid projections to the Nacc stem almost exclusively from the BLA (Kelley et al. 1982; McDonald 1991b) and the BLA also has reciprocal connections with the prefrontal cortex (McDonald 1991a), both of which are considered important structures in the mediation of cocaine reinforcement (Schenk et al. 1991; Weissenborn et al. 1997; Hurd et al. 1997; Pierce et al. 1998). It was anticipated that 6-OHDA lesions of the amygdala would attenuate the reinforcing effects of cocaine in a similar manner to D_1 receptor blockade, but this lesion actually

increased BP at the highest dose of cocaine tested, under a PR schedule. In an attempt to reconcile the discrepancy between these studies, McGregor et al. (1994) hypothesised that responding under a CRF schedule was dependent on an animals ability to monitor optimal plasma levels of cocaine, through interoceptive drug cues, whereas performance under a PR schedule necessitated the translation of this experience to motivational goal directed behaviour, in which D_1 receptor blockade, within the amygdala, produced no significant effect. It was concluded therefore, that the amygdala was involved in the interoceptive stimulus properties of cocaine but not the motivation to self-administer cocaine (McGregor et al. 1994).

Further support for this hypothesis was derived from a study by Deminiere et al. (1988) who reported that 6-OHDA lesions of the amygdala sensitised animals to the reinforcing properties of amphetamine and that these lesioned animals acquired amphetamine self-administration at doses which would not sustain significant self-administration in sham-operated controls. Deminiere et al. (1988) investigated the acquisition of amphetamine self-administration under CRF schedule and explained their findings in terms of an increase in mesolimbic transmission resulting from the amygdala lesion, hence facilitating the acquisition of lower doses of amphetamine. However, it is difficult to compare these studies directly with those of McGregor et al. (1994) as the latter investigated the impact of 6-OHDA lesions of the amygdala in drug-experienced animals trained to self-administer cocaine under a PR schedule, whereas Deminiere et al. (1988) studied the acquisition of amphetamine self-administration.

Currently there is considerable evidence to indicate that the development of behavioural sensitisation to psychomotor stimulants can be dissociated temporally and anatomically into two components which have been termed initiation and expression (Leith and Kuczenski 1982; Kalivas and Stewart 1991; Cador et al. 1995). The ventral tegmental area (VTA) is considered important in the initiation of behavioural sensitisation (Vezina and Stewart 1990; Kalivas and Aldesdatter 1993; Kalivas 1995; Wolf and Xue 1998) whereas the Nacc and dorsal prefrontal cortex (Pfc) are considered important in mediating the expression of behavioural sensitisation (Wolf et al. 1995; Pierce and Kalivas 1997; Pierce et al. 1998). It is of interest to note that the central nucleus of the amygdala (CeA) projects to the VTA (Wallace et al. 1992) while the dorsal BLA projects heavily to the shell of the Nacc and the BLA also has reciprocal connections with the dorso-lateral Pfc (McDonald 1991a). These anatomical connections might indicate that disruption of function within the CeA or BLA may also produce distinct effects, depending on phase of initiation or expression of behavioural sensitisation.

However, the limitations of intra-cranial infusions must also be considered. Caine et al. (1995) demonstrated that under a CRF schedule of cocaine self-administration intra-cranial infusions of SCH 23390 produced greater rate increasing effects on self-administration when infused intra-Nacc than identical infusions intra-amygdala. Furthermore, the concentration of intra-amygdala infusions of radioactive labelled [^{3}H]SCH 23390, halved within 10 mins of infusion, at the site of injection, and diffused significantly to encompass the entire amygdala within 40

min post-infusion. These findings are also supported by the results of an in vivo voltammetric study (Jones et al. 1995) which found endogenous DA-uptake within the BLA to be relatively insensitive to uptake inhibitors, including cocaine, at concentrations which produced dramatic inhibition within the striatum. In addition, DA uptake within the BLA was also shown to be significantly slower than that within the Nacc and caudate putamen i.e., the half-life of DA within the extracellular space maybe longer in the BLA. Together, these findings would indicate that the specificity of intra-cranial infusions are both regionally and temporally dependent, and that unless suitable precautions are taken, neighbouring structures may be influenced as each infusion dissipates. The findings of Jones et al. (1995) also indicate that intra-amygdala infusions of DA antagonists might produce greater disruption of DA function within the CeA than the BLA.

The effect of BLA lesions on the acquisition of cocaine self-administration at a high dose, reported in this thesis (Expt. 1; Chapter 3) may support the conclusion of McGregor and Roberts (1993) that amygdala lesions increase the rate of cocaine self-administration by diminishing the interoceptive stimulus of cocaine, under a CRF schedule of cocaine self-administration. Yet, these findings are not consistent with those of Deminiere et al. (1988) who reported that 6-OHDA lesions of the amygdala enhanced the acquisition of amphetamine self-administration at low doses. BLA-lesioned animals in this thesis (Expt. 1; Chapter 3) showed enhanced acquisition of a high dose of cocaine self-administration (0.5 mg/ infusion) relative to control animals, but there was no significant difference between the groups' acquisition at the lower doses. It seems unlikely that the contrast in these effects

stem from differences between the actions of amphetamine and cocaine, and would indicate instead, that the neuronal basis of these effects differ. Care was taken in the experiments presented in this thesis to exclude animals sustaining neuronal damage to the CeA. In most instances, lesions were restricted to the basal and lateral nuclei of the amygdala as the CeA appears to be relatively insensitive to quinolinic acid and the toxin tended to diffuse laterally from the BLA towards the cortex. However, intra-cranial infusions used by McGregor and Roberts (1993) would undoubtedly encompass both the BLA and CeA within a very short time and it is possible that the 6-OHDA lesions employed by both McGregor et al. (1994) and Deminiere et al. (1988) also destroyed DA terminals within both the CeA and BLA. The extent of their lesions were also recorded as HPLC assays of endogenous dopamine and dihydroxyphenylacetic acid (DOPAC) found in the amygdala. DOPAC is a metabolite of DA and the DA/ DOPAC ratio found in a tissue sample is considered an index of dopaminergic activity, although this gives no indication of the exact source of the neurotransmitter other than that it is endogenous.

As mentioned above, the development of sensitisation to psychomotor stimulants are considered to be temporally and anatomically distinct. Therefore, it is also possible that lesion effects produced during the acquisition of drug self-administration may differ from those produced once this behaviour is established and an animal has gained significant drug experience. Experiment 3 (Chapter 3) of this thesis indicated that excitotoxic lesion of the BLA made following the acquisition of cocaine self-administration did not affect self-administration rates,

seen during the acquisition of this behaviour. In an attempt to clarify the findings reported by McGregor et al. (1993, 1994) and Deminiere et al. (1988) this chapter aims to investigate the effect of quinolinic acid lesions of the BLA on cocaine self-administration under a PR schedule of reinforcement. First, to assess the importance of BLA efferents unconfounded by the possible disruption of CeA neurotransmission, therefore to differentiate whether or not the origin of McGregor and Roberts findings arise primarily through the disruption of CeA or BLA neurotransmission. Second, to investigate whether the motivation to self-administer cocaine is altered in BLA-lesioned animals while under the influence of cocaine. Deficits in the acquisition of a second-order schedule of cocaine self-administration seen in BLA-lesioned animals (Expt. 5; Chapter 4) and the findings of Meil and See (1997) indicate that the BLA is an important component in the neural mechanism underlying cue-elicited drug-seeking behaviour. Similar lesions of the BLA have been shown to selectively diminish responding for a conditioned reinforcer, following intra-Nacc microinjections of amphetamine (Cador et al. 1989). Furthermore, in response to a low dose cocaine challenge, Fontana et al. (1993) demonstrated that a cocaine associated environment elicits conditioned increases in mesolimbic DA overflow, and with cocaine self-administration, and Ranaldi and Roberts (1996) reported that contingent presentation of a conditioned stimulus also enhanced the motivation to initiate and maintain cocaine self-administration. Taken together, these findings may indicate that cocaine associated cues also contribute to the reinforcing power of cocaine. McGregor et al. (1994) proposed that under a PR schedule of cocaine self-administration BP represents a measure of the translation of subjective experience into motivational action. If

conditioned associations are selectively impaired by lesions of the BLA and these conditioned associations also contribute to the reinforcing effects of cocaine, it is predicted that lesions of the BLA would also produce reductions in the BP measured under a PR schedule of cocaine reinforcement.

First, Expt. 7 tested the hypothesis of McGregor and Roberts (1993) which suggest that the BLA mediates the interoceptive stimulus properties of cocaine. If this is the case, it would be expected that under a PR schedule of cocaine self-administration, animals with lesions of the BLA should be relatively insensitive to alteration in drug dose. Secondly, those animals which complete the PR schedule in Expt. 7 were trained under a fixed-interval 15min fixed-ratio 10, schedule of cocaine self-administration FI15(FR10:S) and, in Expt. 8 the impact of CS omission on self-administration maintained under this schedule was assessed. Contrary to the conclusions of McGregor and Roberts (1993) it is proposed that the interoceptive stimulus properties of cocaine are not mediated by the BLA, but instead that the BLA is important in the formation of conditioned associations and it is the loss of such conditioned associations in BLA-lesioned animals, which interfere with motivational aspects of cocaine self-administration. As a result, it was predicted that under FI15(FR10:S) schedule of cocaine self-administration, animals with lesions of the BLA would be relatively insensitive to the omission and reinstatement of a cocaine associated cue.

Experiment 7: Lesions of the BLA and performance under a progressive ratio schedule of cocaine reinforcement.

Eighteen animals began this experiment. Eleven were given excitotoxic lesions of the BLA while the remaining seven were given sham-lesions with phosphate buffer replacing the toxin. Three days after lesion surgery the animals were surgically prepared with chronic IV catheters (for surgical procedure see General Methods p28 and p32). These animals were allowed to recover in their home cages for a minimum of seven days, before self-administration training began. All rats were then allowed to acquire cocaine self-administration (0.25 mg/infusion) 2hr/ day for seven days, on a fixed-ratio 1:1 (FR1:1) schedule of reinforcement.

Once the rats achieved a stable rate of self-administration on (FR1:1), they progressed onto an arithmetic PR schedule of cocaine reinforcement. The formula used to calculate the progressive response requirement in this experiment produced a series similar to the exponential schedule employed by McGregor and Roberts (1993) such that the response requirements for the fifth, 10th and 20th infusion were similar. In this experiment, the response requirement incremented by one for each successive infusion, and this value doubled after every third infusion. Therefore, the response requirement increased as follows:

Infusion in series		Response requirement per infusion	Response increment/ triad of infusions
1-3	→	|1, 1, 2 |	1
4-6	→	| 4, 6, 8 |	2
7-9	→	| 12, 16, 20 |	4
10-12	→	| 28, 36, 44 |	8
13-15	→	| 60, 76, 92 |	16
16-18	→	| 124, 156, 188 |	32
19-21	→	| 252, 316, 360 |	64
22-24	→	| 508, 636, 764 |	128

Table 1. Response requirements under the progressive-ratio schedule of cocaine reinforcement used in Expt. 7.

The response requirement for both the first and second infusion of each session was one lever response, because the program was written to include a 'priming' infusion which was *never* administered. Both BLA-lesioned and control animals were run on the PR schedule for 14 consecutive daily sessions. During this time, all parameters remained constant, except for drug dose, which was changed systematically over successive three and two day periods as outlined below. The overall number of responses made on both the drug and non-reinforced levers and the total number of infusions earned (BP) during each session were recorded for each rat. Statistical analyses of the behaviour over the first five days (acquisition training) were analysed separately.

PR Test Day	Dose of cocaine (mg/ infusion)
1-5	0.25
6-8	**0.083**
9-10	0.25
11-13	**0.5**
14	0.25

Table 2. The pattern of cociane dose alterations during the 14 day progressive-ratio trial (Expt. 7).

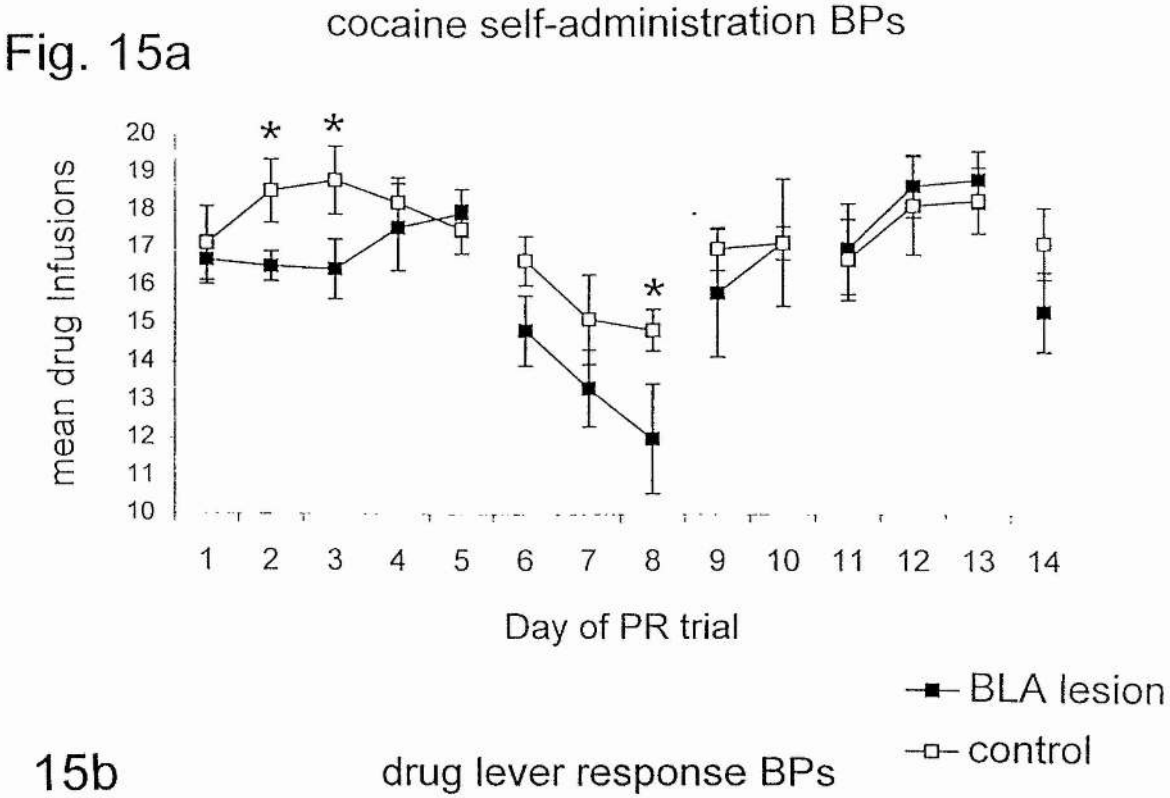
Statistical analyses

Initially, performance on the first five days under the PR schedule (0.25 mg cocaine/ infusion) was analysed using a two-way analysis of variance with factors: Group (BLA-lesioned, control) and Day (1, 2, 3, 4, 5). A second analysis was then carried out using a three-way analysis of variance to assess the effect of dose alterations on BP with factors: Dose (medium, low, high), Day (1,2,3) and Group (BLA-lesioned, control). Baseline days 3-5 of the PR trail were compared with days 6-8 and 11-13, the lower and higher test doses (0.083 and 0.5 mg cocaine/ infusion respectively). Post-hoc analyses were carried out using the Student-Newman-Keuls test, where applicable.

Results

One lesioned rat was discarded prior to analysis of the behavioural data because histological analysis showed there was considerable cell sparing in the basal and lateral nuclei of the amygdala, unilaterally. Two further lesioned animals were discounted during the acquisition of self-administration on continuous reinforcement, because of catheter failure. Of the rats which began the PR schedule eight BLA-lesioned and seven sham-operated controls were included in the first five days of PR acquisition. However a further two lesioned animals were subsequently lost due to catheter related problems, therefore, six BLA-lesioned animals and seven control animals were included in the final analysis of overall dose effects. The main results are shown in Fig. 15a, b. On each day of the PR trial BP was recorded separately for both the total number of infusions administered and the overall number of drug-lever responses. As the response requirements increase under a PR schedule, the overall number of drug-lever responses made may represent a more accurate measure of reinforcement because it includes behaviour for infusions that were not delivered (Woolverton 1995). Non-reinforced lever responding was also recorded on each day of the PR trail to provide a measure of basal activity. Fig 15a shows mean BPs for the number of infusions earned on each of the 14 days for BLA-lesioned and sham-operated control animals. Fig. 15b shows mean values for total number of drug lever responses made and Fig. 15c shows the mean number of non-reinforced lever responses made by each group on each day. Data for the total number of drug lever responses were square-root transformed before analyses to reduce overall variability, but in the interest of clarity only the non-transformed data are plotted (Fig. 15b).

Fig. 15a



15b

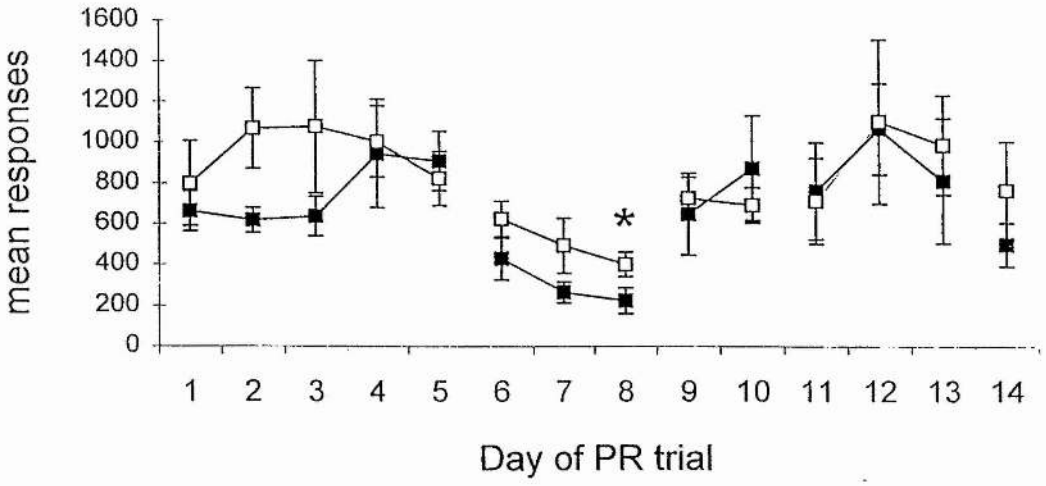


Fig. 15 a, b, c Depict the pattern of responding seen in BLA-lesioned and sham-operated control animals on each day of the 14 day progressive-ratio (PR) trial as the dose of cocaine was systematically lowered and then increased from baseline. Mean infusion break-points (Fig. 15a); mean drug lever responding (Fig. 15b); mean non-reinforced lever responding (Fig. 15c, over page). Baseline days 1-5 inclusive (0.25mg cocaine/ infusion); low dose, days 6-9 inclusive (0.083mg cocaine/ infusion); high dose, days 11-13 inclusive (0.5mg cocaine/ infusion); days 9, 10 and 14 repeat baseline days (0.25mg cocaine/ infusion). *=$p<0.05$

Fig. 15c

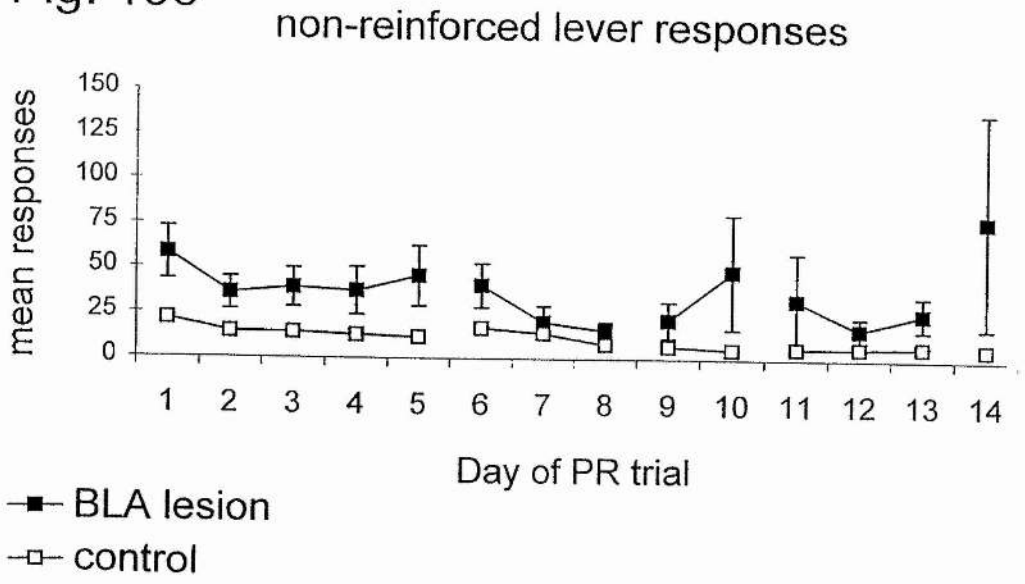


Fig. 15c The pattern of responding on the non-reinforced lever on each day of the 14 day PR trial in BLA lesioned and sham-operated control rats (see previous page for details).

a) Baseline performance under a progressive-ratio schedule of self-administration (days 1-5)

Analyses of the BPs recorded over the first five days of the PR trial (0.25mg cocaine/ infusion) showed that the BLA-lesioned group did not differ significantly from the control animals. There was no main effect of Group [$F(1,13)=1.15$, p=NS] or Day [$F(4,52)=0.49$, p=NS]. However, there was a significant Group x Day interaction [$F(4,52)=2.89$, $p=0.031$] and further post-hoc analyses revealed that sham-operated control animals earned significantly more infusions than the BLA group on the second and third days ($p<0.05$) although BP for both groups were equivalent by days four and five (Fig. 15a).

Total drug lever responses over the first 5 baseline days did not differ between the groups or days and there was no main effect of Group [$F(1,13)=0.97$, p=NS], Day [$F(4,52)=0.68$, p=NS] and there was no significant Group x Day interaction [$F(4,52)=1.91$, p=NS] (Fig. 15b).

For non-reinforced lever responding (Fig. 15c), BLA-lesioned animals showed approximately twice the number of responses as control animals on each of the first five days under the PR schedule. Statistical analyses showed a clear and highly significant effect of Group [$F(1,13)=24.45$, $p<0.001$]. The difference between the groups remained consistent over the five days, and correspondingly there was no main effect of Day [$F(4,52)=0.62$, p=NS] or Group x Day interaction [$F(4,52)=0.17$, p=NS].

b) Effect of cocaine dose under a progressive-ratio schedule of self-administration

The effect of cocaine dose was examined by comparing the mean number of infusions earned over the last three baseline days (days 3-5; 0.25 mg cocaine/ infusions) with those earned over the three days at the lower dose (days 6-8; 0.083mg cocaine/ infusion) and the three days at the higher dose (days 11-13; 0.5mg cocaine/ infusion). Days 9 and 10 were 'change over' days when the animals were exposed to the same dose of cocaine as the baseline days (0.25mg cocaine/ infusion) this was done to minimise contrast effects between the low and high doses (see Table 2 and Fig. 15a).

A three-way analysis of the number of infusions earned at the three different doses of cocaine showed a significant effect main effect of Dose [$F(1,11)=62.56$, $p<0.001$] and Dose x Day interaction [$F(4,44)=10.02$, $p<0.001$] (Fig. 15a). For both groups, BPs decreased and then increased as the drug dose was first lowered and then raised above the training dose, but the BLA-lesioned group appeared comparatively more sensitive to reductions in drug dose and this was reflected as a significant Group x Dose interaction [$F(2,22)=6.52$, $p<0.01$]. Student-Newman-Keuls post-hoc analyses revealed that BLA-lesioned animals self-administered significantly fewer infusions on the third day at the low test dose ($p<0.05$) but there was no main effect of Group [$F(1,11)=0.28$, p=NS] or Day [$F(2,22)=0.76$, p=NS].

Statistical analyses based on the overall number of drug lever responses at each of the three doses tested (Fig. 15b) showed no main effect of Group [$F(1,11)=0.25$,

p=NS], or Group x Dose interaction [F(2,22)=1.78, p=NS] but a significant effect of Dose [F(2,22)=32.09, p<0.0001], and a Dose x Day interaction [F(4,44)=3.14, p=0.24]. Student-Newman-Keuls post-hoc analyses also revealed that the groups differed significantly in the number of responses emitted on the third day at the low dose ($p<0.05$) when BLA-lesioned animals responded significantly less than control animals. However, there was no main effect of Day [F(2,22)=1.73, p=NS] or Group x Day interaction [F(2,22)=0.18, p=NS], and no overall Group x Dose x Day interaction [F(4,44)=1.13, p=NS].

Statistical analyses of the non-reinforced lever responding over the three doses of cocaine showed that there was again, a significant effect of Group [F(1,11)=33.81, $p\leq0.001$] but no effect of Day [F(2,22)=0.66, p=NS]. BLA-lesioned animals responded at a significantly higher level than the sham group on each of the test days and this non-reinforced lever responding was not sensitive to alterations in drug Dose [F(2,22)=2.04, p=NS] and there were no significant interactions (Fig. 15c).

Discussion

This experiment demonstrates that both BLA-lesioned and control animals acquired cocaine self-administration under a PR schedule of reinforcement. Sham-operated control animals self-administered significantly more infusions on the second and third baseline days, but BPs for the overall number of drug lever responses were not significantly different between the groups over the first five baseline days. However, lesioned animals did respond approximately twice as much as control animals on the non-reinforced lever on each baseline day.

Reducing the self-administered dose of cocaine by a third (0.25⇒0.083mg cocaine/ infusion; days 6-8) resulted in a decrease in the BPs of BLA-lesioned and control animals, measured either as the total number of infusions self-administered or the overall number of drug lever responses made. However, by the third day at the low dose, BLA-lesioned animals self-administered significantly fewer infusions and responded significantly less on the drug lever than control animals, which might indicate that BLA-lesioned animals were more sensitive to reductions in cocaine dose than control animals.

Returning the dose of cocaine to the training dose (0.25mg cocaine/ infusion; days 9-10) restored the BPs of lesioned and control animals to values similar to those recorded on the fourth and fifth baseline days at 0.25 mg/ infusion of cocaine. But, increasing the dose of cocaine self-administered by a factor of two (0.25⇒0.5mg cocaine/ infusion; days 11-13) did not significantly increase BPs above those recorded during the baseline training period (0.25mg cocaine/ infusion) in either group. These results suggest that animals with lesions of the BLA are more sensitive to reductions in the dose of cocaine than control animals, but that higher doses of cocaine do not significantly increase BPs in either lesioned or control groups.

McGregor et al. (1993, 1994) reported that the rewarding properties of cocaine may be dissociated by different schedules of reinforcement. Their findings indicated that D_1 receptor blockade within the amygdala significantly increased the rate of

cocaine self-administration under a CRF schedule when compared with identical antagonist infusions intra-Nacc. However, the reverse was true under a PR schedule of reinforcement, in which BP was more sensitive to D_1 receptor blockade within the Nacc than identical infusions intra-amygdala. These authors concluded that the interoceptive stimulus properties of cocaine were more important in determining self-administration under a CRF schedule of reinforcement and that the motivation to self-administer cocaine was more important in determining BP under a PR schedule of reinforcement. As a result, McGregor et al. (1994) proposed that D_1 receptor blockade within the amygdala diminished interoceptive cocaine cues and consequently enhanced the rate of cocaine self-administration under a CRF schedule, whereas D_1 receptor blockade within the Nacc diminished the motivation to self-administer cocaine and therefore reduced BPs under a PR schedule of reinforcement.

In the present study, in which BP was taken as the last infusion self-administered in each session (as in the McGregor et al. studies 1993, 1994), reductions in the dose of cocaine produced significant reductions in the BPs of animals with lesions of the BLA, which would indicate that these lesioned animals were more sensitive to alterations in cocaine dose, a finding opposite to the conclusions of McGregor et al. (1993, 1994). BLA-lesioned animals self-administered significantly fewer infusions when compared to sham-operated controls on both the second and third days under the PR schedule, and following a reduction in cocaine dose (0.25⇒0.083mg cocaine/ infusion), decreased BPs significantly by the third day at the low dose.

As the dose of cocaine was increased however (0.25⇒0.5mg cocaine/ infusion), BPs did not differ significantly between the groups on any day and it could be argued that this finding does support the hypothesis put forward by McGregor et al. (1994) that the amygdala is important in determining self-administration rates under a CRF schedule but not a PR schedule. In the present study, BLA-lesioned animals acquired cocaine self-administration (0.5mg cocaine/ infusion) under a CRF schedule at a significantly faster rate than control animals (Expt. 1 Chapter 3), but failed to show a significant alteration in BP at this dose (present study). Together these findings might support the hypothesis of McGregor and Roberts (i) that the rewarding properties of cocaine can be dissociated by different schedules of reinforcement or (ii) that lesions of the BLA selectively diminish the interoceptive stimulus properties of cocaine.

However, the findings of McGregor et al. (1994) with 6-OHDA lesions of the amygdala suggest that the above explanations are unlikely. They reported that under a PR schedule of self-administration at 0.5mg cocaine/ infusion, animals with 6-OHDA lesions of the amygdala produced significantly higher BPs than in control animals - directly contradicting their earlier findings with intra-amygdala infusions of SCH 23390. From this study, McGregor et al. (1994) concluded that 6-OHDA lesions of the amygdala may attenuate an aversive component of cocaine's action, therefore increasing the drugs reinforcing effects and hence BPs at this dose. Interestingly, this hypothesis is supported by more recent CRF self-administration data (Caine et al. 1995) which indicated that SCH 23390, infused either intra-Nacc

or intra-amygdala produced effects opposite to those described by McGregor and Roberts (1993) with SCH 23390. The main difference between these studies was that Caine et al. (1995) used a dose of cocaine exactly half that used in the McGregor and Roberts study (1993). Caine et al. (1995) reported that D_1 receptor blockade within the amygdala reduced CRF self-administration, while similar D_1 receptor blockade within the Nacc enhanced CRF self-administration. Thus, D_1 receptor blockade within the amygdala selectively enhanced self-administration of a higher but not a lower dose of cocaine, which might support a possible role of the amygdala in the mediation of aversive effects associated with higher doses of cocaine.

It is possible that lesions of the BLA may selectively mitigate an aversive component of cocaine's action at higher doses, but it would appear that the present PR results do not support the contention of amygdaloid involvement in the processing of interoceptive cocaine cues (McGregor et al. 1993, 1994). Several factors make the studies of McGregor and Roberts difficult to interpret and compare with the present findings, but it is likely that the effects observed in their studies arose through disruption of neurotransmission within multiple amygdaloid nuclei.

Intra-cranial infusions of SCH 23390 have been shown to produce regionally specific attenuation of the reinforcing effects of cocaine self-administration under a CRF schedule, similar to that seen by reducing the unit dose of cocaine (Caine et al. 1995). In contrast, intra-cranial infusions of SCH 23390 made prior to a PR

schedule of self-administration are unlikely to reflect the direct attenuation of a drugs rewarding properties. This is due to the fact that as a PR schedule progresses the number of responses, and hence the temporal delay, between successive infusions increases. As cocaine has a relatively short half-life, basal plasma levels of cocaine may fall substantially between infusions, therefore, the reinforcing effect of successive infusions under a PR schedule may diminish for reasons that are unrelated to the antagonist pretreatment. Furthermore, unlike a CRF schedule, PR schedules of reinforcement terminate once a period (e.g. 1hr) of non-reinforcement elapses. As a result, a PR schedule of cocaine self-administration may last as long as 4-5 hours, exceeding the duration and specificity of intra-cranial antagonist pretreatments and making comparisons between these schedules very difficult. Caine et al. (1995) demonstrated that intra-cranial infusions of ^{3}[H]SCH 23390 decreased in concentration and diffused relatively rapidly away from the initial injection site, when administered intra-amygdala. Combined with the results of an *in vivo* voltammetry study (Jones et al. 1995) which indicated that within the amygdala the BLA is relatively insensitive to the actions of DA uptake inhibitors (including cocaine), these findings would imply that the effects seen by Caine et al. (1995) and McGregor and Roberts (1993) resulted from a blockade of DA function within the central nucleus of the amygdala (CeA).

Differences between break-point as total infusions or total responses

Woolverton (1995) suggested that under a PR schedule of drug self-administration the BP of drug lever responses made may provide a more accurate measure of reinforcing strength, than the number of infusions administered. The findings of

the present study upheld this supposition in that differences in response BP between the groups were less pronounced than those produced with infusion BP, however the significant difference between the groups on the third day at the low dose was reflected in the BPs of both infusion number and responses made.

In addition to reductions in BP seen in the number of infusions self-administered by BLA-lesioned animals these animal responded significantly more on the non-reinforced lever on every day of the PR trial. This difference was not sensitive to alterations in drug dose, and may represent a loss of action-outcome associations. Indiscriminate responding or 'frustrative' emotionality, have been reported in rats with amygdala lesions (Henke and Maxwell 1973).

Progressive-ratio versus second-order schedules of cocaine self-administration

The ability of both BLA-lesioned and sham-operated control animals to acquire a PR schedule of cocaine self-administration is of particular interest because these results contrast markedly with those of Expt. 5 (Chapter 4) in which animals with lesions of the BLA were significantly impaired in their acquisition of a second-order schedule of cocaine self-administration. The striking difference between these two schedules is that one (the second-order schedule) relies on the formation of conditioned associations and the acquired incentive motivational capacity of drug-related conditioned stimuli, whereas the other (the PR schedule) relies on the repetition of an instrumental response, maintained initially, by the stimulant properties of cocaine. The fact that BLA-lesioned animals had significantly lower BPs when the dose of cocaine was lowered under the PR schedule, and responded

approximately twice as much on the non-reinforced lever as control animals under the PR schedule, suggests that the performance of BLA-lesioned animals was more under the control of the primary reinforcer and less under the control of secondary conditioned reinforcers.

Although increasing the dose of cocaine to the higher dose (0.5mg cocaine/ infusion), failed to increase the BPs of lesioned animals under the PR schedule significantly, Ranaldi and Roberts (1996) reported that contingent presentation of a conditioned stimulus enhanced the motivation to initiate and maintain cocaine self-administration. Therefore, the lack of effect on BP at the high dose of cocaine may reflect an impairment in the contribution of conditioned associations to the motivation of BLA-lesioned animals. In the present experiments, BLA-lesioned animals acquired cocaine self-administration at the high dose, at a significantly faster rate than control animals (Expt. 1; 0.5mg cocaine/ infusion) but failed to reach significantly higher BPs under a PR schedule at this dose (present study). Together, these findings may support the premise that PR schedules of reinforcement depend more on incentive motivational factors than schedules of continuous reinforcement (McGregor and Roberts 1993). In addition, these findings may also indicate that lesions of the BLA impair the motivational impetus of self-administration behaviour that is both drug-free (Expt. 5; Chapter 4) and under the influence of cocaine (present study).

Experiment 8: BLA lesions on the effect of CS omission under an FI schedule of cocaine reinforcement.

Those animals which successfully completed the 14 day PR trial (Expt. 7) were then placed under a FI 15 (FR10:S) schedule of cocaine self-administration. Under this schedule successive infusions of cocaine were only available once a fixed time interval had elapsed (in this case 15 min). However, throughout each interval every 10th response on the CR lever produced a 1 second presentation of the light stimulus (CS) which had been paired with each drug infusion during the earlier acquisition of cocaine self-administration, under both the FR and PR schedules.

Therefore, the rate and number of CS light presentations earned by an animal in each interval was used to assess the acquired incentive motivational value of the CS. Studying the pattern of responding during the 1st and 2nd 15 min intervals also allows for a direct comparison of drug-seeking behaviour under both drug-free and drug-driven conditions.

In this experiment all animals were allowed to self-administer a maximum of five infusions/ session/ day, under a FI 15 /FR10:S schedule, with 0.25 mg cocaine/ infusion reinforcement. Therefore, under this schedule, the minimum duration of each session was 75 min. Each animal was given five baseline sessions followed by three test sessions in which the CS light was never presented (CS–) and another three sessions in which CS presentation was re-instated (CS+).

Statistical analyses

All data are presented as a percentage of the corresponding baseline values of each animal. The percentage of baseline was then compared between the last three baseline days, three CS– days and three CS+ days. This was analysed using a three-way analysis of variance, with factors: Group (BLA-lesioned, control), Day (1, 2, 3) and CS condition (baseline, CS–, CS+). The first and second intervals were analysed separately in all cases.

Results

The catheters of two control animals blocked during the first days of Expt. 8. Therefore, the final statistical analyses for this experiment included six BLA-lesioned animals and five sham-operated controls. Mean responses for BLA-lesioned and control animals during baseline, CS– and CS+ conditions are shown for the first and second intervals in Fig. 16a and 16b respectively.

a) Baseline data

Initially, statistical analyses of the three baseline days alone showed that there was no significant difference between the groups during either the first or second intervals [$F(1,9)=0.18$, p=NS] and [$F(1,9)=0.10$, p=NS] respectively. There was also no effect of Day in either the first or second intervals [$F(2,18)=1.89$, p=NS] and [$F(2,18)=0.68$, p=NS] respectively, and there were no significant Group x Day interaction.

Fig. 16a

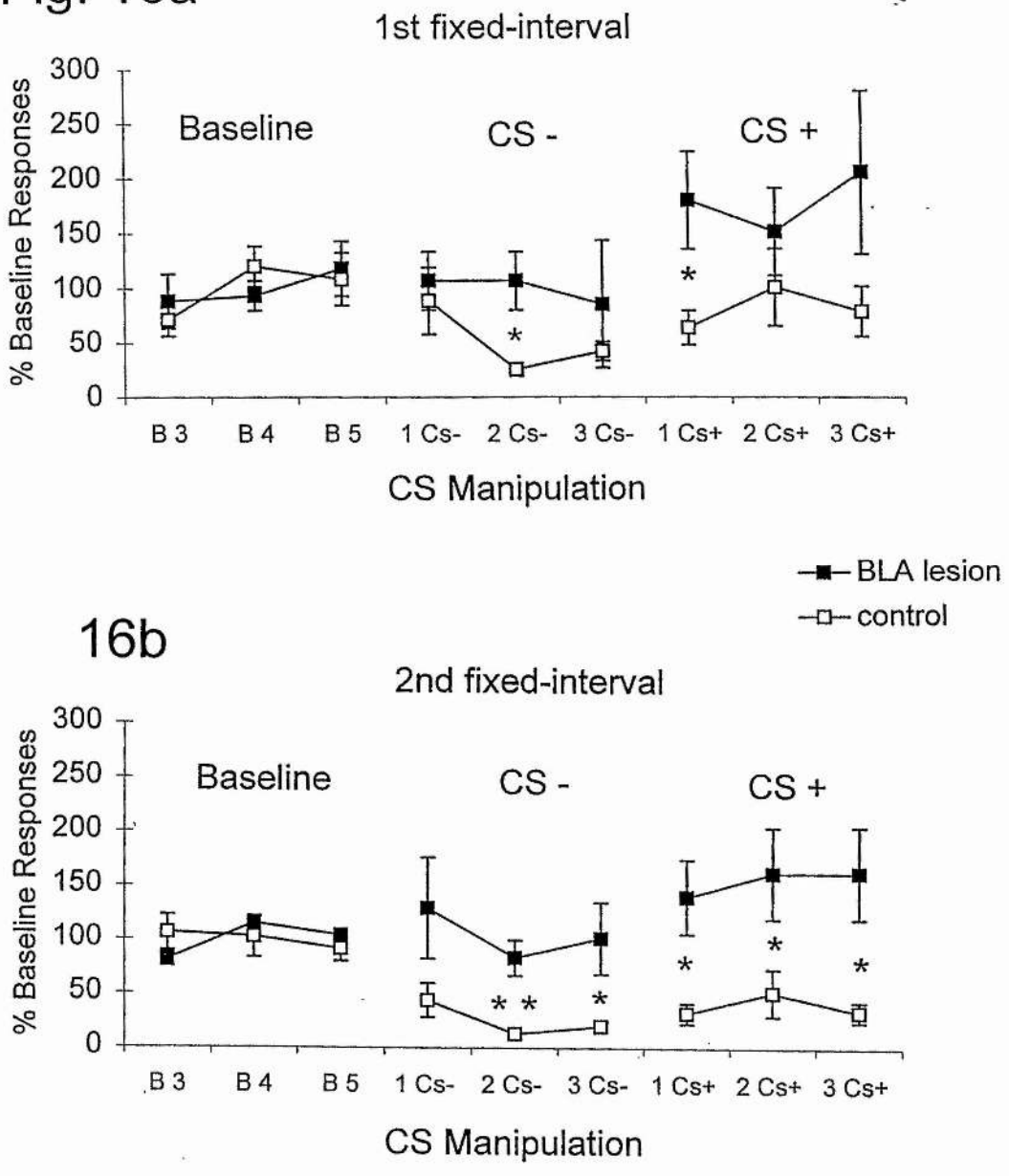


Fig. 16 a, b The percentage of baseline responses for BLA-lesioned and sham-operated controls, under an FI 15(FR10:S) schedule of reinforcement (0.25mg cocaine/ infusion), following CS omission (CS-) and reinstatement (CS+). Fig.16a illustrates responding during the first 15 min interval (drug-free). Fig.16b illustrates responding during the second 15 min interval (drug-driven). * = $p<0.05$, ** = $p<0.01$

b) CS manipulations

Analyses of the BLA-lesioned and sham groups' responding during the first interval (i.e. drug-free; Fig. 16a), revealed a significant main effect of CS [$F(2,18)=4.47$, $p=0.027$] and a significant effect of Group [$F(1,9)=9.88$, $p=0.012$]. BLA-lesioned animals were relatively unaffected by the initial CS omission, compared with sham-operated controls (Fig. 16a, CS–), but showed a marked increase in responding when the CS was reinstated (Fig. 16a, CS+). In contrast, control animals showed a decrease in responses relative to baseline when the CS was omitted and failed to make a full recovery when the CS was re-instated (Fig.16a, CS+). This difference between the groups was reflected in a significant Group x CS interaction [$F(2,18)=4.32$, $p=0.029$]. Student-Newman-Keuls post-hoc test revealed significant differences between the BLA and control groups on day two of CS omission ($p<0.05$) and day one of CS re-instatement ($p<0.05$). Overall, there was no significant effect of Day [$F(2,18)=0.24$, p=NS] and no other interactions were significant.

Analysis of responding during the second 15 min interval (i.e. drug-driven Fig.16b) also showed a significant main effect of CS [$F(2,18)=3.55$, $p=0.05$], and Group [$F(1,9)=8.86$, $p=0.016$]. BLA-lesioned animals showed a small elevation from baseline response levels on the first CS omission day followed by a small reduction on the second day and partial recovery of responding by the third day. When the CS was re-introduced the lesioned animals showed a marked increase in responding on day one and this persisted on the two following re-instatement days. Control animals showed a reduction in responding during the CS omission days and

recovered only slightly when the CS was re-introduced. This was reflected as a significant Group x CS interaction [$F(2,18)=8.33$, $p<0.05$] and post-hoc tests showed that the groups differed significantly on the second CS omission day ($p<0.005$) and each day thereafter ($p<0.05$) as BLA-lesioned animals responded more than control animals on each day. There was no main effect of Day [$F(2,18)=0.06$, p=NS], and no other interactions were significant.

There was no difference between the groups in their non-reinforced lever responding during the first [$F(1, 9)=2.11$, p=NS] and second [$F(1,9)=0.00$, p=NS] intervals respectively. Only three animals in total made over 12 non-reinforced lever responses/ interval, and there was no effect of CS [$F(2, 18)=0.75$, p=NS]; [$F(2, 18)=0.46$, p=NS] or Day [$F(2, 18)=0.21$, p=NS]; [$F(2, 18)=0.78$, p=NS] in the first and second intervals respectively.

Discussion

Under this fixed interval schedule, each drug infusion was made available once a fixed 15 min time interval elapsed, however, during each interval, every 10th drug lever response produced a 1s presentation of the CS previously paired with each drug infusion during the initial acquisition of cocaine self-administration and PR schedule. Therefore, the number of drug lever responses (CS presentations) made by each animal in each interval could be used as an index of the acquired incentive value of the CS. In addition, as each drug infusion was only available following a fixed time interval it was possible to compare drug-free responding (during the first interval) with drug-driven responding (during the second interval). In this way the

extent to which behaviour was governed by the motivational properties of a drug associated cue can be contrasted with the direct stimulant properties of cocaine.

The results of this experiment clearly show that BLA-lesioned animals were relatively insensitive to the omission and reinstatement of a drug associated cue. Omission of the CS significantly reduced the percentage of baseline responses made by sham-operated control animals during the first and second intervals, yet this manipulation produced no significant alteration in the responding of animals with lesions of the BLA. CS reinstatement on the other hand, produced a non-significant elevation in responding in the sham-operated group which neared baseline levels during the first interval, whereas the lesioned group showed a non-significant elevation from baseline, during both the first and second intervals. The groups also differed in the pattern of responding between the first and second intervals. Following CS omission, control animals consistently responded less during the second interval (drug-driven) than the first (drug-free). In contrast BLA-lesioned animals responded equally or more during the second, drug-driven interval.

These findings would indicate that BLA-lesioned animals were relatively indifferent to CS manipulations and therefore support the findings of Expt. 5 (Chapter 4) which demonstrated that BLA-lesioned animals were impaired in their acquisition of a second-order schedule of cocaine self-administration, and furthermore, confirm the findings of Meil and See (1997) who reported that excitotoxic lesions of the BLA severely impaired the impact of stimulus-cued

recovery in the self-administration of cocaine. In their study, Meil and See (1997), assessed the impact of CS reinstatement following a three week extinction phase in which responding no longer resulted in either drug infusions or CS presentations. They reported that in comparison to sham-operated controls, excitotoxic lesions of the BLA made after seven or 14 days of cocaine self-administration experience, severely impaired the capacity of a cocaine associated stimulus to reinitiate responding on both the first and 21st day of extinction training, in the absence of cocaine reinforcement. The results of the present study were not as clear as those reported by Meil and See, but two major differences between the studies may account for this. Firstly, each infusion of cocaine in the present study consisted of 0.25mg cocaine/ 0.1ml over 4s, whereas Meil and See used a dose of 0.33mg cocaine/ 0.05ml over 2.7s. The exact dose of cocaine used in each study does not differ greatly but the volume and speed of delivery in the Meil and See (1997) experiment may have significantly enhanced the development of conditioning to drug associated stimuli in the sham-operated control group. Secondly, in the present study, CS omission was assessed independently of drug infusions. Under the FI 15 schedule, a maximum of five infusions were available on each day of the trial, irrespective of CS condition, and the overall number of responses made did not correlate with the delivery of each drug infusion. Control animals responded at a significantly lower level than BLA-lesioned animals from the second CS omission day which may indicate these animals adapted more quickly to the limited demands of the schedule, and perhaps were not as prone as lesioned animals to the development of habitual responding (McDonald and White 1993).

Fixed-interval versus second-order schedule of cocaine self-administration

The results of the present experiment showed that animals with lesions of the BLA could acquire a fixed-interval schedule of cocaine self-administration, in which every 10th response on the reinforced lever resulted in the presentation of the drug-related conditioned stimulus, previously paired with each drug infusion during training. These results may appear surprising, in light of the findings of Expt. 5 (Chapter 4) in which animals with similar BLA lesions were severely impaired in the acquisition of a second-order schedule of cocaine self-administration. However, it is possible that the results of the present experiment were influenced by the self-administration history of each animal. Only those animals successfully completing the 14 day PR trial (Expt. 7) went on to be assessed in their responding under the fixed-interval schedule of cocaine self-administration. Therefore, all animals in the present study had extensive PR training and were accustomed to emitting hundreds of responses during each self-administration session. Significantly, in Expt. 5, all drug-seeking behaviour (the second-order component of the test) was drug-free, prior to the first infusion of each session. Once each animal completed the second-order requirement for the first infusion of each session, the following nine infusions were made contingent upon each subsequent lever response. As a result, the animals in Expt. 5 never responded for extended periods under the influence of cocaine, and it is less likely that this schedule favoured the development of habitual responding. With extended instrumental training, habitual (stimulus-response) responding becomes increasingly probable (Dickinson et al. 1995). Therefore, it is likely that the BLA-lesioned animals in the present study acquired a fixed-interval schedule of self-administration because their

lever responding had become a habitual to some degree, following extended PR training in Expt. 7.

Conclusions

The experiments reported in this chapter have demonstrated that animals with lesions of the BLA are capable of acquiring cocaine self-administration under both progressive-ratio (PR) and fixed-interval (FI) schedules of reinforcement. Rats with lesions of the BLA were more sensitive to reductions in the dose of cocaine under a PR schedule and less sensitive omission of a drug-related conditioned stimulus under an FI schedule. These findings may indicate that the drug-seeking behaviour of BLA-lesioned animals is determined both more by the primary reinforcer and less by secondary or conditioned reinforcers. The BLA has been implicated in the formation of conditioned associations and animals with lesions of the BLA were severely impaired in the acquisition of a second-order schedule of cocaine self-administration (Expt. 5; Chapter 4), which is thought to rely on the formation of drug-related conditioned associations. Ranaldi and Roberts (1996) recently suggested that conditioned drug cues may contribute to the motivation to self-administer cocaine. As a result, it is possible that lesions of the BLA which appear to disrupt mechanisms underlying conditioned reinforcement also interfere with the potential reinforcing properties of self-administered cocaine. The present findings indicate that the BLA provides a specific and significant contribution to the psychological factors underlying the propensity and motivation to self-administer cocaine.

Chapter 6: Neurochemical correlates of excitotoxic lesions of the BLA following IV or intra-Nacc cocaine administration using *in vivo* microdialysis

Introduction

The experimental results discussed in Chapters 4 and 5 are consistent with many studies implicating amygdaloid function in aspects of associative learning or conditioned reinforcement (Gaffan and Harrison 1987; Cador et al. 1989; Everitt et al 1989; Robbins et al. 1989; Burns et al. 1993, 1994; Wilson et al. 1994; Hatfield et al. 1996; Meil and See 1997; Hitchcott and Phillips 1997; Hitchcott et al. 1997). Ventral-striatal DA transmission has been implicated strongly in the mediation of psychomotor stimulant action and appetitive reinforcement (Taylor and Robbins 1986; Wise 1987; Koob and Bloom 1988) and it has been proposed that excitatory limbic afferents from the prefrontal cortex, ventral subiculum and amygdala are particularly important in the expression of conditioned behavioural responses (Everitt et al. 1991). The notion that limbic cortical afferents provide a dynamic influence on Nacc efferent projections, thereby affecting the selection and adaptation of behaviour in response to environmental stimuli, was first proposed by Mogenson et al. (1980). This theory proposed that the Nacc, modulated by mesolimbic DA transmission, was critical for the translation of motivation to behavioural output and has been supported by many studies, particularly with reference to basolateral amygdaloid function (Yim and Mogenson 1982; Taylor and Robbins 1984, 1986, Cador et al. 1989; Everitt et al. 1989, 1991; Robbins et al. 1989; Burns et al. 1993). Amygdaloid projections to the ventral striatum and Nacc arise almost exclusively from the BLA (Kelley et al. 1982; McDonald 1991b) and

are thought to be excitatory, probably glutamatergic (Burns et al. 1993). However, the precise mechanism by which limbic afferents arising in the BLA interact with ventral striatal DA neurons to bring about conditioned reinforcement is unclear and remains the focus of many behavioural and neurochemical investigations. Three main theories have been proposed and these will be discussed briefly. Firstly, the model proposed by Mogenson et al. (1980) assumed that mesolimbic DA modulated the activity of excitatory limbic afferents, such as those originating in the BLA, ventral subiculum and prefrontal cortex (Yim and Mogenson 1989). This hypothesis is supported by a recent study (Reid et al. 1997) which reported that acute IP injections of either cocaine or amphetamine enhanced extracellular levels of Glu measured by microdialysis within both the Nacc and Pfc but not within the striatum. Furthermore, the stimulant-enhanced Glu transmission within the Nacc and Pfc was blocked completely in animals with 6-OHDA lesions of the Nacc, suggesting that these effects were dependent upon intact Nacc DA function. Reid et al. (1997) concluded that psychomotor stimulants selectively enhance Glu release within limbic structures. In light of the behavioural findings of Chapters 4 and 5, which indicate an important role for the BLA in the formation of conditioned associations and cue-elicited drug-seeking behaviour, the findings of Reid et al. (1997) may also have relevance for the acquisition and maintenance of drug-taking and drug-seeking behaviour.

A second theory suggests instead that limbic afferents presynaptically stimulate the release of mesolimbic DA (Imperato et al. 1990; Moghaddam et al. 1990). This hypothesis has been supported by reports that local infusion of Glu receptor

agonists (PDC, NMDA and AMPA/ Kinate) enhance extracellular DA release within the striatum (Keefe et al. 1992; Segovia et al. 1997). However, the converse was not found with striatal infusions of Glu receptor antagonists (CPP and CNQX). Whereas the higher doses tested enhanced striatal DA release, lower doses (≤1mM) produced no effect on extracellular DA levels within the Nacc (Keefe et al. 1992). While these findings suggest that Glu may not play a critical role in the modulation of striatal DA release, it should be noted that the experiments of Keefe et al. (1992) were carried out in resting animals and thus do not preclude a role for Glu modulation of striatal DA release during specific reinforced or conditioned behaviours. To investigate the hypothesis that glutamatergic limbic afferents may be involved in the mediation of conditioned reinforcement, Burns et al. (1994) assessed the effect of Glu agonists and antagonists infused intra-Nacc in rats trained to associate the presentation of 10% sucrose with a discrete light stimulus. Microinfusions of amphetamine intra-Nacc have been shown selectively to enhance responding for presentation of a sucrose-associated light (CR) in the absence of reward (Taylor and Robbins 1984). However, this effect was abolished by both Glu agonists and antagonists co-infused with amphetamine intra-Nacc (Burns et al. 1994). One interpretation of these findings would suggest that exogenous alterations in Glu transmission may be associated with non-specific effects which do not reflect normal release patterns (Moghaddam et al. 1990) and therefore disrupt the ability of amphetamine to potentiate responding on a lever producing a CR (Burns et al. 1994). Such effects may also explain why both Glu agonists and high doses of Glu antagonists enhanced DA transmission in the Keefe et al. (1992) study.

In an attempt to address the issue of differences between endogenous and exogenous Glu transmission on striatal DA release Segovia et al. (1997) investigated the effect of the selective Glu uptake inhibitor L-trans-pyrrolidine-3,4-dicarboxylic acid (PDC) at concentrations which enhanced Glu stimulation within the physiological range normally observed in the striatum (i.e. mimicking endogenous release). In contrast to the results of Keefe et al. (1992) it was found that PDC-stimulated Glu release correlated strongly with increases in extracellular DA concentration within the striatum, lending support to the hypothesis that meso-accumbens DA is tonically regulated by excitatory limbic afferents (Segovia et al. 1997).

The third viewpoint on glutamatergic/ dopaminergic interaction within the striatum is based primarily on anatomical evidence suggesting that both Glu and DA terminals converge onto common medium spiny output neurons (Smith and Bolam 1990; Sesack and Pickel 1990) and that there are no direct synaptic connections between Glu and DA terminals within the striatum (Bouyer et al. 1984). Although more recent ultrastructural studies have indicated that Glu receptors may be located on DA axonal processes, supporting the premise that Glu transmission may modulate Nacc DA release (Gracy and Pickel 1996). It is likely that dopaminergic/ glutamatergic interactions are more complex than simple presynaptic modulation of Glu or DA terminal fields and it is possible that another neurotransmitter (possibly GABA) moderates both excitatory limbic afferents and dopaminergic release within the striatum (Smolders et al. 1995; Segovia et al. 1997).

Most of the experiments described above employed *in vivo* microdialysis which involves the implantation of a dialysis probe into specific brain sites permitting the sampling of extracellular fluid. Exchange of molecules occurs at the tip of the probe which consists of a microfine semipermeable membrane (approx. 2 mm long) and this is perfused at a constant rate within a closed system with an iso-osmotic solution (Ringers solution). Neurotransmitters circulating in the extracellular space diffuse across the dialysis membrane along a concentration gradient. The dialysate is then collected at precisely timed, consecutive intervals (e.g. 10 min) in dialysate vials resting on the probe outlet. With the appropriate high performance liquid chromatography (HPLC) electrochemical or fluorescence detection system, each dialysate sample can be analysed for virtually every substance existing within the extracellular fluid. The concentration of differing neurotransmitters can then be quantified and correlated temporally with pharmacological treatments administered to the rat (Di Chiara et al. 1996).

The experiments in the present chapter sought to assess Nacc neurochemical correlates of excitotoxic lesions of the BLA in response to both local (intra-Nacc) and IV acute infusions of cocaine. Each dialysate sample was divided as it was collected and analysed with both HPLC electrochemical, and HPLC fluorescence detection for catecholamine and amino acid contents respectively. The extracellular concentrations of DA, and the amino acids Glu and also taurine (Trn) were then quantified for each animal.

Taurine (Trn) is a sulfonated amino acid that has been linked with osmoregulation within the brain (Wade et al. 1988). Cellular swelling is known to be characteristic of early neurotoxicity and such effects, induced by Glu agonist have been related to increases in the extracellular level of Trn. As a result it has been suggested that enhanced Trn release may provide an index of early excitatory amino acid (EAA) induced neurotoxicity, *in vivo* (Menéndez et al. 1989). More recently, Trn has also been shown to function in a neuroprotective manner against the development of catalepsy, observed in rats following a two week DA-antagonist treatment with intra-peritoneal (IP) haloperidol. Animals co-administered with Trn (IP) were significantly less cataleptic than animals treated with haloperidol alone (Lidsky et al. 1995). Interestingly, haloperidol treatment has also been associated with enhanced Glu transmission within the striatum (Yamamoto and Cooperman 1994), and haloperidol induced catalepsy can also be attenuated by the co-administration of MK-801, a non-competitive Glu antagonist (Elliott et al. 1990). Taken together, these findings suggest that Trn release may be related to, and may also act to limit, glutamatergic stimulation and excitability. As limbic afferents arising in the BLA are considered to be glutamatergic in nature (Burns et al. (1994) and Trn release appears to be linked to glutamatergic activity (Yamamoto and Cooperman 1994), it was considered of interest to investigate the effect of excitotoxic lesions of the BLA on the release on both the amino acids Glu and Trn, within the Nacc.

The results of Expt. 1 (Chapter 3) indicated that during the initial acquisition of cocaine self-administration, BLA-lesioned animals responded significantly more than sham-operated controls for higher doses of cocaine (i.e. the dose-response

function of lesioned animals was transiently shifted to the right). Subsequently, sham-operated control animals self-administering the high dose of cocaine gradually increased the overall number of infusions administered per session, until they plateaued at a rate of self-administration that did not differ from BLA-lesioned animals, self-administering the same dose. Following stabilisation of cocaine self-administration, a within-session dose-response test (Expt. 6; Chapter 4) demonstrated that there was no longer a significant difference between the group's responses to alterations in cocaine dose, as both groups produced clear inverted U-shaped dose-response functions, which did not shift to the left or right of each other. However, despite evident equivalence between the reinforcing effects of cocaine in the two groups, BLA-lesioned animals were significantly impaired in their acquisition of a second-order schedule of cocaine self-administration (Expt.5; Chapter 4). This apparent behavioural dichotomy may reflect a selective disruption of the mechanisms underlying conditioned reinforcement which are thought to be necessary for the successful acquisition of a second-order schedule of reinforcement. Therefore, formation of conditioned associations may also depend upon intact functioning within the BLA and the associated basal amygdaloid afferent projections to the ventral striatum and Nacc.

Investigation of spontaneous and cocaine-stimulated neuronal release of DA, Glu and Trn, within the Nacc of BLA-lesioned and sham-operated control animals may provide a more precise interpretation of the behavioural findings summarised above. Identifying whether lesions of the BLA are associated with a selective reduction in extracellular Glu or Trn transmission, and by contrasting both local

versus IV cocaine-stimulated DA-release, it may be possible to determine whether lesions of the BLA also affect extracellular DA levels within the Nacc.

This chapter reports the use of *in vivo* microdialysis in three separate experiments. Experiment 9 examines the Nacc neurochemical response to intra-Nacc infusion by reverse dialysis of three cocaine doses (5, 20, 50μM) and a single K^+ (60mM) infusion. K^+ stimulation causes the depolarisation of neurons and thereby gives an indication of the potential (maximal) neurotransmitter release. This is an important test as it confirms that the preceding cocaine infusions produce dose-dependent effects that are not restricted by a 'ceiling effect'. It was predicted that lesions of the BLA would not interfere with the dopaminergic response to intra-Nacc infusions of cocaine but may be associated with a reduction in Nacc glutamate levels, indicative of the loss if excitatory Nacc afferents in BLA lesioned animals. However, both lesioned and control groups should respond similarly to infusion of K^+ as lesions of the BLA should not interfere with the synthesis or neural availability of DA.

In Expt. 10 a separate group of animals was used to investigate the effect of single IV infusions of cocaine, identical in dose and volume to those self-administered by rats in the earlier experiments described in this thesis (0.25mg and 0.5mg cocaine/ infusion respectively). This experiment explored possible differences between the groups in response to IV cocaine. Again, it was predicted that lesions of the BLA would not interfere with the dopaminergic response to IV cocaine because deficits

in glutamatergic neurotransmission may be masked following IV cocaine as other limbic afferents are simultaneously stimulated.

Finally a third group of animals were used to assess the response to tetrodotoxin (TTX; 1μM) infusion by reverse dialysis, intra-Nacc (Expt. 11). TTX is known to block sodium conductance in neurons at nanomolar concentrations and contrary to K^+ stimulation produces neuronal hyperpolarisation. Significant reductions in the concentration of extracellular DA measured following co-perfusion with TTX verifies that the composition of the dialysate reflect neuronally-released, action-potential-dependent DA, as opposed to action-potential-independent DA release. This is also an important validation of the neurochemical correlations made using *in-vivo* microdialysis (Westerink et al. 1987), as this technique samples receptor overflow, not receptor occupation (Burechailo and Martin-Iverson 1996).

Methods

All experiments were carried out in BLA-lesioned and sham-operated control animals with no previous drug treatment or cocaine self-administration experience. At the time of initial lesion surgery all animals weighed between 330-360 g. Twenty-four BLA lesioned and 17 sham-operated control animals were prepared as described in General Methods (p28). On each testing day, three animals were dialysed simultaneously using three separate stereotaxic frames. Animals were first injected with Urethane (0.1g/ kg), a long-acting anaesthetic, sufficient to render them anaesthetised for the entire duration of the experiment (6-8 hrs). The heads were then shaved, swabbed with 70% ethanol and placed in the stereotaxic frames.

Special care was taken to ensure that the rats stayed warm by placing each animal on an electric thermal pad while in the stereotaxic frame, and using overhead heating lamps and insulating blankets when necessary. Body temperatures were regularly monitored with rectal thermometers and stabilised at 37°C. Nacc probe placements were calculated from Bregma [co-ordinates: AP: +1.7; L: ± 1.5; V: –7.8 from dural surface ; incisor bar: –3.3].

Concentric design microdialysis probes were used in each experiment and the construction of these are described in General Methods p35; the component parts are listed in Appendix 2(c). Prior to connection, each microdialysis probe was held in ultra pure water to soften and 'prime' the microbore membrane. When the probe was fully 'primed' it was connected to the micro-infusion flow line. Microdialysis flow was then established using artificial CSF (1µl/ min) and the probe was carefully implanted into one side of the Nacc, this was counterbalanced between subjects. Once implanted, the probe was tested to ensure that the correct volume (1µl/ min) was delivered into the dialysate collection vial. Had the probe been damaged during implantation, or simply faulty in construction, the volume delivered into the vial would be reduced and a new probe was 'primed' and implanted in the opposite Nacc. When the microdialysis flow was established, the probe was left in position for 3hr to allow the surrounding neural tissue to recover and stabilise neurochemically. Dialysate collection vials were changed regularly during this period but these initial samples were not saved.

Collection vials were pre-prepared with 2µl perchloric acid (PCA) in each. Care was taken to ensure that the duration of each sample was precisely 10 min, and that the sample collection vials were renewed directly one after the other. The full volume of each sample was therefore, 12µl (1µl/ min dialysate and 2µl PCA/ vial). On collection, each sample was immediately divided into two 6µl volumes using a pipette. These were placed directly onto dry ice before being stored at –80 °C until required for analysis. Two separate HPLC analyses were carried out on each sample: fluorescence detection of EAAs and electrochemical detection of DA.

Following the 3hr stabilising period, the collection of dialysate samples began. Each experiment began with the collection of six baseline samples (1hr). The microdialysis infusion line for each rat was connected via a three-way liquid switch, allowing the micro-syringes to be interchanged easily during the dialysis session. When all drug treatments were completed and the final dialysate sample had been collected all animals were sacrificed by trans-cardial perfusion with 4% PFA. The brains were then collected for lesion assessment and probe placement (see General Methods p37-39).

HPLC Analysis

Dopamine was determined in brain dialysates by high performance liquid chromatography (HPLC) and electrochemical detection (see General Methods p42). Samples were separated on a microbore C18 column (Sep Stik 50DS 100 x1mm) and detected by oxidation (+750 mV). The absolute detection limit of dopamine was approximately 1 fmol.

Dialysate levels of Trn and Glu were determined by conventional HPLC and fluorescence detection (254nm). All samples were derivitised prior to injection, with an equal volume of β-mercaptoethanol /OPA working reagent (6μl). Chromographic data were acquired on-line using Gynkcosoft software (version 4.1, HPLC Technology, Stockport, UK) and peak areas were used to quantify levels of each substance in dialysate fluid.

Histological assessment

Representative photomicrographs of the probe placement in two animals are shown in Plate 4a, b. A schematic representation of all probe placements are shown in Fig. 17a, b. Lesion assessments are reported in each experiment.

Experiment 9: Intra-Nacc cocaine infusion

Eight BLA-lesioned animals and six sham operated controls were used in this experiment. Following 1hr of baseline samples each animal was infused with cocaine by reverse dialysis, (i.e. directly intra-Nacc within the dialysis probe) with three doses of cocaine (5, 20, 50μM) and a potassium pulse (K^+ 60mM).

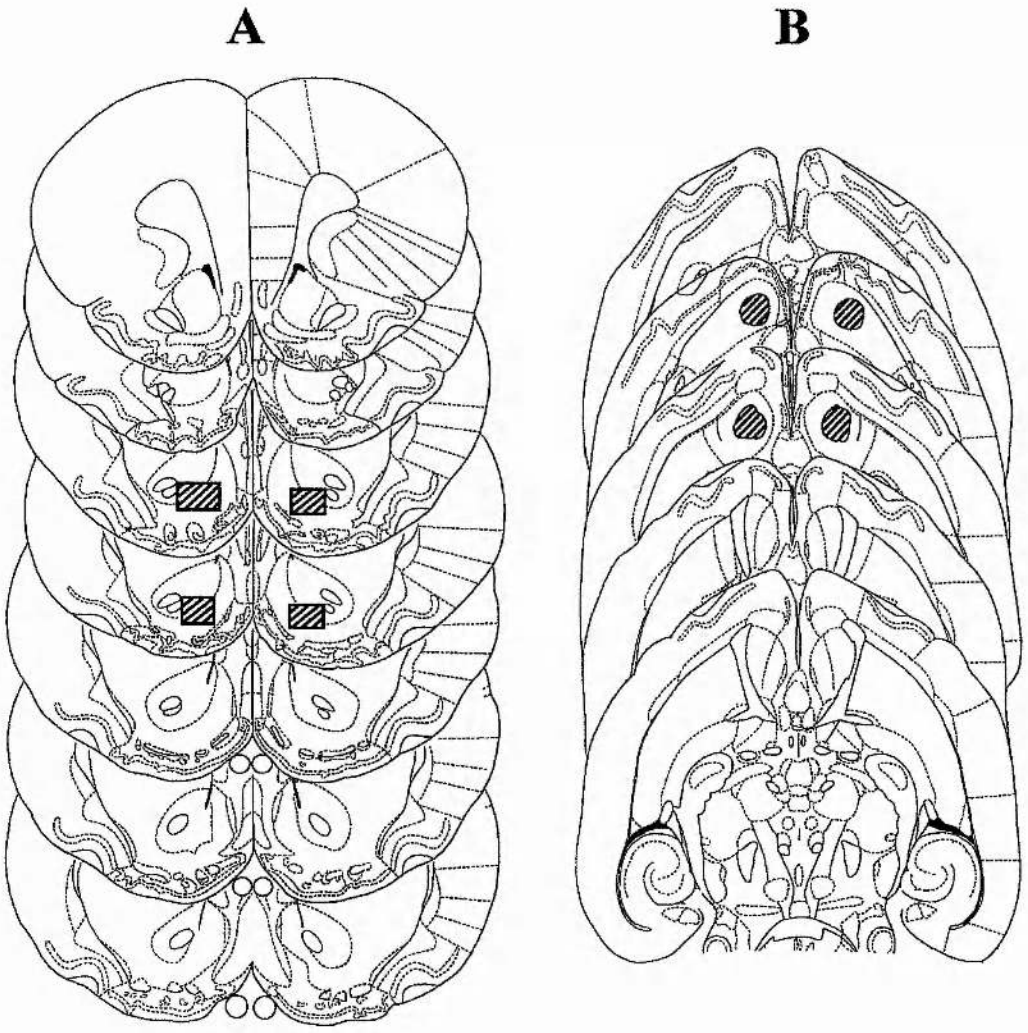
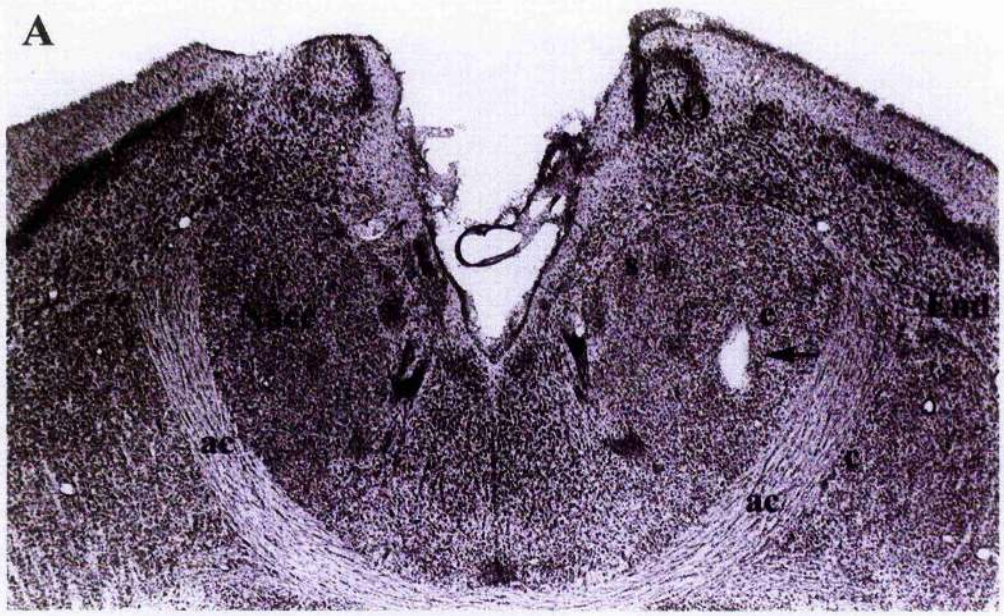
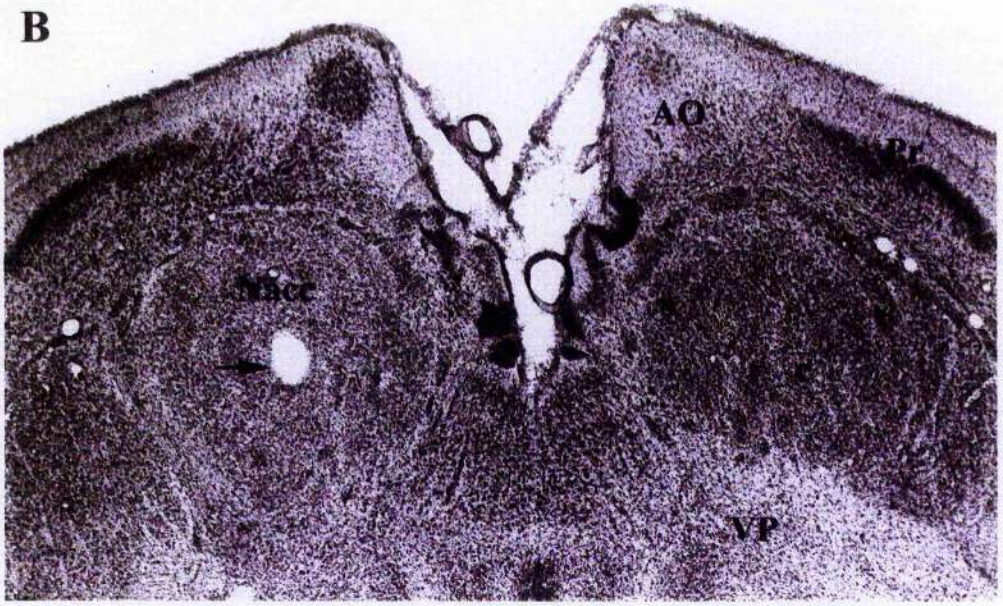


Fig. 17a, b Schematic representation of microdialysis probe placements within the nucleus accumbens. The shaded areas show the location of the tip of the microdialysis probe for all rats included in the dialysis experiments. Adapted from Paxinos and Watsons Brain Maps (1992) showing coronal sections (a) +2.70 to +0.07 and horizontal sections (b) -8.10 to -7.0 from Bregma, respectively.

Plate 4



Approximately -7.0mm from Bregma



Approximately -7.6mm from Bregma

Photomicrographs of horizontal (60μm) sections through the nucleus accumbens showing representative dialysis probe placements in 2 different animals. Section A is approximately -7.0mm from Bregma, section B is approximately -7.6mm from Bregma. The dialysis probe tract is indicated by the arrow in each section. Nacc = nucleus accumbens, s = shell region of nucleus accumbens, c = core region of nucleus accumbens, ac = anterior commisure, AO = anterior olfactory nucleus, CP = caudate putamen, VP = ventral pallidum, En_d = dorsal endopiriform nucleus, Pr = piriform cortex.

Each cocaine infusion lasted for exactly 10 min (one sample) and the K^+ infusion lasted for exactly 5 min (half a sample). Each dose of cocaine and K^+ was held in a separate syringe in the multi-syringe microdialysis pump and a CMA three-way liquid switch was used to alternate between syringes and drug doses. Following six baseline samples with aCSF infusion the first drug syringe (5μM cocaine) was switched into the flow position for 10 min (1 sample). Infusion then reverted to aCSF for 50 min (five more samples). and the pattern then repeated for the other two cocaine doses. All drug doses were administered in an ascending order. Finally, the K^+ pulse was switched to flow for 5 min, and then switched back to aCSF for a further 55 min. Overall, dialysate samples were collected once every 10 min for 5hr (30 samples).

Statistical analyses

Basal DA, Glu and Trn levels were assessed initially using two-way analyses of variance for BLA-lesioned and control animals over the first six 10 min time bins, prior to drug treatment: between subject factor Group (2 levels: BLA-lesioned, control), within-subject factor Time (6 levels: 6x10min time bins). Extracellular DA, Glu and Trn release in response to cocaine infusions were analysed using three-way analyses of variance with repeated measures: between subject factor Group (2 levels: BLA-lesioned, control), within-subject factors Dose (4 levels: baseline, cocaine infusions 5μM, 20μM, 50μM) and Time (6 levels: 6x10min time bins, following each cocaine infusion). Extracellular DA, Glu and Trn release in response to a single K^+ infusion (60mM) were analysed separately using a two-way analysis of variance with between subject factor of Group (2 levels: BLA-lesioned,

control) within-subject factor Time (6 levels: 6x10 min time bins, following K^+ infusion). Student-Newman-Keuls Post-hoc tests were carried out where applicable. Linear associations between extracellular Trn and Glu release were examined using Linear Regression analysis (SPSS).

Results: Exp. 9

Histological assessments confirmed that all probe placements were within the medial core of the Nacc. Two lesioned animals were discarded because the lesions sustained were incomplete; both showed unilateral patchy sparing of caudal magnocellular and parvocellular basal amygdaloid neurons. The samples from one control animal were also discarded because of a leaking syringe, which resulted in an inconsistent perfusate flow rate. Therefore, six BLA-lesioned and five sham-operated control animals were used in for the analysis of extra-cellular Glu and Trn in response to intra-Nacc cocaine and K^+ infusion. An additional two animals; one BLA-lesioned and one sham-operated control were also assessed in their DA response, to intra-Nacc cocaine infusions and K^+. The final group sizes analysed for DA-efflux following intra-Nacc cocaine were therefore seven BLA-lesioned and six shams-operated controls.

Intra-Nacc cocaine infusion: Dopamine-efflux

Mean DA-efflux in BLA-lesioned and sham-operated control animals is shown in Fig. 18a. A two-way analysis of variance of baseline samples only showed that the groups did not differ in their basal extracellular DA release. There was no significant effect of Group [$F(1,11)=0.72$, p=NS] or Time [$F(5,55)=1.39$, p=NS]

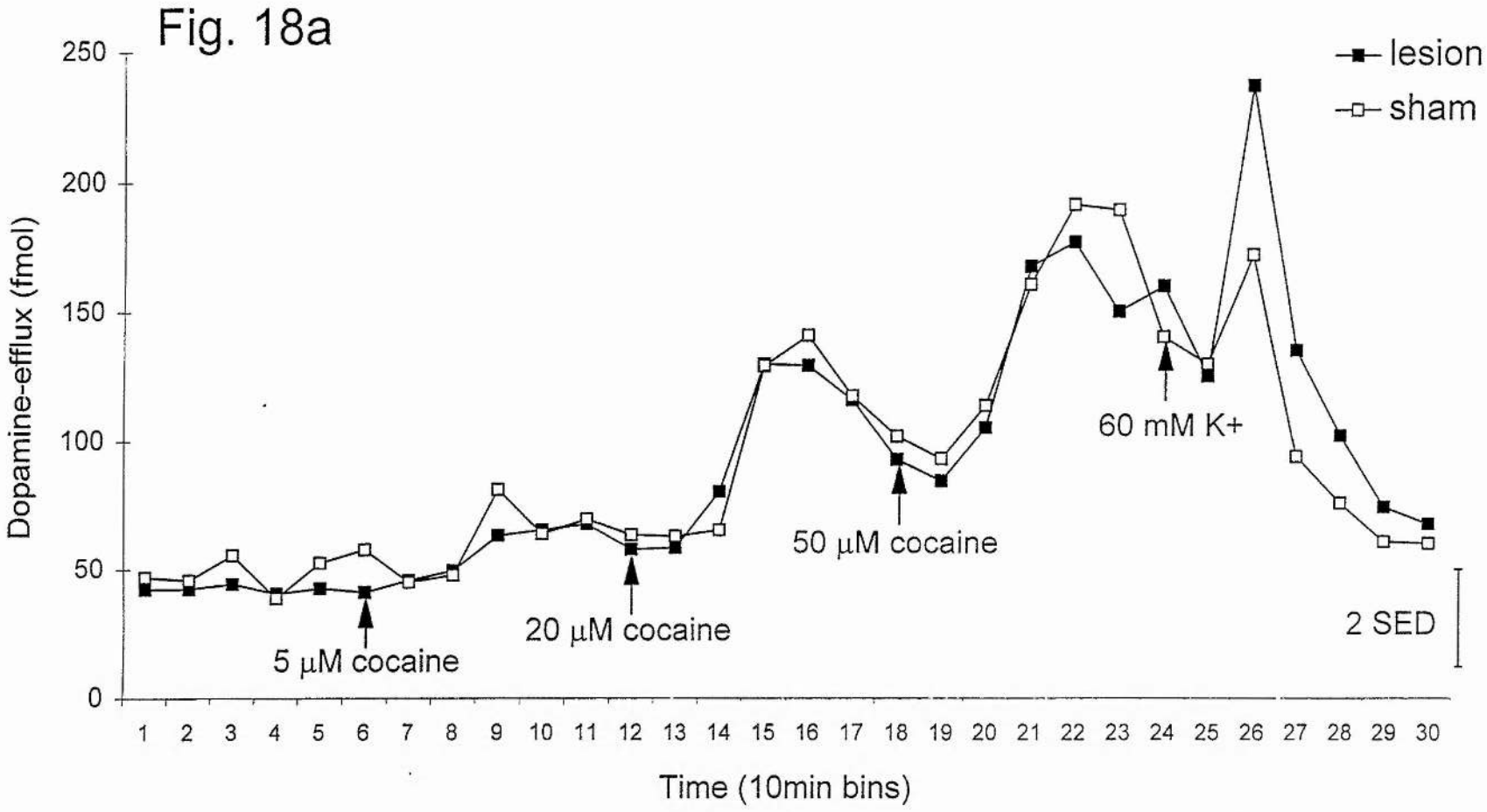


Fig. 18a Mean dopamine-efflux in BLA-lesioned and control rats following intra-Nacc infusions of cocaine (5, 20, 50 μM) and K^+ (60mM). Each cocaine infusions lasted for exactly 10 min (1 dialysate sample) following the collection of 6 baseline samples. The K^+ infusion lasted for exactly 5 min, following the collection all intra-Nacc cocaine infusions. Each animal received all infusions in the order illustrated in the figure.

and there was no Group x Time interaction. Both groups of animals showed a clear dose-dependent increase in extracellular DA levels following infusions of cocaine (5, 20, 50μM). A three-way analysis of variance showed significant main effects of Dose [$F(3,33)=46.57$, $p<0.001$], and Time [$F(5,55)=23.19$, $p<0.001$], and a significant Dose x Time interaction [$F(15,165)=5.89$, $p<0.001$]. There was no significant effect of Group [$F(1,11)=0.15$, NS] and Group x Dose, Group x Time and Group x Dose x Time interactions were all non-significant.

Intra-Nacc cocaine infusion: Glutamate-efflux

Mean Glu-efflux in both BLA-lesioned and sham-operated control animals are shown in Fig. 18b. A two-way analysis of variance of the baseline samples only showed that groups differed in their basal Glu-efflux. There was a significant effect of Group [$F(1,9)=6.27$, $p<0.05$ and Time [$F(5,45)=2.73$, $p<0.05$] but no significant Group x Time interaction [$F(5,45)=2.12$, p=NS]. Student-Newman-Keuls post-hoc test revealed that the groups differed during the second10 min time bin ($p<0.05$) in which extracellular Glu release was significantly lower in BLA-lesioned animals. In response to cocaine infusions, a three-way analysis of variance with repeated measures showed a significant main effects of Group [$F(1,9)=5.88$, $p<0.05$], Dose [$F(3,27)=3.24$, $p<0.05$] and a significant Group x Dose x Time interaction [$F(15,135)=2.28$, $p<0.01$]. There was no significant main effect of Time [$F(4,45)=2.25$, p=NS], Dose x Time [$F(15,135)=1.29$ p=NS] or Group x Time interaction [$F(5,45)=0.55$, p=NS]. Post-hoc tests revealed that extracellular Glu-efflux was significantly lower in BLA-lesioned animals on the 2nd, 7th, 15th, 16th time bins ($p<0.05$) and the 8th, 10th and 20th time bins ($p<0.01$).

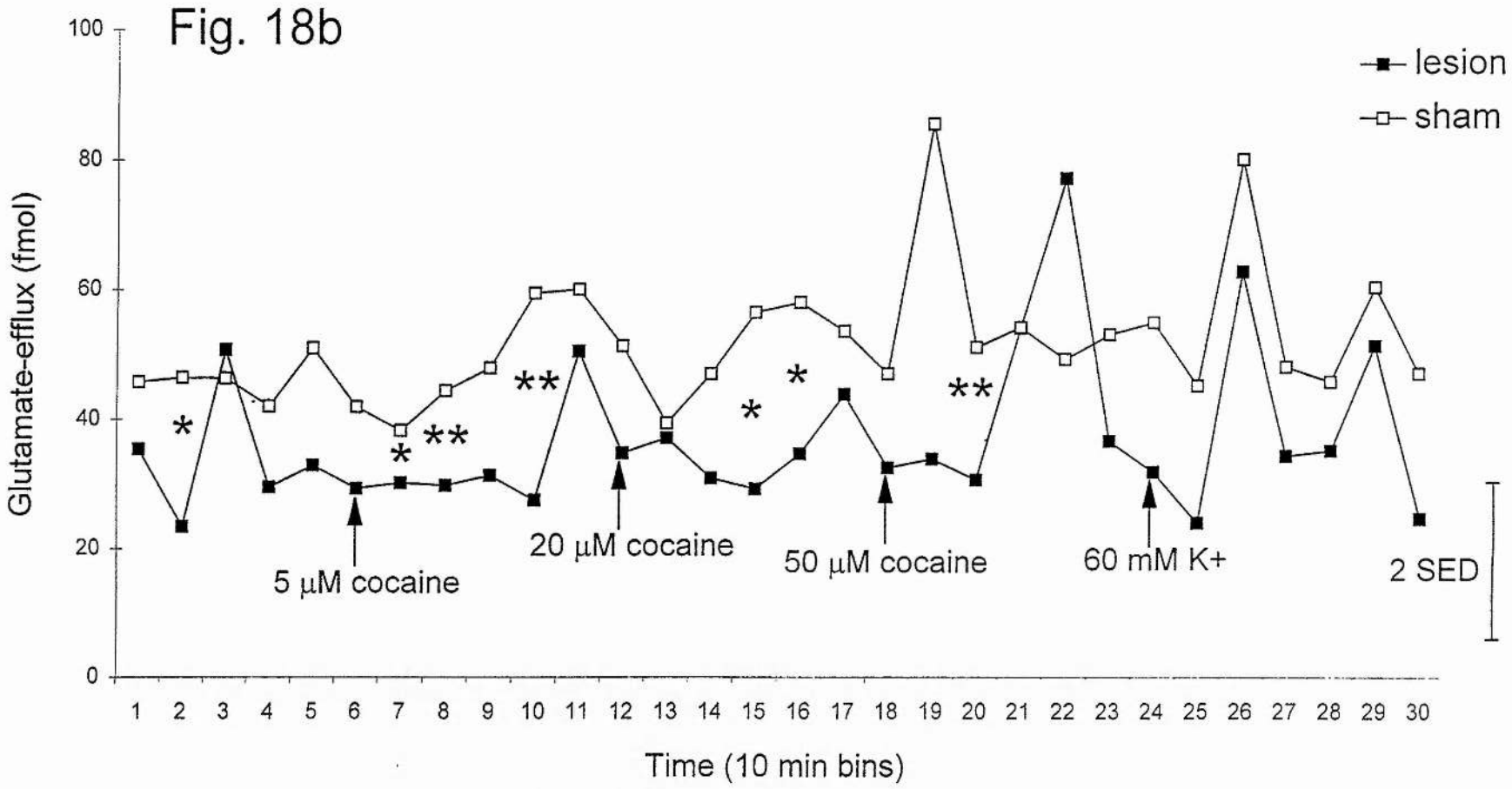


Fig. 18b Mean glutamate-efflux in BLA-lesioned and control rats following intra-Nacc infusions of cocaine (5, 20, 50 μM) and K^+ (60mM). Each cocaine infusions lasted for exactly 10 min (1 dialysate sample) following the collection of 6 baseline samples. The K^+ infusion lasted for exactly 5 min, following the collection all intra-Nacc cocaine infusions. Each animal received all infusions in the order illustrated in the figure. * = $p < 0.05$, ** = $p < 0.001$.

In the control group there was a cocaine-dependent pattern of Glu release which peaked between the 5th and 6th samples following the first cocaine infusion (5μM) and between the 3rd and 4th sample following the second cocaine infusion (20μM). The highest dose of cocaine (50μM) produced an instantaneous increase in extracellular Glu within the first 10 min post-infusion. Relative to controls, BLA-lesioned animals showed a significant reduction in extracellular Glu levels, and the pattern of release following each intra-Nacc cocaine infusion appeared to be delayed. Glu-efflux in BLA-lesioned animals peaked approximately 2-3 samples after the control group at each dose of cocaine infused.

Intra-Nacc cocaine infusion: Taurine-efflux

Fig. 18c shows the extracellular Trn-efflux in response to intra-Nacc cocaine infusions in BLA-lesioned and sham-operated control animals. A two-way analysis of variance of the baseline samples only showed that the groups did not differ in their basal Trn release. There was no significant effect of Group [$F(1,9)=4.14$, p=NS] or Time [$F(5,45)=1.32$, p=NS] and no Group x Time interaction [$F(5,45)=0.67$, p=NS]. Infusions of cocaine were characterised by an initial reduction in extracellular Trn levels followed by an elevation which peaked between the 3rd and 6th sample in both lesioned and control groups. A three-way analysis of variance with repeated measures showed significant main effects of Dose [$F(3,27)=6.38$, $p<0.005$] and Time [$F(5,45)=13.64$, $p<0.001$] and a significant Dose x Time interaction [$F(15, 135)=3.22$, $p<0.001$]. There was no significant main effect of Group [$F(1,9)=3.20$, p=NS] and Group x Dose, Group x Time and Group x Dose x Time interactions were all non-significant.

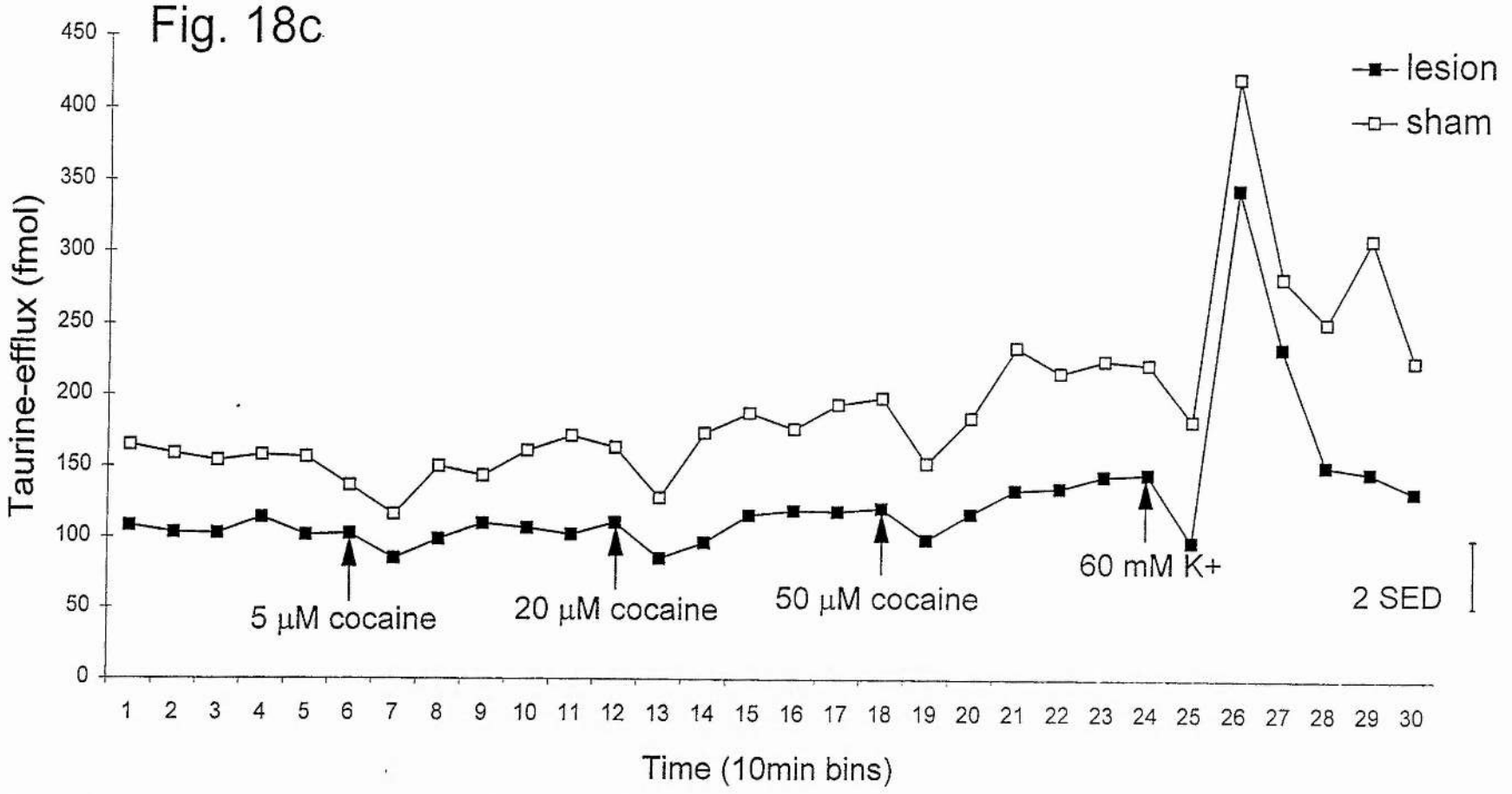


Fig. 18c Mean taurine-efflux in BLA-lesioned and control rats following intra-Nacc infusions of cocaine (5, 20, 50 μM) and K^+ (60mM). Each cocaine infusions lasted for exactly 10 min (1 dialysate sample) following the collection of 6 baseline samples. The K^+ infusion lasted for exactly 5 min, following the collection all intra-Nacc cocaine infusions. Each animal received all infusions in the order illustrated in the figure.

Intra-Nacc K+ infusion: dopamine-, glutamate- and taurine-efflux

Differences between BLA-lesioned and sham-operated control animals in response to intra-Nacc K^+ infusion (60mM) were examined individually for DA, Glu and Trn, using two-way analyses of variance. Analysis of DA-efflux showed that there was no significant effect of Group [$F(1,11)=2.17$, p=NS] but a significant main effect of Time [$F(1,11)=19.35$, $p<0.001$] and a significant Group x Time interaction [$F(5,55)=2.47$, $p<0.05$]. Extracellular levels of DA peaked in the second sample post-infusion for both lesioned and control animals, but this response also diminished more rapidly in control animals.

Intra-Nacc infusion of K^+ produced an initial reduction in Glu release followed by an increase, which also peaked in the second sample post-infusion in lesioned and control animals. Analysis of variance showed that there was a significant main effect of Time [$F(5,45)=8.14$, $p<0.001$] but no significant effect of Group [$F(1,9)=2.56$, p=NS] or Group x Time interaction [$F(5,45)=0.30$, p=NS].

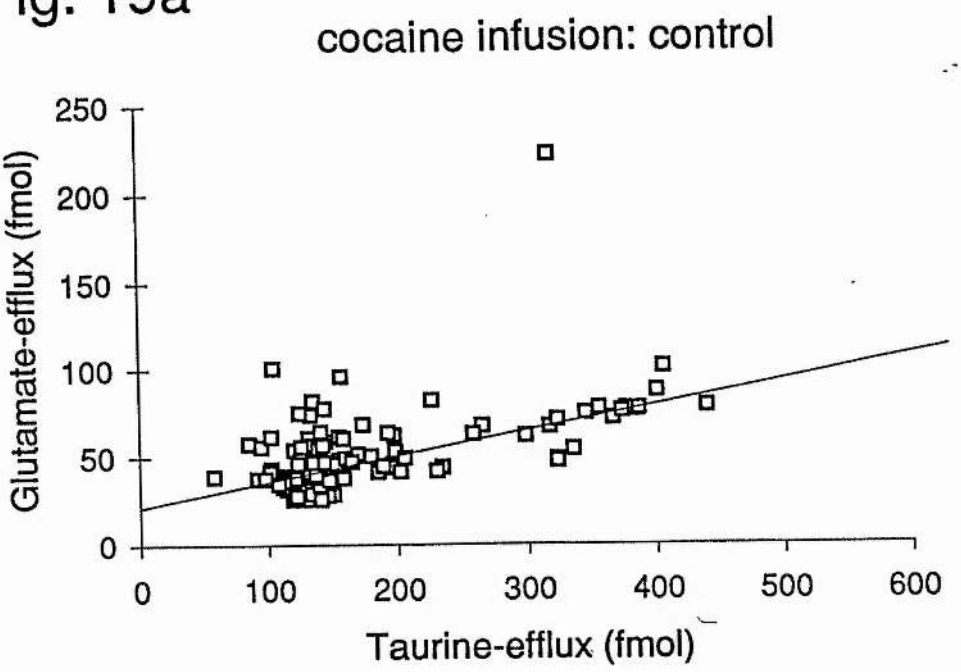
Both lesioned and control groups also responded similarly in their Trn response to intra-Nacc K^+ infusion, characterised by an initial reduction in extracellular levels followed by a marked increase, which peaked in the second sample post-infusion. Analysis of variance showed there was also a significant main effect of Time [$F(5,45)=15.8$, $p<0.001$] but no significant effect of Group [$F(1,9)=4.32$, p=NS] or Group x Time interaction [$F(5,45)=0.80$, p=NS].

Relationships between taurine- and glutamate-efflux

It appeared from examination of Figs 18b and 18c that the patterns of Glu and Trn release in response to cocaine infusions were similar for the control group but not for the BLA-lesioned group, whereas both groups appeared to respond similarly to infusions of K^+. To investigate this observation the relationship between Trn and Glu release in both lesioned and control animals were examined using Linear Regression analysis. These correlations were divided into separate tests. First, the cocaine-dependent relationship between Trn and Glu was assessed in each group (18 samples/ animal, excluding baseline and K^+-stimulated data). Secondly, the K^+-dependent relationship between Trn and Glu was assessed in each group (6 samples/ animal, excluding all non-K^+ samples).

Figure 19a and 19b show scatterplots of the extracellular concentrations of Trn and Glu release following intra-Nacc cocaine infusions in sham-operated and BLA-lesioned animals respectively. Control animals showed a significant correlation between Trn and Glu release ($r=0.52$, $p<0.001$) and a highly significant linear relationship between the two amino acids ($b=0.14\pm0.022$, $p<0.001$) (Fig. 19a). In contrast, cocaine-induced Trn and Glu release showed no significant correlation in BLA-lesioned animals ($r=0.17$, p=NS) and there was no significant linear regression ($b=0.11\pm0.069$, p=NS) (Fig. 19b).

Fig. 19a



19b

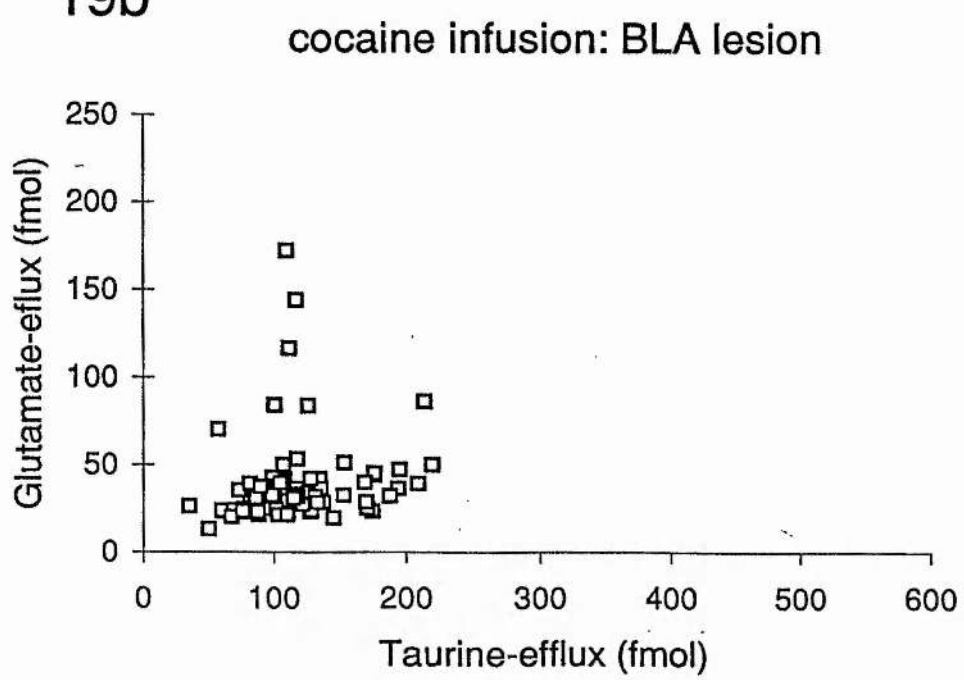
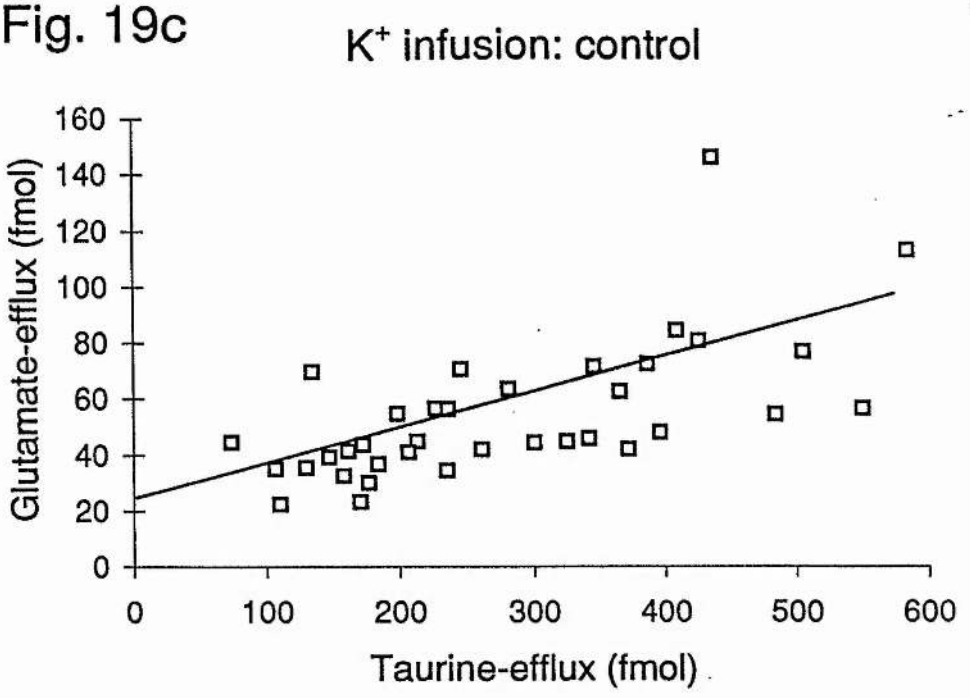


Fig. 19 a, b Linear regression between glutamate- and taurine-efflux in response to intra-Nacc cocaine infusions (excluding baseline samples and samples following K^+ infusion) in control and BLA-lesioned animals respectively. Control animals showed a significant correlation ($r=0.52$, $p<0.001$) and linear relationship ($b=0.14\pm0.022$, $p<0001$) (Fig. 19a). In contrast BLA-lesioned animals showed no correlation ($r=0.17$, $p=$NS) and no linear relationship ($b=0.11\pm0.069$, $p=$NS) (Fig. 19b).

Fig. 19c



19d

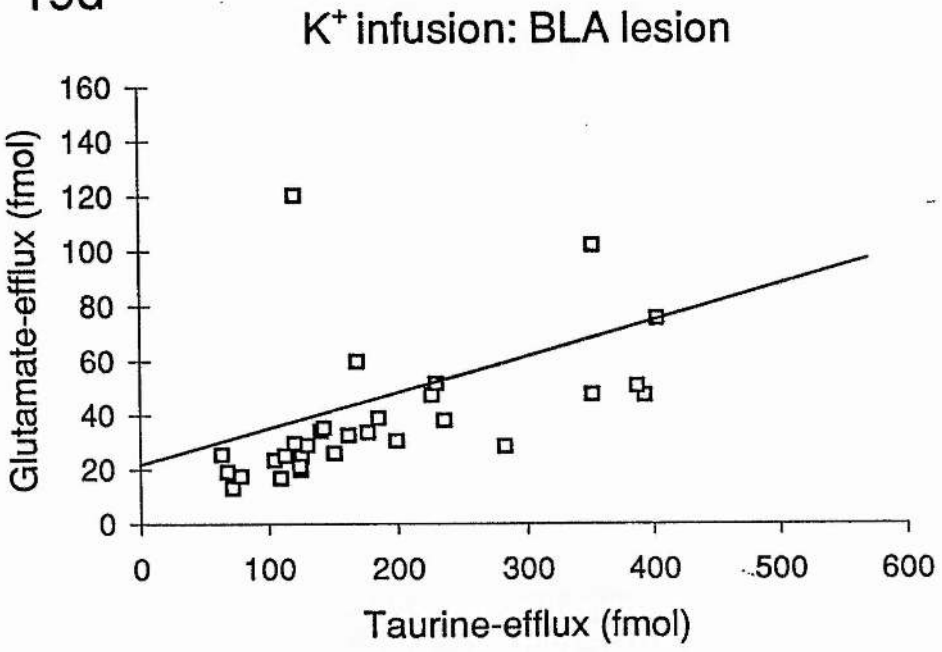


Fig. 19 c, d Linear regression between glutamate- and taurine-efflux in response to K^+ infusions only in control and BLA-lesioned animals respectively. Both groups showed significant positive correlations between the release of the two amino acids ($r=0.65$, $p< 0.001$) control animals (Fig. 19c) and ($r=0.51$, $p<0.01$) lesioned animals (Fig. 19d). Control animals and lesioned animals also showed significant linear relationships ($b=0.12\pm0.024$, $p<0.001$) and($b=0.12\pm0.04$, $p<0.01$) respectively.

Taurine and glutamate regression coefficient: Intra-Nacc K^+

Figure 19c and 19d show scatterplots of the extracellular concentration of Trn and Glu release following intra-Nacc K^+ infusion in sham-operated and BLA-lesioned animals respectively. Taurine and Glu release was significantly correlated in sham-operated control animals ($r=0.65$, $p<0.001$) and BLA-lesioned animals ($r=0.51$, $p<0.01$) and there was also a significant linear relationship between the amino acids in both the control and lesioned groups ($b=0.12\pm0.024$, $p<0.001$) and ($b=0.12\pm0.04$, $p<0.01$) respectively.

Summary

Relative to controls, BLA-lesioned animals were impaired in their basal and cocaine-induced Glu-efflux. However, these lesioned animals did not differ significantly from controls in their basal or cocaine-induced DA- and Trn-efflux, and both groups also responded similarly to intra-Nacc K^+ infusion. Glutamate and Trn release were significantly correlated in both groups following intra-Nacc K^+ infusion, but in response to intra-Nacc infusions of cocaine only control animals showed a significant correlation between the extracellular levels of Trn and Glu.

Experiment 10: Intravenous infusions of cocaine

Following initial lesion surgery, all rats in this experiment underwent IV catheterisation surgery (see General Methods p32). Twelve BLA-lesioned rats and six sham-operated controls were prepared and dialysed in exactly the same manner as described in Expt. 9, except that cocaine infusions were given IV rather than intra-Nacc. The neurochemical response to cocaine was measured following IV

infusions at doses levels of 0.25 and 0.5 mg cocaine/ infusion. After an hour of baseline sampling (six samples) the first cocaine dose was administered IV (0.25 mg/ infusion/ 4s) followed by the collection of six more samples at 10 min intervals. The second dose of cocaine (0.5 mg/ infusion/ 4s) was then administered IV and a further six dialysate samples collected. Each sample was handled in exactly the same way as in Expt. 9, being immediately divided for separate DA and amino acid analyses and stored on dry ice until transfer to a storage freezer. Overall, dialysate samples were collected exactly once every 10 min for 3hr (18 samples/ rat).

Statistical Analyses

Basal extracellular DA and Glu content were analysed initially with a two-way analyses of variance for BLA-lesioned and control animals over the first six 10 min time bins prior to drug treatment: between subject factor Group (2 levels: BLA-lesioned, control), within-subject factor Time (6 levels: 6x10min time bins). Three-way analyses of variance with repeated measures: with between subject factor of Group (2 levels: BLA-lesioned, control), and within-subject factors Dose (3 levels: baseline, 0.25, 0.5mg cocaine/ infusion) and Time (6 levels: 6x10 min time bins, following each infusion) were used to assess the extracellular DA and Glu response to IV infusions of cocaine. Student-Newman-Keuls Post-hoc tests were carried out were applicable.

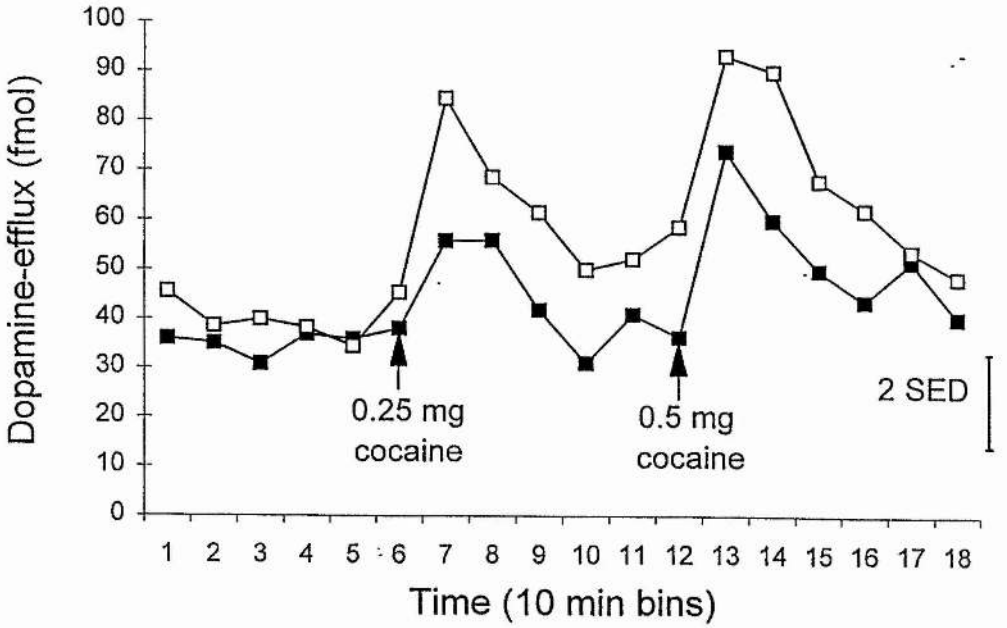
Results: Exp. 10

Histological assessment confirmed that all probe placements were within the medial core of the Nacc. One lesioned animal was excluded from the final analysis because of incomplete (unilateral) neuronal damage of the BLA and one sham-operated animal was discounted because of catheter failure. Group numbers in the final statistical analyses were therefore eleven BLA-lesioned and five sham-operated controls.

IV cocaine infusion: Dopamine efflux

Fig. 20a shows the DA efflux in response to IV administration of cocaine in both BLA-lesioned and sham-operated control animals. A two-way analysis of variance showed that the groups did not differ significantly in their basal DA release. There was no significant effect of Group [$F(1,14)=0.57$, p=NS] or Time [$F(5,70)=0.81$, p=NS] and no Group x Time interaction. In response to cocaine, a three-way analysis of variance showed significant main effects of Dose [$F(2,28)=17.30$, $p<0.001$] and Time [$F(5,70)=11.77$, $p<0.001$] and a significant Dose x Time interaction [$F(10,140)=4.22$ $p<0.001$]. There was no significant main effect of Group [$F(1,14)=1.88$, p=NS] and Group x Dose, Group x Time and Group x Dose x Time interactions were all non-significant. Both BLA-lesioned and control groups showed a dose- and time-dependent increase in extracellular Nacc DA-efflux following IV infusions of cocaine.

Fig. 20a



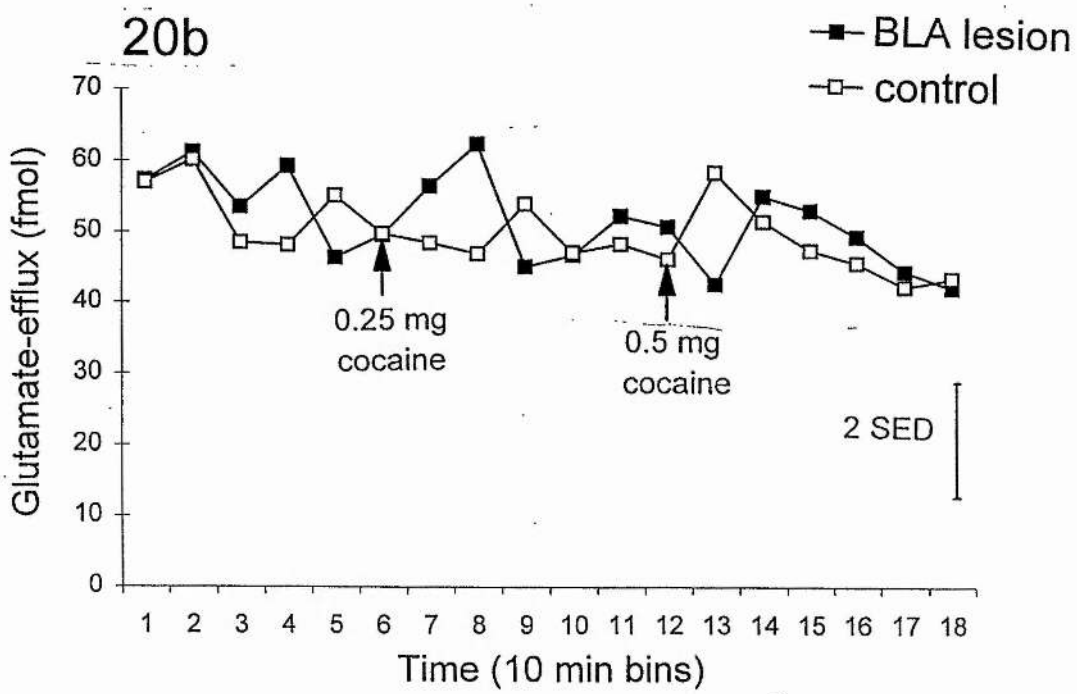


Fig. 20 a, b Mean dopamine- and glutamate-efflux (Fig. 20a and 20b respectively) within the Nacc of BLA-lesioned and sham-operated control rats in response to two single, IV infusions of cocaine. All rats were administered the infusions in ascending dose order. Error bars represent standard error of the difference.

IV cocaine infusion: Glutamate efflux

Fig. 20b shows Nacc Glu efflux in response to IV administration of two cocaine infusions in both BLA-lesioned and sham-operated control animals. It became clear during the HPLC analysis for Glu that there was no relationship between IV cocaine infusions and Nacc Glu-efflux in sham-operated control animals. Therefore the analysis was terminated after six of the initial eleven BLA-lesioned animals had been processed. The data in Fig 20b relates to six BLA-lesioned animals and five sham-operated controls.

A two-way analysis of variance showed that the groups did not differ in their basal Glu-efflux. There was no significant effect of Group [$F(1,9)=0.02$, p=NS] or Time [$F(5,45)=0.91$ p=NS] and no significant Group x Time interaction. A three-way analysis of variance also confirmed that there was no dose- or time-dependent relationship between IV cocaine infusions and Nacc Glu-efflux in either group. There were no significant main effects of Group [$F(1,9)=0.07$, p=NS], Dose [$F(2,18)=1.12$, p=NS] or Time [$F(5,45)=2.23$, p=NS], and there were no significant interactions between these factors. Extracellular Trn-efflux in response to IV infusions of cocaine were not calculated due to the evident lack of effect observed with Glu release.

Summary

Both BLA-lesioned and control animals showed similar dose-dependent increases in extracellular DA-efflux in response to IV infusions of cocaine. Neither group showed a significant correlation between IV cocaine infusions and extracellular Glu-efflux.

Experiment 11: Intra-Nacc tetrodotoxin infusion

This experiment was carried out to confirm that samples of DA-efflux were neuronal in origin. As Glu release is known to be tetrodotoxin (TTX) insensitive (Shiraishi et al. 1997) extracellular concentrations of Glu and Trn were not assessed. Five BLA-lesioned and five sham-operated control rats were dialysed as described in Expt. 9. Following 1hr baseline sample collection (six samples), the dialysate syringe was switched from CSF to TTX (1μM) and this was left in flow for 2hr (12 samples). Each sample was handled in exactly the same way as in Expt. 9 and 10. Overall, dialysate samples were collected once every 10 min for 3hr (18 samples/ rat).

Statistical analyses

Extracellular DA-efflux was calculated as a percent of baseline for each group and analysed. Basal DA-efflux was initially analysed using a two-way analysis of variance with betweeen subject factor of Group (2 levels: BLA-lesioned, control) and within subject factor of Time (6 levels: 6x10min time bins). The DA response to TTX infusions was also assessed using a two-way analysis of variance with repeated measures: between subject factor of Group (2 levels: BLA-lesioned, control) and within-subject factor Time (18 levels: 18x10 min time bins).

Results Expt. 11

Histological assessment confirmed that all probe placements were within the medial core of the Nacc. One BLA-lesioned animal was discounted from the analysis because of insufficient neuronal damage to the full antero-posterior extent of the BLA.

Fig. 21

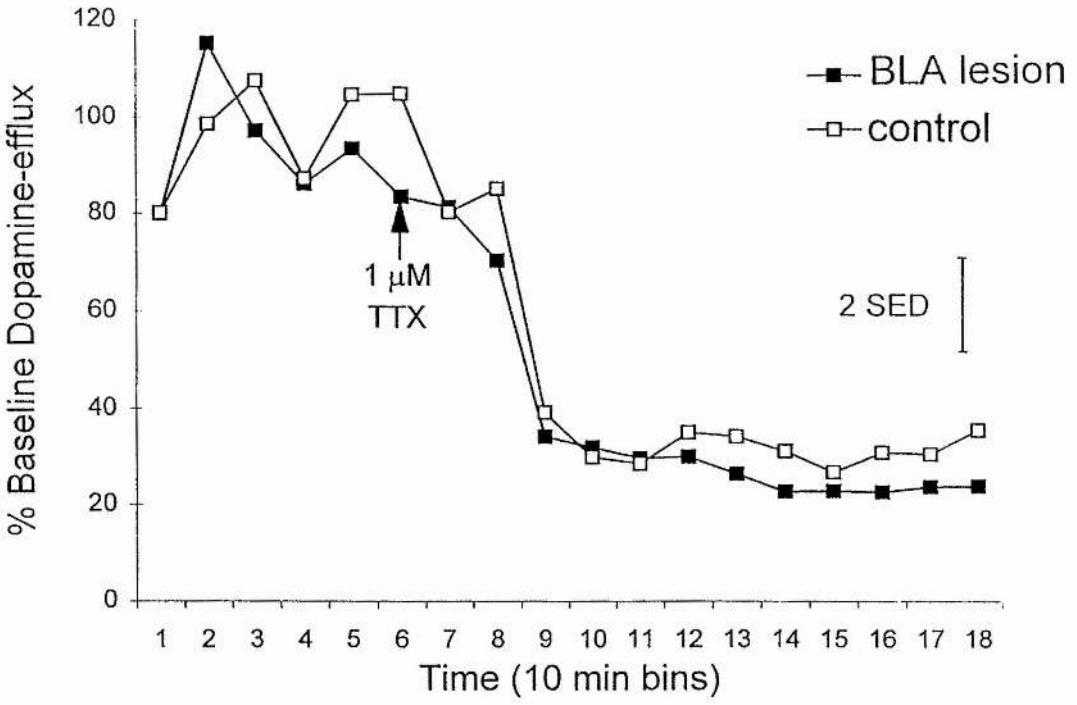


Fig. 21 Mean percent of baseline DA-efflux in BLA-lesioned and control animals in response to TTX (1μM). Following 6 baseline samples TTX was infused continuously for the remainder of the dialysis test session.

Therefore, final group numbers included in the statistical analyses were four BLA-lesions and five sham-operated controls.

Intra-Nacc TTX infusion: Dopamine efflux

Fig. 21 shows the DA response to intra-Nacc infusion with TTX in BLA-lesioned and sham-operated control animals. Values are given as the a percentage change from the mean baseline DA-efflux. Consistent with the observations in Expts. 9 and 10, a two-way analysis of variance showed that BLA-lesioned and sham-operated control animals did not differ in their basal DA efflux. There was no significant effect of Group [$F(1,7)=0.00$, p=NS], Time [$F(5,350=4.28$, p=NS] and no significant Group x Time interaction. Infusion of 1μM TTX produced comparable reductions in extracellular DA in both groups (30-20% of baseline). A two-way analysis of variance with repeated measures showed a significant main effect of Time [$F(17, 119)=34.69$, $p<0.0001$] but no significant effect of Group [$F(1,7)=1.69$, p=NS] and no significant Group x Time interaction [$F(17,119)=$ 0.56, p=NS].

Discussion: Experiments 9, 10 and 11

The results of these experiments demonstrate that following a period of post-lesion recovery, BLA-lesioned and sham-operated control animals did not differ significantly in their spontaneous DA-efflux. Both groups were also equally sensitive to TTX (1μM), which produced a 70-80% reduction in the concentration of DA collected within 30 min of infusion, indicating that the DA content of each sample was mostly neuronal in origin, and both groups were equally sensitive to K^+ infusion. In

response to intra-Nacc infusions of cocaine, control animals showed dose-dependent enhancement in the extracellular levels of DA, Glu and Trn. BLA-lesioned animals also showed a dose-dependent increase in DA-efflux, identical to that observed in controls, but concomitant increases in Glu-efflux were attenuated, while Trn release was consistently lower relative to control animals. In control animals, extracellular levels of Trn and Glu were linearly correlated at all times following intra-Nacc cocaine and K^+ infusion. However, in BLA-lesioned animals a linear relationship between Trn and Glu release was only observed following intra-Nacc K^+ infusion. Taurine and Glu-efflux were not significantly correlated in response to intra-Nacc infusions of cocaine in lesioned animals. In contrast, both BLA-lesioned and sham-operated controls responded similarly to IV infusions of cocaine. Although DA-efflux appeared to be lower in the lesioned group this effect was not statistically significant.

These findings suggest that projections from the BLA to the Nacc modify Glu transmisson and that Trn-efflux may be positively related to increments in extracellular Glu release. Excitotoxic lesions of the BLA did not disrupt cocaine-induced enhancements of DA-efflux indicating that the response of Nacc DA neurons to cocaine are not directly impaired by lesions of the BLA. In the present experiments, BLA efferent projections do not appear to be involved in the mediation of DA responses to either local or IV infusions of cocaine. However, it should be noted that these experiments were carried out in anaesthetised rats with no prior drug experience and that the drug infusions were administered non-contingently. It is

possible these findings may differ considerably in conscious animals with prior cocaine self-administration experience.

The results of Chapters 3, 4 and 5 have indicated that lesions of the BLA do not interfere with the primary reinforcing or stimulant effects of cocaine, administered either contingently (IV) or non-contingently (IP), yet animals with lesions of the BLA were severly impaired in their acquisition of a second-order schedule of cocaine self-administration, and were relatively unaffected by the omission and reinstatement of a cocaine-related conditioned stimulus, under a fixed-interval schedule of cocaine self-administration. These behavioural findings suggest that lesions of the BLA may selectively impair the formation and utilisation of cocaine-related conditioned associations. The primary reinforcing properties of psychomotor stimulants are thought to be mediated via dopaminergic transmission within the ventral stiatum and Nacc (Roberts et al. 1980) and the present neurochemical findings indicated that BLA-lesioned and control animals produced similar elevations in extracellular DA release within the Nacc, following both intra-NAcc, and IV infusions of cocaine. These findings therefore lend support to the conclusions of the earlier behavioural studies (Chapters 3-5) that lesions of the BLA do not interfere with the primary reinforcing properties of cocaine. The present neurochemical results also indicated that animals with excitotoxic lesions of the BLA were impaired relative to controls, in their Nacc glutamatergic response to local cocaine infusions. As the present dialysis experiment was carried out in anaesthetised animals it is impossible to identify a neurochemical cause and behavioural effect relationship, but it is possible that the selective reduction in extracellular Nacc Glu-efflux may have mediated the

behavioural deficits observed in BLA-lesioned animals (Expt. 4, 5) which were attributed to an impairment in the neural mechanisms underlying conditioned reinforcement. However, the present findings fail to shed light on the rapid acquisition of a high dose of cocaine (Expt. 3). It has been suggested (Chapter 3) that lesions of the BLA may mitigate an aversive component of cocaine's actions, which acts to limit the acquisition of cocaine self-administration in control animals, at higher doses (McGregor et al. 1994) but the present neurochemical results can only verify that the rapid acquisition observed in lesioned animals was not due to a reduction in DA transmission, and hence the reinforcing properties of cocaine, at this dose (Roberts et al. 1980).

Anatomical tracing studies suggest that the Nacc receives regionally specific, excitatory afferent projections from several limbic structures including the BLA, prefrontal cortex, subiculum and hypothalamus (Kelley et al. 1982; Kelley and Domesick 1982; McDonald 1991a, b; Kirouac and Ganguly 1995). The BLA also appears to be involved in what McDonald (1991b) has described as 'triangular-relationships' between the BLA, other specific limbic structures and the Nacc. Separate BLA efferents, project to and topographically overlap with, both the origin and striatal target sites of Nacc afferent projections arising in the ventral subiculum, medial prefrontal and lateral prefrontal cortices (McDonald 1991a, b). This may indicate that the BLA is in a good position to influence the overall activity of different limbic inputs to the Nacc, but the lack of a neurochemical correlation between IV cocaine infusions and Nacc Glu-efflux in either sham-operated controls or BLA-lesioned animals would suggest this is unlikely.

In a recent electrophysiological study Korzeniewska et al. (1997) demonstrated that the strength of connections between the BLA, ventral subiculum, Nacc and subpallidal area varied depending upon the emotional and motivational context of a familiar behavioural task. Using the analysis of coherences in EEG signals recorded within each of these structures, rats were initially trained to walk along a runway from an exposed platform to a safe goal box. Variations of this basic task by the addition of positive and negative reinforcement (e.g. the presence of food or sexual reinforcement within the goal box or the loud ringing of a bell for the duration of the rats locomotor activity) reliably produced alterations in the strength of these connections which, due to the design of the experiment, could be differentiated from alterations in locomotor activity. In addition, connections between the BLA and the ventral subiculum were strengthened in all emotionally engaging situations at the two highest frequency bands recorded, suggesting that the activity of these structures are dynamically influenced by environmental contingencies.

Blaha and co workers have also employed *in vivo* chronoamperometry to investigate the relationship between specific limbic structures and the Nacc. Short bursts of electrical stimulation were applied either to the ventral subiculum (Blaha et al. 1997) or the BLA (Floresco et al. 1998) while DA oxidation currents within the Nacc were taken, as an indicator of DA terminal-efflux. This technique allows numerous samples to be taken within a very short period of time (e.g. every 30s) and therefore makes it possible to plot the activity of Nacc DA neurons with an extremely high degree of resolution. The pattern of dopaminergic oxidation currents within the Nacc

following electrical stimulation of both the subiculum and the BLA could be divided into three components depending upon the nature and duration of electrical stimulation: a brief initial increase from baseline, followed by a drop below baseline levels and finally a prolonged rise above baseline. Repetitive low frequency stimulation (20 Hz for 10s) applied to the BLA was characterised by all three components, time-locked to the initiation of electrical stimulation. In contrast, bursts of high frequency stimulation (100Hz, 5 pulses/ burst, 1s interburst interval for 40s s) within the BLA were associated with only the first and third components. In each study both the first and third components of the Nacc DA response were dose-dependently blocked or attenuated by infusions of ionotropic Glu antagonist administered intra-Nacc, whereas the second suppressive component of the DA response was blocked by metabotropic Glu antagonists. Similar Glu and DA antagonist infusions intra-VTA produced no effect on Nacc DA oxidation currents (Floresco et al. 1998) while 6-OHDA lesions of DA cell bodies within the VTA completely eliminated the accumbal DA oxidation component of the Nacc signal (Blaha et al. 1997). Together these findings strongly support the argument for glutamatergic modulation of ventral striatal DA release.

Using *in vivo* electrochemical measurements within the Nacc of conscious rats Richardson and Gratton (1996) reported that exposure to sensory stimuli associated with primary rewards (food or sexual activity) also evoked rapid and prolonged increases in Nacc DA oxidation currents, similar to those observed in response to high frequency stimulation of the ventral subiculum and BLA (Blaha et al. 1997; Floresco et al. 1998). Furthermore, within the amygdala, individual neurons have

been shown to respond selectively during positive or negative auditory discrimination (Muramoto et al. 1993; Ono et al. (1995). Neurons within the corticomedial amygdala (CmA) responded indiscriminately to both positive and negative conditioned and unconditioned stimuli, whereas BLA neurons were found to respond selectively to positive conditioned and unconditioned stimuli. It was concluded (Muramoto et al. 1993) that the CmA functions non-specifically in arousal and attention whereas the BLA is important in discriminating and learning conditioned associations.

Blaha et al. (1997) hypothesised that ventral subicular activity may act to attenuate inputs from other limbic structures which compete functionally for the selection and initiation of specific patterns of behaviour. Similarly, Floresco et al. (1998) concluded that higher frequency transmission from the BLA to the Nacc may preferentially activate specific Nacc output pathways that result in preparatory behaviours directed towards reward related stimuli, or that augmenting DA-efflux presynaptically may facilitate the influence of BLA inputs relative to other Nacc inputs, thereby ensuring that approach behaviour is directed towards salient environmental stimuli. Clearly Nacc afferent projections arising in the subiculum, BLA and prefrontal cortex, communicate in a complex manner and the present findings may support the notion that these Nacc afferents functionally 'compete' for expression within the Nacc. For instance, the results of the present experiment showed that BLA-lesioned animals were significantly impaired relative to controls, in their Nacc Glu response to local infusions of cocaine, yet these lesioned animals were not impaired in their Nacc DA response to cocaine.

Given the findings reported by Floresco et al. (1998) it might be predicted that lesions of the BLA in the present study should disrupt cocaine-induced DA release, as well as Nacc Glu release within the Nacc. However, significant differences between *in vivo* microdialysis and *in vivo* electrochemical detection may provide some insight into these discrepancies. *In vivo* chronamperometric recordings can sample neuronal activity as frequently as every 30s and the modified stearate electrochemical probe has been shown to be selective for DA within the striatum (Blaha 1996). Dialysate samples on the other hand, can be processed for the extracellular concentration of numerous neurotransmitters although, if both monoamine and amino acid HPLC analyses are to be conducted, samples can be taken only once every 10 min. As a result, dialysate samples reflect a cumulative neuronal response whereas electrochemical detection provides a dynamic measure of neuronal activity with a high degree of temporal resolution. Thus, it is possible that dialysate samples do not reflect the complexity of neurochemical alterations produced by excitotoxic lesions of the BLA.

Blaha and Winn (1993) compared differences between the results measured with *in vivo* microdialysis and *in vivo* chronoamperometry under identical experimental conditions. Both systems detected enhanced DA transmission within the striatum of rats administered infusions of neostigmine (a cholinesterase inhibitor) intra-substantia nigra, but the magnitude of this response was significantly greater when measured by *in vivo* chromamperometry than by *in vivo* microdialysis. These authors concluded that the greater surface area of the microdialysis probe resulted in a less specific sampling

area within the striatum, which combined with the long sample duration (10 min versus 30s with *in vivo* chronoamperometry) distorted and ultimately reduced the overall dopaminergic response. Blaha and Winn (1993) also pointed out that the DA baseline taken with *in vivo* chronoamperometry was exceptionally (approximately 100%) sensitive to TTX infusion, while identical TTX infusion lowered the microdialysis baseline by only 75% (similar to the present results in Expt. 11). Therefore, differences in the non-neuronal DA content of dialysis samples may also contribute to differences observed between these detection systems (Blaha and Winn 1993). These findings also suggest that the present results with microdialysis may not have exposed the potential neurochemical deficits associated with excitotoxic lesions of the BLA.

BLA efferent projections which terminate in the Nacc and ventral striatum are thought to convey associative information about environmental stimuli which predict biologically relevant events, such as the delivery of water or food and the opportunity to copulate or self-administer cocaine (Robbins et al. 1989; Everitt et al. 1989; Hatfield et al. 1996; Meil and See 1997). The BLA has been shown to be selectively involved in working memory but not reference memory tasks (Ohno et al. 1993) and the firing rate of neurons within the BLA have also been shown to increase in rats presented with stimuli associated with primary reward (Ono et al. 1995). The animals used in the present set of experiments had no prior drug self-administration experience and were anaesthetised at the time of dialysis. Therefore, the findings of the present experiment cannot reflect glutamatergic activity, or lack of activity (in BLA-lesioned animals) presumed to convey associative information about pertinent

environmental stimuli to the mesolimbic DA system. Furthermore, striatal Glu transmission in anaesthetised rats is approximately 45% less than in conscious rats (Shiraishi et al. 1997).

Several reports have utilised microdialysis in freely-moving animals to assess the neurochemical correlates of specific behaviours (Hemby et al. 1997; Hurd et al. 1989) and it has been reported that in the absence of cocaine, drug-associated cues can elicit conditioned mesolimbic DA-overflow in freely-moving rats with prior drug experience (Fontana et al. 1993). Experiment 5 demonstrated that rats with lesions of the BLA were impaired in the acquisition of second-order schedules of cocaine self-administration and similar lesions have also been shown to impair cue-elicited reinstatement of self-administration behaviour, following repeated extinction trials in which cocaine was not available (Meil and See 1997). It seems likely therefore, that differences observed between the groups in the present dialysis experiment do not reflect the potential neurochemical deficits which may be associated with lesions of the BLA, particularly in reference to the disruption of behaviours which are thought to rely on the formation of conditioned associations.

Several studies have also demonstrated that the expression of behavioural sensitisation to psychomotor stimulants is enhanced when repeated drug injections are administered in the same environmental context (Gold et al. 1988; Ahmed 1996; Hemby et al. 1997) and behavioural sensitisation has also been shown to occur following a limited number of cocaine self-administration sessions (Phillips and Di Ciano 1996). Pierce and Kalivas (1997) have proposed that behavioural sensitisation

to psychomotor stimulants may consists of two components (i) a non-associative element which depends on enhanced DA transmission and (ii) an associative component which is dependent upon enhanced EAA activity, underlies conditioned sensitisation effects, and may rely heavily on glutamatergic limbic afferents. More recently, the effects of dorsolateral prefrontal cortex (Pfc) lesions on the development of behavioural sensitisation to cocaine were investigated by Pierce et al. (1998) and these authors concluded that excitatory amino acid projections from the dorsolateral Pfc to the Nacc facilitated cocaine-induced sensitisation by enhancing glutamatergic transmission within the core of the Nacc. Interestingly, lesions of the BLA have also been reported to inhibit behavioural sensitisation to cocaine (Wolf et al. 1995) and it has been suggested that the BLA participates in sensitisation via projections to the Pfc. However, these authors proposed that such effects were mediated indirectly via Pfc projections onto DA neurons within the VTA (Wolf et al. 1995; Wolf 1998).

Hooks et al. (1993) reported that in conscious animals, repeated IP injections of cocaine administered in the context of specific environment produced environmentally dependent sensitisation to a subsequent IP injections of cocaine (i.e. conditioned enhancement of the drugs stimulant effects). In contrast, similar context-specific intra-Nacc cocaine infusions produced environmentally independent sensitisation to subsequent intra-Nacc cocaine infusions (i.e. unconditioned stimulant effects). Furthermore, an acute systemic cocaine injection failed to elicit a sensitised response in animals that had been pretreated with intra-Nacc cocaine infusions. These authors concluded that the environment paired with the drug's effects may also be involved in the neuronal sensitisation to the drugs actions, and that this effect

relies on structures other than the Nacc. As far as the present results can be compared with those of Hooks et al. (1993), the fact that both BLA-lesioned and control animals showed similar dose-dependent DA-efflux following intra-Nacc and IV infusions of cocaine would indicate that environmental contingencies do not influence the Nacc DA response in anaesthetised animals, but it would appear very likely that Nacc afferents originating within the BLA and dorsolateral Pfc may play an important role in context specific behavioural sensitisation to cocaine.

Hemby et al. (1997) reported that not only was the context in which cocaine was administered an important determinant of Nacc DA-efflux, but that the Nacc DA-response was significantly enhanced when the delivery of drug was contingent upon the behaviour of the animal (i.e. self-administered). It might be concluded therefore, that in conscious animals, systemic or IV injections of cocaine selectively enhance the formation of conditioned associations within the BLA, while concomitant DA stimulation within the Nacc facilitates the behavioural control exerted by such conditioned associations.

Conclusions

Relative to sham-operated controls, animals with lesions of the BLA were significantly impaired in their Nacc Glu response to intra-Nacc infusions of cocaine. However these BLA-lesioned animals were not impaired in their Nacc DA response to cocaine, and both control and lesioned groups produced similar dose-dependent elevations in DA-efflux in response to intra-Nacc, and IV, infusions of cocaine. Both lesioned and control groups also responded similarly to

intra-Nacc TTX and acute K^+ infusion. These findings lend support to the behavioural data which suggest that lesions of the BLA do not interfere with the primary reinforcing properties of cocaine (Chapter 3). Although the rats in the present experiments had no prior cocaine experience and were anaesthetised at the time of dialysis testing, it would appear possible that the behavioural deficits observed under a second-order schedule of cocaine self-administration (Chapters 4) were related to a loss of glutamatergic transmission within the Nacc of BLA-lesioned animals.

Chapter 7: General Discussion

This thesis reports the use of second-order schedules of reinforcement which have been combined with intravenous cocaine self-administration to provide a method for examining the effect of conditioned stimuli on the acquisition of drug-seeking behaviour in rats. The basolateral amygdala (BLA) is thought to be involved in the formation of conditioned associations between motivationally neutral environmental stimuli and biologically significant events. Lesions of the BLA have been shown to disrupt the acquisition of behaviour which is maintained by the presentation of conditioned stimuli associated with natural rewards such as water, food, and sexual activity. Experiments within this thesis have assessed the effect of excitotoxic lesions of the BLA on the acquisition of cocaine-seeking behaviour in rats and have demonstrated that lesions of the BLA severely impair drug-seeking behaviour under a second-order schedule of cocaine self-administration.

Experiment. 1 compared rats with excitotoxic lesions of the BLA with sham-operated controls in their pattern of responding during the acquisition of cocaine self-administration at three different doses (0.5, 0.25, 0.083 mg cocaine/ infusion). At the highest dose of cocaine, lesioned animals were found to self-administer significantly more than controls, from the first day of acquisition. However, this group difference was no longer apparent by the fifth day of testing and no difference betweeen groups were seen at the lower doses of cocaine. Experiment 2 demonstrated that during the acquisition of cocaine self-administration, pairing a discrete light stimulus with each infusion of cocaine significantly enhanced the rate

at which rats acquired self-administration behaviour. This indicated that the light stimulus provided additional discriminative properties and thereby facilitated associative learning between the instrumental lever response and each contingent infusion of cocaine. Lesions of the BLA made following the acquisition and stabilisation of cocaine self-administration in Expt. 3 did not affect the subsequent rate of cocaine self-administration. In addition, Expt. 4 and Expt. 6 demonstrated that BLA-lesioned and sham-operated control animals did not differ either in their locomotor response to IP injections of cocaine (10, 20, 30 mg/ kg) or in their rate of self-administration during a within-session dose-response test, following the initial acquisition of self-administration. These findings suggest that lesions of the BLA produce transitory alterations in the rate of cocaine self-administration that are evident only during the initial acquisition of a relatively high dose of cocaine. Thus, the BLA does not appear to be involved in mediating the primary reinforcing effects of cocaine. Yet despite self-administering more cocaine and therefore experiencing more CS-drug pairings during the initial acquisition of cocaine self-administration (0.5 mg/ infusion), lesions of the BLA severely impaired the ability of rats to acquire cocaine self-administration under a second-order schedule of reinforcement in Expt. 5. The acquisition of a second-order schedule of reinforcement depends upon the formation of conditioned associations, such that motivationally neutral environmental stimuli gain motivational significance by repeated, contingent presentation with a primary reward. The present findings confirm basolateral amygdaloid involvement in the formation of conditioned associations and furthermore suggest that such associations also underlie cocaine-seeking behaviour as assessed in this procedure. Thus it would appear that the

BLA is critically involved in the process by which cocaine-related cues elicit cocaine-seeking behaviour in rats and it is possible that this structure also contributes to similar mechanisms in human subjects, attempting to give-up their drug-taking habit.

Experiment 7 investigated the effect of lesions of the BLA on the acquisition of a progressive-ratio schedule of cocaine self-administration in order to assess the effect of BLA lesions in animals under the influence of cocaine. Under this schedule the response requirement for successive infusions began at a nominal low level and systematically increased throughout each session. Rather than providing a measure of drug-seeking behaviour in the absence of cocaine, this schedule reflects behaviour that is under the influence of cocaine and measures the motivation to continue responding for cocaine as the response requirement increases progressively. The present findings demonstrated that at the initial training dose (0.25 mg/ infusion) both BLA-lesioned and control animals had similar break-points as measured by the overall number of infusions earned or responses made. However, on lowering the dose of cocaine from the training dose, lesioned animals had significantly lower break-points and consequently self-administered fewer infusions of cocaine than controls. Increasing the dose of cocaine from the training dose produced concomitant increases in the break-points of both lesioned and control animals, but these were not significantly higher than those produced at the training dose. Therefore, BLA-lesioned animals appear to be more sensitive to reductions in the dose of cocaine, which might indicate that the

drug-seeking behaviour of lesioned animals is determined more closely by the primary reinforcer than is the case in control animals.

Following the completion of Expt. 7 the same lesioned and control animals were used in Expt. 8 to assess responding for cocaine under a fixed-interval 15 min/ fixed-ratio 10 schedule of cocaine self-administration. Under this schedule each successive infusion of cocaine was available only once a fixed 15 min interval had elapsed, but during this interval every 10th response on the drug-lever resulted in the brief presentation of the light stimulus (CS) previously paired with each drug infusion. The number of responses recorded during the first and second intervals of each session could be attributed to drug-seeking behaviour that was either drug-free or under the influence of cocaine, respectively - before and after, the first infusion of cocaine. Once animals acquired this schedule of self-administration, differences in responding between the first and second intervals were assessed following omission and reinstatement of the drug-related CS. Control animals significantly decreased their overall number of responses during both the first and second intervals following CS omission whereas lesioned animals remained relatively unaffected. Re-instating the CS increased the number of responses made in both groups but lesioned animals responded significantly more during the second interval than sham-operated controls. These findings indicate that the behaviour of BLA-lesioned animals is not only governed more closely by the primary reinforcer, but that drug-seeking behaviour in lesioned animals is also less under the control of conditioned reinforcers.

Finally, Expts. 9, 10 and 11 used *in vivo* microdialysis to assess the neurochemical correlates of excitotoxic lesions of the BLA in response to intra-nucleus accumbens or intravenous infusions of cocaine. Although lesions of the BLA were associated with a significant reduction in accumbens glutamate levels these animals were not impaired in their dopaminergic response to local or intravenous infusions of cocaine. These findings lend further support to the behavioural data and suggest that excitotoxic lesions of the BLA do not interfere with the reinforcing properties of cocaine *per se*. They suggest also that behavioural deficits associated with lesions of the BLA may be related to the disruption of glutamatergic projections to the Nacc.

The findings reported in this work are consistent with, and extend previous studies implicating the BLA in the formation of conditioned associations between motivationally neutral stimuli and primary reinforcers (Burns et al. 1993; Cador et al. 1984; Everitt et al 1989, 1991; Muramoto et al. 1993; Ono et al. 1995; Hitchcott and Phillips 1997; Killcross et al. 1996; Hatfield et al. 1997). These findings also have implications for addiction research, specifically in the areas of drug-seeking behaviour and the propensity of abstinent drug-users to relapse to drug-related activities (Childress et al. 1987, 1988; Ehrman et al. 1992; Grant et al 1996; O'Brien and McLellan 1996; Volkow et al. 1996). The use of a second-order schedule of cocaine self-administration as a valid method for the investigation of cocaine-seeking behaviour in rats has recently been confirmed (Weissenborn et al. 1997; Arroyo et al. 1998) and direct support for the present work can be derived from a recent study in which excitotoxic lesions of the BLA significantly impaired

the capacity of a cocaine-related cue to re-instate drug-seeking behaviour in rats, following a period of non-reinforced self-administration experience (extinction) (Meil and See 1997).

In summary, excitotoxic lesions of the BLA did not interfere with the reinforcing effects of cocaine in rats. Intra-peritoneal injections of cocaine produced similar locomotor responses in both lesioned and control animals and both groups also produced equivalent dose-response functions in response to a within-session dose-response test. Similarly, lesioned and control animals acquired cocaine self-administration under both continuous and progressive-ratio schedules of reinforcement. However, BLA-lesioned animals were (i) severely impaired in the acquisition of second-order schedules of cocaine self-administration; (ii) more sensitive than control animals to reductions in drug dose under a progressive-ratio schedule of cocaine self-administration and (iii) less sensitive than control animals to the omission of a drug-related CS, under a fixed-interval schedule of self-administration. Taken together, these findings suggest that drug-seeking behaviour in animals with lesions of the BLA is more under the control of the primary reinforcer and concomitantly less under the control of secondary, conditioned reinforcers. It is clear from these studies that the BLA is significantly involved in the development and maintenance of drug-seeking behaviour in the rat and, by inference, this structure may also play an important role in the development of problem drug-use in humans.

Experimental validity

It is important to appreciate the limitations of the present studies and to recognise that a considerable amount of work remains to be done to clarify the precise neurochemical interactions that underlie the development and persistence of drug-seeking behaviour in the rat and in humans. For instance, the axon-sparing properties of excitotoxic lesions have recently been called into question. Excitotoxins induce discrete cell death by binding with glutamate receptors and stimulating mass calcium influx, thus resulting in fatal hyperpolarisation of neurons, and it has been reported that such lesions produce transient demyelination of fibres of passage which may interfere with communication between distant structures, unrelated to the lesion site, by slowing down electrical impulses (Brace et al. 1997). It is also clear that damage to fibres that pass through the amygdala can have functional consequences. For example, Dunn and Everitt (1988) reported that electrolytic but not excitotoxic lesions of the amygdala abolished conditioned taste aversion in rats. These authors concluded that the lesions differed in their modes of action and suggested, as a result, that electrolytic lesions arose through the destruction of fibres of passage that crossed through the amygdala, while excitotoxic acid produced discrete axon-sparing lesions of amygdaloid neurons (Dunn and Everitt 1988). Support for the hypothesis that excitotoxic lesions of the amygdala do not have 'non-specific' effects can be drawn from a recent behavioural study which showed that excitotoxic lesions of the BLA made after the acquisition of cocaine self-administration did not impair cue-elicited reinstatement of responding for cocaine, but that lesions made prior to self-administration training severely impaired cue-elicited responding (Meil and See 1997). Had

excitotoxic lesions generated more global 'non-specific' impairment, both types of responding should have been impaired. These findings suggest that the effects of excitotoxic lesions of the amygdala are behaviourally quite specific.

In the present work histological assessments were carried out to ensure that animals included in statistical analyses sustained damage to the basal and lateral nuclei of the amygdala and particular care was taken to exclude animals with damage to the central nucleus of the amygdala. In most instances this did not prove to be a problem as neuronal damage observed in the majority of lesioned animals spread laterally towards the cortex, away from the central nucleus. These observations have been confirmed in a recent study by Killcross et al. (1997) who reported that quinolinic acid produced reliable, discrete, lesions of the BLA whereas ibotenic acid was better suited for lesions of the central nucleus of the amygdala.

Another important factor concerns differences between different drugs of abuse and their potential reinforcement mechanisms. For instance, the present work supports the hypothesis that the BLA is involved in the process by which cocaine-related stimuli maintain cocaine-seeking behaviour, but similar lesions of the BLA do not appear to be involved in the acquisition of a second-order schedule of heroin self-administration (Alderson et al. 1997). Within psychomotor stimulants, cocaine and amphetamine are also known to differ significantly in their mode of action and these differences may have implications for the development and maintenance of drug-seeking behaviour. Cocaine acts by binding with the dopamine transporter molecules to inhibit the reuptake of neuronally released dopamine (Ritz et al. 1987)

whereas amphetamine directly stimulates the release of dopamine. Cocaine also clears rapidly from the brain and has a half-life approximately half that of amphetamine. Following a series of positron emission topography studies (PET) in baboons and human cocaine addicts Volkow et al. (1996) proposed that the pharmacokinetic properties of cocaine, particularly the speed with which cocaine clears from the brain, promotes repeated and frequent self-administration which, in turn, facilitates compulsive drug-use and addiction. This may also have significance for the findings of the present work. BLA efferent projections are thought to convey associational information to the nucleus accumbens and ventral striatum regarding salient environmental contingencies. Therefore as cocaine acts directly on dopamine transporter molecules to enhance naturally occurring dopamine release (Ritz et al. 1997) cocaine may also act directly to enhance the potency of these associations such that drug-related stimuli elicit robust drug-seeking behaviour, irrespective of the development of tolerance to the primary reinforcing effects of the drug (Robinson and Berridge 1993; Carey and Gui 1998).

The validity of microdialysis in anaesthetised rats must also considered when interpreting the data from Chapter 6, particularly in the extrapolation of neurochemical findings to earlier behavioural data. The process of dialysis depends on the flow of substances along their concentration gradients, across a microbore membrane and into the dialysate solution (artificial CSF). Two factors can significantly alter the concentration of neurotransmitters recovered within each dialysate sample for reasons unrelated to the neurochemical activity of the animal; (i) the polarity of the aCSF and (ii) the dialysis flow rate. Artificial CSF is made

up of the few ions necessary for neurotransmitter release - calcium, sodium, potassium and chloride but alterations in the balance of these ions may alter the polarity of the solution and therefore distort the composition of the dialysate recovered. The composition of dialysate may also be distorted if the dialysis flow-rate is too fast. This is because the probe can act as a 'sink' drawing neurotransmitters along their concentration gradients from a larger area of surrounding tissue which may invalidate the anatomical specificity of neurotransmitter release. To limit these potential hazards the aCSF used in the present dialysis experiments was made up freshly each day from a refrigerated stock solution, which itself was renewed weekly. Special FEP tubing which has an exceptionally small dead volume was also used to connect the aCSF syringes to the dialysis probes. Combined with a constant flow rate of 1µl/ min it is likely that the dialysate samples collected were anatomically specific and that the composition of each sample was not artificially distorted.

However, the fact that animals in the present dialysis experiments were anaesthetised at the time of testing does limit the scope of interpretation. It has been reported that glutamate release measured by microdialysis in anaesthetised rats can be as much as 45% below that of conscious freely-moving rats (Shiraishi et al. 1997). Although it is likely that these effects are caused by a loss of motor activity in anaesthetised animals, one can only speculate about the pattern of neurochemical activity in freely-moving animals from studies using anaesthetised rats. Very little can be inferred about the neurochemical bases of the behavioural deficits observed in BLA-lesioned animals in the present study, although it is clear

that these lesioned animals are not impaired in their dopaminergic response to acute injections of cocaine, despite marked reductions in Nacc glutamate levels relative to control animals.

In vivo microdialysis samples the neurochemical composition of extracellular fluid over specific time periods and these measurements are considered to provide a correlate of neurochemical activity. In a recent study, Burechailo and Martin-Iverson (1996) reported that receptor occupancy may be functionally more relevant than extracellular overflow measured by *in vivo* dialysis because unbound neurotransmitters (i.e. circulating in the extracellular fluid) have no function and therefore may not provide an accurate correlation of neurochemical activity. They propose that receptor occupancy depends on several factors: neurotransmitter concentration, receptor density and receptor affinity, all of which may influence neurochemical function, but may not be reflected by *in vivo* microdialysis. Burechailo and Martin-Iverson (1996) utilised a novel procedure to determine receptor occupancy using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) a substance which irreversibly inactivates monoamine receptors, but can only denature receptors that are not already bound by their respective neurotransmitters. Tissue sample analyses carried out 24 hr following treatment with EEDQ provides an indication of dopamine and serotonin receptors occupancy at the time of treatment. A major drawback with this technique however, is that it cannot provide a dynamic measure of neurochemical activity and therefore it is impossible to make within-subject comparisons (i.e. baseline measurements versus consecutive treatments in a single animal). Furthermore, the 30 min delay between

experimental treatment and EEDQ administration used in the study of Burechailo and Martin-Iverson (1996) may be too long to give an accurate indication of neuronal activity during the first minutes post-experimental treatment.

The rate with which neurotransmitters are synthesised may also affect their extracellular levels measured by dialysis as well as receptor occupation. Baumann (1993) demonstrated that repeated cocaine treatment ameliorates the initial reduction in DA synthesis observed in many brain regions, including the BLA and Nacc, following an acute injection of cocaine. These authors proposed that this effect may have arisen through the desensitisation of DA autoreceptor regulation induced by repeated cocaine administration. All dialysis experiments reported in the present work were carried out in drug naive rats with no prior self-administration experience, so it is unlikely that differences in sensitisation would pose a problem in these studies. *In vivo* microdialysis may provide only a correlation of neurotransmitter release, but with appropriate control groups or within subject comparisons it remains a versatile and powerful tool with which to assess neurochemical activity *in vivo*.

Future perspectives

As mentioned above, dialysis experiments carried out in anaesthetised animals cannot provide information regarding the neurochemical bases of behaviour. In light of the behavioural deficits observed in BLA-lesioned rats under a second-order schedule of cocaine self-administration (Chapter 4) it would be interesting to assess the neurochemical activity within the Nacc of freely-moving rats, using *in vivo* microdialysis, in response to drug-related conditioned reinforcers.

Di Ciano et al. (1998) recently reported the use of *in vivo* chronoamperometry to quantify DA oxidation currents within the Nacc of freely moving rats either self-administering or passively receiving (yoked) infusions of amphetamine. Both groups were presented with a 5s conditioned light stimulus paired with the onset of each drug infusion, and during each 3hr session Nacc DA oxidation currents rose significantly in both amphetamine groups, relative to yoked, vehicle-administered controls. Following seven days of drug administration training, animals were tested in the absence of amphetamine. During the test session, all drug infusions were replaced with vehicle and the previously drug-related light stimulus was presented once every 30 min as well as with each vehicle infusion administered.

Di Ciano et al. (1998) reported that in the absence of amphetamine, presentation of the drug-related conditioned stimulus significantly increased Nacc DA oxidation currents in both yoked and self-administering rats with prior amphetamine experience. Surprisingly, these findings did not suggest significant differences in the Nacc DA responses of yoked and self-administering animals. Several studies

(discussed in Chapter 3) have suggested that the firing pattern of Nacc DA neurons increase significantly in response to self- but not passive administration of cocaine (Carelli et al. 1993; Carelli and Deadwyler 1994) and that contingent administration may be important during the acquisition of cocaine self-administration (Wilson et al. 1994). Furthermore, Hemby et al. (1997) recently reported that Nacc DA-efflux was significantly enhanced in rats self-administering cocaine relative to rats receiving yoked-cocaine infusions, which also oppose the findings of Di Ciano et al. (1998).

Differences in the reinforcing actions of amphetamine and cocaine are unlikely to explain the discrepancies between the Hemby et al. (1997) and Di Ciano et al. (1998) studies, but it is possible that procedural differences, such as the *in vivo* techniques employed and the duration of pre-test training, may account for some inconsistencies. Both the extended period of drug training and the fact that animals lived permanently in their self-administration chambers may have enhanced the salience of drug-related conditioned stimuli for self-administering animals in the Hemby et al. (1997) study. In addition, these conditions may have simultaneously interfered with the reinforcing effects of cocaine in yoked animals, as a result of stress associated with non-contingent drug administration and because these yoked animals lived permanently in the drug-related environment (Maier and Seligman 1976; Dworkin et al. 1995). On the other hand, it is possible that the effects observed by Di Ciano et al. (1998) reflected enhanced dopaminergic activity within the VTA, rather than conditioned enhancements in Nacc DA-efflux in response to a drug-related conditioned stimulus. An increase in the firing rate of VTA DA neurons have been reported in the rat and monkey following the presentation of unpredicted stimuli (Ono et al. 1995;

Mirenowicz and Schultz 1996) and omission of amphetamine reinforcement on the test day in the Di Ciano et al. (1998) study may have operated as such an unpredictable stimulus, concomitantly enhancing Nacc DA-efflux in both self- and passively administered rats, in the absence of amphetamine.

Assuming that the BLA is involved in the formation of conditioned associations which also underlie cocaine-seeking behaviour, an investigation of the neurochemical response within the Nacc of conscious BLA-lesioned and sham-operated control rats, previously trained to self-administer cocaine, may elucidate the precise neurochemical correlates of cue-elicited cocaine-seeking behaviour. Furthermore, to examine whether contingent and non-contingent cocaine administration produce different neurochemical responses, the pattern of neurochemical activity within the Nacc of conscious drug naive rats could be assessed following self- or passive-administration of cocaine. Parallel assessment of the corticosteroid levels found in the blood of these animals might also provide an index of the possible anxiogenic properties of cocaine reinforcement. Enhanced corticosteroid release is highly correlated with the autonomic response to stress (Piazza et al. 1991). Therefore, if lesions on the BLA evoke anxiolytic-like effects, the corticosteroid response of BLA-lesioned animals may be lower than sham-operated controls self-administering the same dose of cocaine. Likewise, if non-contingent (passive) administration of cocaine elicits a greater stress-response than contingent self-administration, the corticosteroid response of self-administering rats may be lower than that found in passively-administered rats.

However, dialysis experiments which attempt to assess cocaine-seeking behaviour in freely-moving rats are inherently difficult to design and execute. First, it is difficult to control for extraneous behaviour often associated with both the direct and conditioned effects of psychomotor stimulants. Secondly, the dopaminergic response of an animal freely self-administering cocaine quickly reaches a ceiling (see Hurd et al. 1997) at which point subtle differences between treatment groups may be masked. Thirdly, to minimise the neuronal damage associated with implanting a dialysis probe it is important to implant the probe at least 24 hours prior to testing. During this time it is relatively easy for the probe itself to become damaged or blocked. Combining these problems with intravenous catheterisation surgery, an extended period of self-administration training prior to testing, and the possibility of excitotoxic lesion variability, the overall number of potential difficulties increases dramatically during freely-moving dialysis.

For the above reasons it may be wise to begin by dialysing freely-moving animals with and without lesions of the BLA and assessing their neurochemical response to the presentation of a conditioned stimulus associated with a natural reward, such as sucrose pellets (Phillips et al. 1993; Richardson and Gratton 1996) before attempting to examine cue-elicited cocaine-seeking behaviour. Rather than employ a second-order schedule of reinforcement to assess conditioned incentive motivation a simpler and equally effective model is the acquisition of a new response paradigm (Mackintosh 1974; Robbins 1975, 1976; Robbins et al. 1983, 1989; Taylor and Robbins 1984, 1986). The initial use of sucrose pellet reinforcement would not only be beneficial in consolidating the optimal training regime but would also produce

data with which to compare neurochemical adaptations specific to cocaine self-administration. As both sugar pellet and cocaine reinforced animals would receive identical training, a pairwise comparison of the groups within each set would expose any basal changes in the neurochemical profile as a direct result of the drugs effects, as well as determine how repeated cocaine administration influences BLA function (Pierce and Kalivas 1997).

Non-dopaminergic involvement in BLA function

The role of both cholinergic and serotonergic involvement in drug-seeking behaviour also deserve further investigation. The amygdala receives a strong serotonergic innervation which appears in the highest concentration in the basal magnocellular and parvicellular neurons of the amygdala (Fallon and Ciofi 1992) and these BLA neurons also receive dense cholinergic innervation from the basal forebrain (in primates the nucleus basalis of Meynert) which provide innervation of the cerebral cortex (Carlsen 1989). Cocaine is known to bind to the dopamine transporter to block reuptake and to prolong normal DA stimulation (Ritz et al. 1987). In addition, cocaine also produces general stimulatory effects and has an affinity for serotonin (5HT), adrenaline, muscarinic M_1 and M_2 receptors as well as the *sigma* receptor (see Walsh and Cunningham 1997). Ritz and George (1997a) report that the 5HT transporter and the $5HT_2$ receptor are important sites in the mediation of cocaine-induced convulsions whereas dopaminergic, muscarinic M_1 and sigma binding sites are significantly correlated with cocaine-induced lethality (Ritz and George 1997b). Eidelberg (1963) concluded that the limbic system, particularly the amygdala, was critically involved in the mediation of cocaine-

induced convulsions, although Ritz and George (1997a) noted that interactions between important neurotransmitters may occur in structures that innervate the amygdala rather than in the amygdala *per se*. For instance, $5HT_2$ receptors are primarily localised on cholinergic neurons in the pontomesencephalic tegmentum (Morilak and Ciaranello 1993) and therefore enhanced serotonergic transmission may stimulate cholinergic activity. Furthermore, a primary function of the muscarinic M_4 receptor is to enhance the release of glycine which in itself is a critical prerequisite for the activation of N-methly-D-aspartate (NMDA) glutamate receptors (Russo et al. 1993) which may elicit convulsions (Ritz and George 1997a). The primary reinforcing properties of cocaine are known to rely on dopaminergic activation (Roberts et al. 1977) but it would appear that serotonergic as well as cholinergic systems may be involved in the actions of cocaine (Walsh and Cunningham 1997; Ritz and George 1997b). In a recent report the development of tolerance to the stimulant properties of cocaine was linked with $5HT_3$ receptor subsensitivity within the nucleus accumbens of rats (Matell and King 1997) and Rocha et al. (1998) demonstrated that 'knockout' mice, lacking the $5HT_{1B}$ receptor subtype, showed both enhanced locomotor response to cocaine and significantly higher break-points than wild-type mice under a progressive-ratio schedule of cocaine self-administration. These findings suggest that the importance of non-dopaminergic contributions to cocaine-seeking behaviour also warrant further investigation.

Many reports have also implicated amygdaloid function in learning and memory (Sarter and Markowitsch 1985; Kesner 1992; McGaugh et al. 1992). As mentioned

above the BLA receives dense cholinergic innervation which arises in the nucleus basalis magnocellularis (NBM) (Carlsen 1989, Kesner et al. 1990; Dalmaz 1993). Degeneration of cholinergic NBM neurons have been linked with dementia and Alzheimer's disease and consequently non-selective lesions of the basal forebrain have been used as an animal model of Alzheimer's disease in tests of learning and memory (Whitehouse et al. 1982). Interestingly, Ohno et al. (1992) reported that in the rat, cholinergic function of the BLA may be selectively involved in a working memory, three-panel runway task. Electrolytic lesions of the BLA were associated with an increase in the number of errors made during the completion of the task, but these deficits were attenuated by intra-peritoneal administration of both cholinesterase inhibitors and the muscarinic receptor agonist oxotremorine. Neither BLA nor corticomedial lesions impaired performance in a reference memory version of the task and it was concluded that lesions of the BLA selectively impaired working memory by reducing cholinergic function. Similar results had been achieved in an earlier study using the NMDA antagonist AP5 in an test of fear potentiated startle. Miserendino et al. (1990) infused AP5 intra-BLA and found that disruption of NMDA activity within the BLA selectively prevented the acquisition but not the retention of a fear potentiated startle response in rats. To assess both cholinergic and NMDA involvement in the mediation of working and reference memory Ohno et al. (1993) investigated the effects of the muscarinic antagonist scoploamine or the NMDA antagonist CPP infused intra-BLA, on performance in their three-panel runway task. Consistent with earlier findings, both the NMDA and muscarinic antagonists were effective in disrupting working memory while producing no effect on a reference memory task. These results

suggest that working memory but not reference memory is mediated by both NMDA and cholinergic function within the BLA (Ohno et al. 1993). These findings may parallel reports which suggest that lesions of the BLA made following the acquisition of cocaine self-administration did not block cue-elicited reinstatement of responding, but that this effect was severely disrupted in animals given BLA-lesions prior to the acquisition of cocaine self-administration (Meil and See 1997).

Other reports have suggested that the amygdala is also an important site in the integration of neuromodulatory influences on memory storage (Introini-Collison et al. 1989). Dalmaz et al. (1993) investigated the interaction between noradrenergic and cholinergic function in memory and concluded that low doses of noradrenergic agonists facilitate memory for an active avoidance task by stimulating the release of acetylcholine (ACh) within the amygdala. Similarly the muscarinic cholinergic agonist oxotremorine, infused intra-amygdala, enhanced memory performance, but these effects were independent of noradrenergic activity. Although this work did not differentiate between specific amygdaloid nuclei it is likely that the BLA was significantly involved, simply because of the high concentration of cholinergic afferents within the basolateral nucleus (Carlsen 1989). It is possible therefore, that deficits in drug-seeking behaviour observed in BLA-lesioned rats in the present studies may have arisen through the disruption of cholinergic function within the BLA.

The amygdala is also richly innervated by gamma amino butyric acid (GABA) and relative to other brain areas $GABA_A$ receptors are found in particularly high concentration within the BLA (Niehoff and Kuhar 1983; Carlsen 1989). GABA receptors are allosteric, with three subunits (GABA, benzodiazepine, barbiturate, steriod and picrotoxin). Binding at any one of these sites facilitates binding of the other ligands, but GABA has been shown to bind more securely when a benzodiazepine is bound to the receptor (Tallman and Gallagher 1985). Benzodiazepines are anxiolytic drugs which are thought to produce tranquillising effects via enhanced GABA-ergic inhibition, and several studies have indicated that the BLA is also an important site in the mediation of these effects (Vellucci et al. 1988; Tomaz et al. 1992; Davis 1992, 1994). In the present thesis excitotoxic lesions of the BLA were associated with accelerated acquisition of cocaine self-administration at a high dose (Expt. 1). The precise reasons for this effect remain unclear, but it has been suggested that cocaine is associated with anxiogenic effects (Ettenberg et al. 1993; McGregor and Roberts 1994; Dworkin et al. 1995). If this is the case, lesions of the BLA may also act selectively to diminish aversive properties of cocaine, possibly by evoking effects similar to enhanced GABA-ergic inhibition within the amygdala.

The interaction between GABA-ergic and cholinergic systems in memory and learning have also been studied extensively. Sarter and Bruno (1994) reviewed some of these studies and discussed the use of trans-synaptic modulation of cortical ACh as a method of investigating the functional significance and activity of intact cholinergic neurons, as opposed to lesion induced correlates of behavioural

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function. GABAergic afferents originating in the Nacc inhibit cholinergic neurons within the basal forebrain and reduction in GABA transmission results in an increase in cortical ACh and facilitation of cognitive function (Sarter et al. 1988). Sarter et al. (1990) proposed that the actions of select $GABA_A$-receptor ligands do not reflect normal GABAergic function whereas benzodiazepine ligands modulate GABA receptors and therefore induce GABAergic activation within a normal physiological range. However, the results of studies investigating the actions of the benzodiazepine agonist chlordiazepoxide (CDP) on cortical ACh release have been mixed (Sarter et al. 1994). Moore et al. (1993) investigated the effect of CDP administration in rats trained to associate sudden darkness with the presentation of food-reward. Interestingly, this behavioural training consistently enhanced cortical ACh release (70-100%) as measured by *in vivo* microdialysis. Furthermore, this behaviourally activated ACh-efflux was completely blocked by both systemic and intra-basal forebrain infusion of CDP. It was therefore hypothesised that cortical ACh is responsible for the mediation and detection of behaviourally significant stimuli, and that these effects can be modulated by endogenous GABAergic activation (Moore et al. 1993; Sarter et al. 1994).

The conclusions of Sarter et al. (1994) have several implications for the findings of the present work and indeed highlight several interesting areas worthy of further investigation. Many studies have reported that the BLA is an important structure in the mediation of conditioned reinforcement and the present work, and that of Meil and See (1997) has demonstrated that the BLA is also involved in cue-elicited cocaine seeking-behaviour. The BLA receives dense cholinergic innervation which

arises in the NBM (Carlsen 1989) and the behavioural function associated with both cortical ACh function and excitotoxic lesions of the BLA suggest that these effects are related. Furthermore, as discussed above, cocaine produces general stimulation of a number of non-dopaminergic systems that may interact to modulate cholinergic, GABAergic, serotonergic or noradrenergic activation. These systems may not mediate the primary reinforcing effects of cocaine; although Robledo et al. (1996) demonstrated that AMPA lesions of the NBM shifted the dose-response function of animals self-administering cocaine to the left of controls, they also point out that the observed effects may have reflected a specific resistance to extinction of responding for lower doses of cocaine in the NBM-lesioned animals. Clearly the involvement of non-dopaminergic systems in cocaine self-administration requires further investigation. It is possible that the effects of discrete excitotoxic lesions of the BLA reported in the present work arose in part, through the disruption of cholinergic, GABAergic or serotonergic function. With the development of specific neurotoxins and receptor ligands it will be easier to investigate the involvement of these systems in cue-elicited drug-seeking behaviour and the development of tolerance to the reinforcing effects of cocaine. Such research will undoubtedly enhance the current understanding of the neural mechanisms underlying compulsive drug-use and addiction.

Conclusions

It is now widely recognised that the BLA plays a significant role in the assimilation of sensory information and the recognition of salient environmental contingencies. These functions are important for a wide range of emotional and social behaviours

which appear to share a common objective - survival. Weiskrantz (1956) hypothesised that the amygdala was involved with the recognition of biologically significant stimuli and more than 40 years on this notion still prevails. The BLA has been associated with the explicit memory of emotional events (Cahill et al. 1994, 1995); emotional salience in face recognition (Morris et al. 1996; Hamann et al. 1996); memory and learning (Meserendion et al. 1990; Ohno et al. 1993); fear and anxiety (Davis et al. 1994; Killcross 1997) and conditioned reinforcement (Cador et al. 1989; Everitt et al. 1989, 1991). The experiments reported in this thesis demonstrate that the BLA is also an important component in the neural mechanisms underlying conditioned cocaine-seeking behaviour. Excitotoxic lesions of the BLA significantly impaired the acquisition of a second-order schedule of cocaine self-administration, which could not be attributed to alteration in the reinforcing efficacy of cocaine. Glutamatergic projections which arise in the BLA are thought to interact with neurons of the mesolimbic dopaminergic system to mediate conditioned drug-seeking behaviour, although it is likely that other structures such as the prefrontal cortex, ventral subiculum and specific thalamic nuclei are also involved in mediation of certain aspects of cocaine self-administration. The BLA receives considerable GABAergic, cholinergic, and serotonergic innervation and further research is required to elucidate the precise neurochemical bases of the BLA lesion-induced, behavioural deficits reported in the present work. Such neurochemical findings will significantly enhance our understanding of the neural bases of compulsive drug-use and addictive disorders.

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Appendix 1: Solutions and Reagents

(a) Phosphate buffer

Sodium dihydrogen orthophosphate, 4.36g (BDH Laboratory Supplies Poole, UK) and di-sodium hydrogen orthophosphate anhydrous, 10.22g (BDH Laboratory Supplies Poole, UK) were dissolved in 1L of ultra-pure water, filtered and refrigerated until required.

(b) Avertin

99% 2,2,2-tribromoethanol, 10g (BDH Laboratory Supplies Poole, UK) was sonicated in a scintilation vial with tertiary amyl alcohol, 5mg (BDH Laboratory Supplies Poole, UK) until dissolved. This mixture was then slowly added to 450ml phosphate buffer with 40ml absolute alcohol. The Avertin was then filtered, buffered with NaOH to pH 7.0-7.4 and refrigerated in a light protected bottle until required.

(c) Timentin

Timentin powder (Beecham Research, Welwyn Garden City, Herts, UK) is composed of potassium clavulante 200g, with ticarcillin 3g. The antibiotic solution was made up by dissolving 65g of the Timentin powder in 1ml 0.9% sterile saline (Animalecare Ltd., Dunnington, UK). This was made freshly for each treatment.

(d) Paraformaldehyde (PFA)

40g PFA powder (Merck Darmstadt, Germany) was dissolved in 1L phosphate buffer while heated and stirred. The temperature of the solution was not allowed to rise above 70°C. Once dissolved, the solution was filtered and refrigerated until needed.

(e) Gelatine solution

3g of gelatine powder (BDH Laboratory Supplies, Poole, UK) was heated and dissolved in 600ml of ultra-pure water. Off the heat, 0.3g $CrK(SO_4)_2$ (BDH Laboratory Supplies, Poole, UK) was added and the solution was then filtered.

Before use, all slides were briefly dipped twice in this solution and left to dry overnight between each coating.

(f) Artificial CSF composition

	Stock Solution	aCSF(nM)
NaCl	1M	147
KCl	1M	3.0
$MgCl_2$	1M	1.0
$CaCl_2$	1M	1.2
$NaH_2PO_4.H_2O$	0.2M	0.2
$NaHPO_4$	0.2M	1.3

Appendix 2: Components and Suppliers

(a) Intravenous catheter

Osteotec Ltd., UK

Silicon tubing (small) code STHT-C-012-0

Silicon tubing (large) code STHT-C-030-0

Semat Technical Ltd., St. Albans, Herts, UK

Guide cannula code C313G 5UP

Associated Dental Products Ltd., Kemdent Works, Purton, Swindon, UK

Dental Cement

Small Parts Inc., 13980 N.W. 58th Court PO Box 4650 Miami Lakes, Florida, USA

Polypropylene marlex mesh code CMP-500-D

(b) Tygon seal and screw cap

Altec PVC tubing, Unit 4 Riverway, Industrial Park, Alton Hants UK

Tygon Tubing

RS Supplies UK

Metal spacers; (screw cap)

tapped by Dan Morely Precision Engineering Ltd., Cambs, UK

(c) Dialysis probe

Hospal Industrie, 69330, Meyzieu, France

Microbore dialysis membrane.'Filtral 12', 20,000 dalton

ELKAY, 800 Boston Turnpike, Shrewsbury, Mass, 01545 USA

'Running-foot' tubing, solvent flexible 0.014 ID Cat No. 116-0537-040

SGE UK, Milton Keynes, UK

Silica glass tubing code: 10vs-40-140-0D

(d) Dialysis equipment

CMA Biotech Instruments Ltd., Biotech House, 75A High St., Kimpton, Herts, UK

FEP tubing dead volume 1.2 µl/100mm Cat No. 3409501

Tubing Kit Cat No. 3408550

Liquid Switch 110 CMA

Harvard Apparatus, Southnatick, Mass, USA

Harvard micropump Model 240-001

Biotech Instruments, Kimpton, Herts, UK.

CMA - 110, 3-way liquid switch