

DOPAMINERGIC CORRELATES OF SENSORY-SPECIFIC SATIETY
IN THE RAT: MODULATION BY INACTIVATION
OF AMYGDALAR SUBREGIONS

By

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Abstract

Sensory-specific satiety is a critical factor in the selection of a varied diet in humans and animals. *In vivo* microdialysis was used to investigate the modulation of dopamine (DA) efflux in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) by the central nucleus of the amygdala (CeN) and the basolateral amygdala (BLA), and to ascertain the influence of such modulation on dopaminergic correlates of sensory-specific satiety in the rat.

In each experiment, rats were given an opportunity to consume a palatable food to satiety. In a subsequent test meal, rats sampled very little of the familiar food but ingested significant amounts of a novel food. Changes in DA efflux in both the NAc and mPFC reflected this difference in food intake, indicating that DA transmission is influenced by changes in deprivation level and sensory incentive properties of food. Sensory-specific satiety was disrupted by inactivation of the CeN. Reverse-dialysis of lidocaine in the CeN resulted in ~20% decrease in basal levels of DA efflux in the NAc. Continued exposure to lidocaine during the first meal disrupted the overall expression of sensory-specific satiety and the associated changes in DA efflux in the NAc and mPFC. In contrast, inactivation of the BLA neither affected sensory-specific satiety nor its dopaminergic correlates in the NAc. Interestingly, lidocaine infusion into the BLA triggered dramatic oscillatory changes in DA efflux in the mPFC.

Sensory-specific satiety was additionally assessed in rats that had been subjected to repeated cycles of food restriction and binge opportunities, a feeding regimen previously reported to lead to hyperphagia (Hagan and Moss, 1997). Cycled rats displayed normal satiety during the first meal but engaged in another vigorous bout of feeding in a second meal

of the same food, which was never seen in control groups. Given that food restriction is considered to be a form of stress, which in turn is associated with sensitization of the DA system, it is conjectured that these results could be related to a similar dysfunction of the DA system, and thus, to an impairment in the control of food intake by the incentive sensory properties of food.

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List of Abbreviations

ANOVA	analysis of variance
BLA	basolateral amygdala
CeN	central nucleus of amygdala
DA	dopamine
g	gram
GABA	gamma-aminobutyric acid
6-OHDA	6-hydroxydopamine
HPA	hypothalamic-pituitary-adrenal
HPLC-ED	high-pressure liquid chromatography with electrochemical detection
hr	hour
kg	kilogram
L-DOPA	L-dihydroxyphenylalanine
mg	milligram
min	minute
ml	millilitre
μl	microlitre
mM	millimolar
mPFC	medial prefrontal cortex

mV	millivolts
NAc	nucleus accumbens
NST	nucleus of the solitary tract
NMDA	N-methyl-D-aspartate
od	outer diameter
nM	nanomolar
SEM	standard error of the mean
SN	substantia nigra
VTa	ventral tegmental area

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GENERAL INTRODUCTION

"Well," said Pooh, "what I like best-" and then he had to stop and think.

Because although Eating Honey was a very good thing to do, there was a moment just before you began to eat it which was better than when you were, but he didn't know what it was called.

(A. A. Milne, 1928, The House at Pooh Corner)

The regulation of feeding behaviour is complex, involving a dynamic interaction of multiple physiological and cognitive influences (Herman and Polivy, 1984). It is fundamental to the immediate survival of an animal and as such requires neural systems that are devoted to processing stimuli of biological relevance and those that organize appropriate actions in responses to such stimuli. The integration of these multiple processes is thought to be mediated by the mesotelencephalic dopamine (DA) system (Fibiger and Phillips, 1986; Blackburn et al., 1992; Salamone, 1996). In fulfilling this role, the DA system must be subject to a variety of internal and external influences that modulate its function; by the same token, the DA system is also vulnerable to numerous dysregulatory influences. In the context of feeding behaviour, important modulatory input to the DA system may arise from the amygdala, a limbic structure that mediates the processing of sensory (e.g., the sight, smell and taste of food) and motivational (e.g., related to an animal's hunger or satiety state) information (Mishkin and Aggleton, 1981; Aggleton, 1992). In the absence of these normal regulatory influences, or when they are

overridden under certain circumstances, the control of feeding behaviour may be impaired.

The primary objectives of experiments in this thesis were first, to examine the role of the mesotelencephalic DA system and its interactions with the amygdala in the control of food intake and second, to examine the consequences of manipulations that disrupt normal patterns of food intake on DA transmission. To address these objectives, *in vivo* microdialysis was used to monitor changes in DA efflux in mesotelencephalic terminal regions while animals were assessed for sensory-specific satiety. Subsequent experiments focussed on the influence of transient inactivations of the central and basolateral nuclei of the amygdala on dopaminergic correlates of sensory-specific satiety. A final experiment examined the effect of food restriction, a form of stress, on sensory-specific satiety and its implications for dysregulation of DA function.

The mesotelencephalic DA system

The mesotelencephalic DA system originates in the mesencephalon, in DA cell bodies of the ventral tegmental area (VTA, the A10 cell group) and substantia nigra (SN, the A9 cell group) (Dahlström and Fuxe, 1964), and projects to telencephalic terminal fields including the ventral striatum, prefrontal cortex and the amygdala (Fallon and Moore, 1978). Although a general topographic order is preserved within this system, VTA and SN projections overlap in some terminal sites (Björklund and Lindvall, 1984; Deutch et al., 1988; Cooper et al., 1996). DA neurons of the retrorubral field (A8 cell group) also project to forebrain sites innervated by the VTA and/or SN (Dahlström and

Fuxe, 1964), as well as sending shorter collaterals to DA neurons in the VTA (Deutch et al., 1988).

Investigations of the mesotelencephalic DA system in the context of motivated behaviour have focussed on DA function in two medial forebrain structures, the prefrontal cortex and the ventral striatum. In the rat, dopaminergic innervation of the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc) has been well described. The mPFC, which consists of the anterior cingulate, the prelimbic region and the infralimbic region, receives projections primarily from DA neurons in the VTA (Divac et al., 1978; Björklund and Lindvall, 1984). Similarly, the shell and core regions of the NAc – which can be distinguished on the basis of histochemical, anatomical and functional characteristics (Zahm and Brog, 1992) – receive differential dopaminergic innervation. The VTA provides substantial input to both the shell and core regions whereas the SN provides specific input to the core region (Björklund and Lindvall, 1984; Deutch et al., 1988; Cooper et al., 1996).

Regulation of DA release

DA release is dependent on depolarization of the nerve terminal and the subsequent influx of calcium. Extracellular concentrations of DA are closely tied to changes in DA cell body firing rates (Kuhr et al., 1987), with burst-firing patterns resulting in higher levels of DA release than single spiking mode (Gonon, 1988). The nonlinear relationship between neuronal firing and magnitude of DA release has led to the suggestion that DA terminals function as a “high pass filter” (Gonon, 1988; Suaud-Chagny et al., 1992). Application of gamma-aminobutyric acid (GABA) in the VTA decreases the firing rate of DA neurons and causes a substantial decrease in basal

concentrations of DA in the striatum (Suaud-Chagny et al., 1992), whereas activation of N-methyl-D-aspartate (NMDA) glutamate receptors in the VTA results in an increase in terminal DA release that is time-locked to burst firing of DA neurons (Suaud-Chagny et al., 1992; Chergui et al., 1993). Both NMDA and GABA receptor modulation of DA neuron firing appear to be driven by cortical areas, including the prefrontal cortex, by way of excitatory amino acid afferents to the VTA (Sesack and Pickel, 1992; Carr and Sesack, 2000).

The effect of DA on postsynaptic neurons depends on the subtype of DA receptor stimulated, as well as the action of other neurotransmitters and neuropeptides that converge on the same receptive neuron. DA receptors are classified into two genetically, biochemically and pharmacologically defined categories, the D₁-like and D₂-like receptors (Kebabian and Calne, 1979; Cooper et al., 1996). Stimulation of D₁-like receptors (the D₁ and D₅) increases adenylyl cyclase activity and cAMP production while stimulation of the D₂-like receptors (the D₂, D₃ and D₄) activates G_i proteins that inhibit cAMP production (Cooper et al., 1996). Both the D₁-like and D₂-like receptors are located on the postsynaptic membrane, potentially modulating feedback pathways to DA cell bodies in the mesencephalon. Although both subtypes of receptors exist in most areas receiving DA terminals, some structures, such as the prefrontal cortex, have higher densities of D₁- than D₂-like receptors (Boyson et al., 1986). In contrast, the ratio of D₁- to D₂-like receptors is more balanced in most regions of the striatum (De Keyser et al., 1988; Monsma et al., 1989).

In addition to existing on the postsynaptic membrane, D₂-like receptors are also located on presynaptic nerve terminals, soma and dendrites (Gonon and Buda, 1985).

These autoreceptors have strong influences on the overall activity of the DA system.

Nerve terminal DA autoreceptors are important in regulating the synthesis and release of DA but somatodendritic DA autoreceptors also contribute to nerve terminal DA release by regulating firing rates of DA neurons (Chiodo et al., 1984; Gonon and Buda, 1985).

The absence of autoreceptors on the nerve terminal and cell bodies of DA neurons projecting to the prefrontal cortex may contribute to the unique characteristics of this DA subsystem (Cooper et al., 1996). For example, prefrontal projecting DA neurons have higher spontaneous rates of spiking activity, as well as more frequent bursting, compared to neurons projecting to the striatum, whose activity is heavily regulated by the presence of autoreceptors.

Regulation of extracellular DA concentration in the NAc and mPFC

DA synthesis begins with the conversion of the amino acid precursor tyrosine by tyrosine hydroxylase into L-dihydroxyphenylalanine (L-DOPA), which is then converted into DA by a decarboxylation step (Cooper et al., 1996). Extracellular concentrations of DA are regulated by both rates of release and reuptake. DA that is released into the synaptic cleft is quickly taken up by a high affinity DA transporter, thereby limiting diffusion of DA from the synaptic cleft (Garris and Wightman, 1994). DA in the extracellular space is also catabolized into its metabolic by-products by catechol-O-methyltransferase and monoamine oxidase (Cooper et al., 1996).

Estimates of extracellular concentration of DA in the rat NAc and mPFC, two major terminal projections of midbrain DA neurons, indicate that *absolute* concentrations are approximately five times higher in the NAc (Garris and Wightman, 1994). However, when the density of DA innervation to each region is taken into account, the *relative*

concentration of DA is approximately ten times higher in the mPFC than in the NAc (Garris and Wightman, 1994). Comparisons of the relative release-to-reuptake rates in the NAc and mPFC suggest that the difference in extracellular concentrations of DA is largely due to the relative dominance of either the release or reuptake mechanisms (Garris and Wightman, 1994). In the NAc, regulation of DA in the extracellular compartment is "uptake dominated"; that is, under low levels of stimulation (i.e., DA cells are in a single spiking mode), reuptake is efficient and steady-state concentrations of DA are observed. As the frequency of action potentials increases (or during burst firing) and more DA is released, less time may be available between action potentials for reuptake mechanisms to have a significant effect and thus, the observed extracellular concentration of DA increases. In the mPFC, however, the ratio of release-to-reuptake rates is 5-10 times higher than in the striatum, and can be considered "release dominated". Consequently, even low frequencies of action potentials can dramatically increase extracellular concentrations of DA in the mPFC.

Rates of reuptake also influence the distance that DA can diffuse from the point of release. In the mPFC, the half-life of reuptake is ~2.0 sec, as compared to <0.06 sec in the NAc. Therefore, DA in the mPFC has a longer duration in which to diffuse further away from the site of release than DA in the NAc (Garris and Wightman, 1994). This is consistent with distal sites of action (<1 mm radius) and the hypothesis that DA efflux in the mPFC is related to extrasynaptic "volume transmission" rather than synaptic "wiring transmission" (Fuxe and Agnati, 1991).

DA content in microdialysate samples represents a portion of DA released into the synaptic cleft, as well as DA that has diffused into the extrasynaptic space. For this

reason, measures of extracellular concentrations of DA in this thesis are described as “efflux” and the term “release” is reserved for specific referrals to the vesicular release of DA from terminals.

Role of DA in food-motivated behaviours

The relationship between DA and feeding behaviour was initially established by the observation that lesions of the mesotelencephalic system by 6-hydroxydopamine (6-OHDA), a DA-specific neurotoxin, cause adipsia and aphagia in rats (Ungerstedt, 1971). Recently, a similar DA-deficiency syndrome was observed in mice lacking the gene for tyrosine hydroxylase (a critical enzyme in the DA synthesis pathway) in DA neurons (DA $-/-$) (Szczypka et al., 1999). These animals did not eat and drink enough to survive and were only kept alive by intragastric or manual feeding. In the case of the DA $-/-$ mice, food intake can be restored by daily administration of the DA precursor L-DOPA (Szczypka et al., 1999).

The aphagia in DA-deficient animals may be related to deficits in motor coordination or general depression. However, a substantial body of evidence suggests that functional changes in the limbic as distinct from the dorsal striatal aspects of the mesotelencephalic DA system primarily affect motivational aspects of behaviour (Salamone et al., 1997; Berridge and Robinson, 1998). DA-deficient animals have the motor capacity to walk, chew, swallow and generate other movements required for eating, but fail to employ these movements to make contact with the food even if it is presented literally under their noses (Berridge et al., 1989; Szczypka et al., 1999). Furthermore, interference with DA transmission in the NAc by DA receptor antagonism

or partial DA-depletions does not alter food or water intake, but does diminish food hoarding and foraging behaviour (Kelley and Stinus, 1985; Whishaw and Kornelsen, 1993). Similar manipulations also bias animals away from working (e.g., lever pressing) to obtain a preferred food and towards consuming less palatable lab chow with no work requirement (Salamone et al., 1991).

DA and incentive motivation

Impairment of feeding behaviour associated with DA-deficiency has been attributed to disruption of the neural substrate of incentive motivation (Blackburn et al., 1992; Phillips et al., 1992; Smith, 1995; Salamone et al., 1997; Berridge and Robinson, 1998). It is postulated that activation of the mesotelencephalic DA system subserves a process by which the sensory properties of biologically relevant stimuli and environmental stimuli associated with them gain the ability to initiate and maintain behaviours (i.e., to serve as incentives) that guide an organism toward a primary reward, such as food, water or a mate (Blackburn et al., 1992; Berridge and Robinson, 1998).

Observed changes in firing rates of DA neurons in the midbrain that coincide with the occurrence of conditioned and primary incentive stimuli are consistent with this hypothesis. In primates, presentation of a food reward shifts the activity of midbrain DA neurons (A8, A9 and A10 cell groups), from single-spike firing to burst-firing patterns (Ljungberg et al., 1992; Schultz et al., 1993). Similar activity also occurs in response to unexpected or omitted reward. In associative learning tasks, DA neurons respond to primary rewards during the learning phase, but as the reward becomes fully predicted by a preceding cue, time-locked neural responses shift forward in time to be coincident with the cue. Schultz and Dickinson (2000) suggest that DA neuronal firing is related to the

detection of *prediction errors* (i.e., differences in the predicted outcome and the actual reward). Such errors are encoded as increased DA activity, which participate in the eventual attainment (and maintenance) of a fully predictable error-free state (i.e., no change in DA activity) through modifying behavioral responses. Thus, DA neurons do not respond to rewards that are reliably predicted by a cue, or to a cue that occurs at regular intervals (Ljungberg et al., 1992). Schultz and his colleagues propose that the DA system has an important role in an animal's ability to adjust to a changing environment rather than in performing well-learned habits (Ljungberg et al., 1992; Schultz et al., 1995).

The distinction between appetitive and consummatory behaviours may provide further insight on the role of DA in food reward (Blackburn et al., 1992). Consummatory responses are described as innate and stimulus-specific behaviours: food elicits food intake and receptive female rats elicit copulatory behaviour in male rats. In contrast, appetitive responses include a wide range of behaviours elicited by incentive stimuli that are voluntary and flexible (e.g., approaching a food hopper or pressing a lever associated with food reward). Thus, animals learn to employ a variety of appetitive (or preparatory/instrumental) responses that culminate in consummatory behaviour.

In vivo microdialysis studies reveal that consumption of a food reward is correlated with robust increases in DA efflux in the NAc and mPFC (Radhakishun et al., 1988; Hernandez and Hoebel, 1988; Cenci et al., 1992; Yoshida et al., 1992; Pothos et al., 1995; Feenstra and Botterblom, 1996; Martel and Fantino, 1996; Taber and Fibiger, 1997). DA efflux is also increased by incentive stimuli, including cues associated with food reward (Phillips et al., 1993) or by the sight, smell or taste of the food itself (Mark et

al., 1994; Wilson et al., 1995; Bassareo and Di Chiara, 1997; Ahn and Phillips, 1998; Bassareo and Di Chiara, 1999). Similarly, increased DA efflux is observed when animals make an instrumental response to obtain delivery of food (Salamone et al., 1994; Ahn et al., 1997). Collectively, these *in vivo* monitoring data suggest that DA activation is not simply related to the ingestion of food but is also involved in appetitive behaviours leading to the consumption of food. In fact, the use of *in vivo* electrochemical techniques, which allows for fine temporal analysis (on the order of seconds) of changes in DA efflux, has provided interesting observations during the transition from appetitive to consummatory phases of feeding. DA efflux in the NAc has been reported to peak just before each lever press and then drop sharply as the food is retrieved and consumed (Kiyatkin and Gratton, 1994; Richardson and Gratton, 1996).

In the context of food reward, it is relevant that different neurotransmitter systems within the NAc appear to influence specifically the consummatory aspects of feeding. Regulation of food consumption is dependent on the interaction of glutamate and GABA neurotransmission in the shell region of the NAc and the subsequent activation of lateral hypothalamic neurons (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997; Stratford and Kelley, 1999). Stimulation of opioid receptors, especially in the NAc (Bakshi and Kelley, 1994), also may mediate consummatory action, as opioid agonists such as morphine can elicit hyperphagia in nondeprived rats (Jackson and Cooper, 1985; Le Magnen, 1990; Bechara and van der Kooy, 1992; Giraudo et al., 1993; Hagan et al., 1997).

“Wanting” and “liking” of food reward

Berridge (1996) has proposed that there are two components of food reward: the palatability or the sensory pleasure of food (“liking”) and the appetite or decision to eat (“wanting”), each of which involves separate neural mechanisms. Neural processing of the “liking” of food begins in brainstem benzodiazepine circuits (Berridge and Treit, 1986) and continues in forebrain circuits involving GABA, glutamate and opiates, as described above. It is conjectured that the “wanting” of food is mediated by the mesotelencephalic DA system.

Most measures of feeding behaviour, such as the amount of food intake, choice of food and performance of lever presses, reflect both the “liking” and “wanting” of food. As such, most manipulations that alter feeding behaviour cannot be attributed exclusively to impairment of only one aspect of food reward. However, in rats, the perceived palatability (“liking”) of food or fluids can be assessed separately from appetite to eat by monitoring affective taste reactions to foods, which are innate, reflexive orofacial responses to various taste stimuli. For example, bitter tastes elicit gapes, chin rubs, headshakes and face/paw wipes whereas sweet tastes elicit rhythmic mouth movements, forward and lateral tongue protrusions and paw licks (Grill and Berridge, 1985). Evidence suggests that this measure is a reliable and specific reflection of the hedonic assessment of the taste stimuli (Grill and Berridge, 1985; Pecina et al., 1997; Berridge and Robinson, 1998).

Under normal circumstances, both “wanting” and “liking” aspects of food motivation are influenced by hunger and satiety. Development of satiety to (and decreased intake of) a particular food also suppresses hedonic reactions to the taste of that

food but not a different food (Berridge, 1991). A parallel effect has been reported using ratings of pleasantness in humans (Johnson and Vickers, 1992; Rolls and Rolls, 1997; Porrini et al., 1997). If rats are treated with furosemide which induces a sodium-appetite, the consumption of, as well as hedonic taste reactivity to, a sodium chloride solution is significantly increased in comparison to non-depleted rats (Berridge and Schulkin, 1989; Galaverna et al., 1993). Manipulations of the DA system, however, alter food intake but do not change taste reactivity. For example, 6-OHDA lesions of the DA system or blockade of DA receptors produces animals that are aphagic but that are still able to show normal palatability-dependent taste reactions to solutions delivered by intra-oral cannulae (Berridge et al., 1989; Pecina et al., 1997). Berridge (1996) has interpreted these findings as evidence that DA is not involved in hedonic reward *per se*, but instead subserves a motivational process that brings the animal into contact with the food.

According to Berridge and Valenstein (1991), the hedonic “liking” of a reward must precede the “wanting” of a reward. These authors propose that incentive motivation occurs in a three-step process: first, neural substrates mediating a hedonic experience are activated; second, the hedonic event is associated with predictive stimuli; and third, salience is attributed to subsequent perceptions of the natural incentive stimulus and the associated environmental cues. In further developing this line of reasoning, Berridge and Robinson (1998) hypothesize that in the final stage, activation of the DA system is required for the *attribution of salience* to the stimuli. These stimuli then have the capacity to act as incentives for behaviour (i.e., to have control over behaviour). This process is believed to change the neural representation of a stimulus from a motivationally neutral source of information into an attractive and “attention grabbing”

incentive which can serve as a goal that animals will work to acquire. Furthermore, with repeated or continued exposure to the incentive stimulus, attribution of salience must reoccur frequently in order to update or re-evaluate the value of the stimulus, which in turn allows for adjustments to behaviour.

Role of DA in behavioural sensitization

As an extension of the incentive salience attribution theory of DA function, Robinson and Berridge (1993) have proposed an *incentive-sensitization hypothesis* that explains how and why addictive behaviours occur, especially related to drugs of abuse such as amphetamine and cocaine. The theory posits that repeated use of addictive drugs produces “incremental neuroadaptations in the [mesotelencephalic] DA system, rendering it increasingly and perhaps permanently, hypersensitive (‘sensitized’) to drugs and drug-associated stimuli” (Robinson and Berridge, 1993). In other words, as a result of these functional changes in the DA system, there is an excessive attribution of salience to incentive stimuli (e.g., drugs and associated stimuli) and ordinary “wanting” becomes intense “craving”.

Behavioural sensitization to psychostimulant drugs and stressors has been attributed to neural adaptations in the mesotelencephalic DA system (Robinson and Berridge, 1993; Prasad et al., 1995; Marinelli et al., 1996). Subsequent encounters with a sensitizing stimulus or another DA-activating stimulus can elicit enhanced DA transmission and potentiated behavioural responses. For example, repeated administration of stressors (such as mild foot-shock or tail-pinches) enhances not only subsequent responses to the stress-causing stimuli. This process additionally sensitizes amphetamine- or cocaine-induced locomotor activity and increases the propensity to

develop intravenous self-administration (Antelman et al., 1980; Deroche et al., 1993; Deroche et al., 1994; Prasad et al., 1995; Marinelli et al., 1996). Conversely, repeated psychostimulant administration can cross-sensitize behavioural responses to natural rewards. Fiorino and Phillips (1999b) observed that repeated administration of amphetamine enhanced the acquisition of sexual behaviour in sexually naïve male rats. They also observed in these sensitized rats, an increase in appetitive and consummatory aspects of sexual behaviour that were accompanied by an enhancement in the magnitude of DA efflux in the NAc (Fiorino and Phillips, 1999a). This augmented DA efflux following amphetamine sensitization was suggested to reflect enhanced attribution of incentive salience to the sensory properties of the receptive females.

It remains to be determined whether the DA system can be sensitized by natural motivational stimuli and thereby alter behaviour controlled by natural or primary rewards. With regard to feeding behaviour, excessive stress has been associated with hypophagia, but mild stresses, including restraint and tail-pinch, have been shown to increase food consumption (Levine and Morley, 1982; Heinrichs et al., 1992; Badiani et al., 1996; Harris et al., 2000). Food restriction, which also promotes food intake, is considered to be a form of stress because it can elicit stress-related physiological responses, such as the release of corticosterone (Broocks et al., 1990). These considerations suggest the possibility that repeated application of stress - whether in the form of restraint, tail-pinch or food restriction - may serve to sensitize the DA system, and thus enhance behaviours motivated by food-related incentives.

As mentioned above, the “liking” of food is an important determinant of whether that food is “wanted” or not (Berridge, 1991). Therefore, a neural circuit must exist for

“liking” to influence “wanting”. In this regard, brainstem and forebrain circuits involved in the hedonic assessment of food (i.e., “liking”) may be functionally and anatomically related to the mesotelencephalic DA system which subserves “wanting”. Current reports on the role of the amygdala in feeding behaviors (Everitt et al., 1999) suggest that amygdalar modulation of DA transmission may be a potential neural substrate for this process.

The amygdalar complex

The amygdala is a critical component of neural networks that allow an animal to evaluate and respond to environmental stimuli that are pertinent to its current biological needs. One well-known symptom of amygdalar damage, as originally described by Klüver and Bucy (1939), involves altered behavioural responses to food-related stimuli and inappropriate feeding responses to non-food stimuli. Amygdala-lesioned primates exhibit less neophobia to novel foods and have an increased tendency to ingest food which are normally avoided; they also orally explore nonfood items (Kling and Dunne, 1976; Aggleton and Passingham, 1982; Murray et al., 1996). These observations suggest that an intact amygdala may facilitate appropriate behavioural responses by a process through which sensory information from the external world gains motivational or emotional significance (Mishkin and Aggleton, 1981; Uwano et al., 1995). Furthermore, lesions of sensory afferents to the amygdala decrease emotional or behavioural responses to stimuli in the sensory modality affected. For example, rats with lesions of projections from the auditory thalamus to the amygdala do not react to auditory stimuli associated with shock (Romanski and LeDoux, 1992). Similarly, in primates, disconnecting visual

input to the amygdala results in deficits in emotional responses to visually arousing stimuli, while reactions to other sensory stimuli are spared (Horel and Keating, 1969).

Neurophysiological data also indicate that the amygdala is a site of convergence for all modes of sensory stimulation, as neurons in this region of the brain respond to single and multiple modes of sensory input (Uwano et al., 1995). Furthermore, some neurons in the amygdala only show changes in activity to sensory stimuli that are associated with primary reward (Uwano et al., 1995), suggesting that neuronal activity within the amygdala may mediate associative learning processes (Nishijo et al., 1988).

Multimodal amygdalar neurons which respond to conditioned cues and primary reward are mainly found in two subregions of the amygdala, the central nucleus (CeN) of the amygdala and the basolateral amygdala (BLA) (Uwano et al., 1995). Recent studies by Everitt and his colleagues (Killcross et al., 1997; Everitt et al., 1999), as well as Holland and Gallagher and their colleagues (Schoenbaum et al., 1998; Holland and Gallagher, 1999), indicate that the CeN and BLA are important in mediating different aspects of associative learning processes and furthermore, that amygdalar modulation of DA transmission may be critical in the behavioural expression of these processes (Everitt et al., 1999; Parkinson et al., 2000c). Current views of the neuroanatomy of the amygdala also support a dissociation of function between the CeN and BLA (Alheid et al., 1995; Swanson and Petrovich, 1998; Pitkänen, 2000). As such, a brief review of the neuroanatomy of the amygdala will precede a review of recent reports on the role of the CeN and BLA in food-motivated behaviours.

Neuroanatomy of the amygdala

Current evidence suggests that the CeN and BLA are highly differentiated regions of the amygdala, with each subregion sharing unique anatomical connections and histochemical properties of distinct regions of the brain. Alheid and Heimer (1988) have proposed that a continuum of neurons, extending from the central and medial nuclei of the amygdala, through the bed nucleus of the stria terminalis, the posterior limb of the anterior commissure and into the NAc shell region comprises the “extended (central) amygdala”. In support of this view, a majority of the neurons in the CeN, like those in the striatum, are peptide-expressing medium-sized spiny GABA-containing neurons (Sun and Cassell, 1993; Meredith et al., 1993; Davis et al., 1994; Swanson and Petrovich, 1998) that are silent or exhibit low levels of spontaneous activity (Veinante and Freund-Mercier, 1998). Furthermore, like the NAc shell, the CeN receives innervation from the BLA and heavy dopaminergic input from the VTA (Fallon et al., 1978b; McDonald, 1991b). Of all the projection neurons in the amygdala, anatomical and electrophysiological evidence indicate that those arising from the CeN are uniquely GABAergic, whereas other projection neurons are likely to be glutamatergic (McDonald, 1992; Davis et al., 1994).

The BLA, consisting of the basal and lateral nuclei of the amygdala, is proposed to be more “cortical” than “striatal” in character (Swanson and Petrovich, 1998). As in other cortical areas, there are two main cell types in the BLA: pyramidal glutamatergic projection neurons and peptide-expressing non-pyramidal GABAergic interneurons. More controversially, the BLA is proposed to be a ventromedial extension of the claustrum underlying the temporal, piriform (the primary olfactory cortex), insular (the

primary taste cortex) and frontal cortices (Swanson and Petrovich, 1998). This suggestion is based largely on embryological data and adult topographical relations, but Swanson and Petrovich (1998) concede that a great deal more needs to be learned about the morphology and connections of neurons in the claustrum and the BLA before their intimate relationship can be considered to be established.

Of central importance for the objectives of the present thesis are the anatomical data that provide support for functional interactions between the CeN and BLA with the mesotelencephalic DA system. The CeN sends inhibitory afferents to the midbrain, with heavier innervation to the SN and retrorubral field than to the VTA (Wallace et al., 1992), but does not project directly to regions receiving DA input. In contrast, the BLA sends excitatory afferents to the NAc and mPFC (McDonald, 1991a; McDonald, 1991b), which are innervated by DA terminals (Fallon and Moore, 1978; Björklund and Lindvall, 1984). Within the NAc, more caudal regions of the BLA synapse in the shell region whereas more rostral regions terminate mainly in the core region (Brog et al., 1993; Shinonaga et al., 1994). In the mPFC, projections from the BLA mainly innervate the prelimbic and infralimbic regions (McDonald, 1991a). The differences in anatomical connectivity of the CeN and BLA with the DA system and the differences in neurotransmitters in projection neurons from the CeN and BLA (inhibitory versus excitatory, respectively), are in agreement with the hypothesis that each amygdalar subregion utilizes different mechanisms to modulate DA transmission in a functionally relevant manner.

Role of the CeN and BLA in food-motivated behaviours

The “striatal-like” and “cortical-like” characterization of the CeN and BLA, respectively, suggests that each may mediate different aspects of appetitively motivated

behaviours. Recent investigations indicate that each subregion subserves a different form of associative learning, namely Pavlovian (or classical) and instrumental (or operant) conditioning, which may mediate different aspects of incentive-motivated behaviours (Bolles, 1972; Dickinson, 1989; Dickinson and Balleine, 1994; Toates, 1998).

Pavlovian conditioning involves learning the predictive value of a previously neutral stimulus like a light cue (the conditioned stimulus, CS) paired with a natural reward such as food (the unconditioned stimulus, US). In an autoshaping task, as a consequence of repeated CS-US pairings, animals learn to approach the CS (conditioned responses, CR) before going to the food hopper where food is actually delivered. Everitt and his colleagues have shown that lesions of the CeN disrupt the *acquisition* of this task whereas lesions of the BLA do not (Everitt et al., 1999; Parkinson et al., 2000b), which indicates that the CeN has an important role in early stages of associative learning (Everitt et al., 1999).

Lesions of the NAc core region also impair the development and performance of Pavlovian approach tasks, suggesting an essential functional link between the CeN and NAc core. The CeN does not have a direct projection to the NAc but does innervate the SN and VTA which contain DA neurons (Phillipson, 1979; Wallace et al., 1992). This information led Everitt and his colleagues (Everitt et al., 1999) to hypothesize that by interacting with the DA system, the CeN can organize behavioural activation directed towards the appropriate CS in the environment. Consistent with this suggestion, blocking DA transmission in the NAc produces the same impairment as CeN and NAc core lesions (Parkinson et al., 1999; Everitt et al., 1999; Parkinson et al., 2000a). Additionally, the CeN may orchestrate arousal mechanisms because a disconnection of the CeN-

striatonigral pathway prevents the development of conditioned orienting responses (Han et al., 1997). It has also been suggested that interactions between the CeN and basal forebrain cholinergic systems may drive attentional mechanisms (Holland and Gallagher, 1999; Holland et al., 2000).

During the process of Pavlovian conditioning, the CS is associated with key properties of the US (Colwill and Motzkin, 1994; Málková et al., 1997) such that presentation of a CS leads to the activation of a US representation, including its incentive motivational properties. As such, a Pavlovian CS has the ability to serve as a conditioned reinforcer of (new) instrumental learning and evidence from a number of research labs suggests that this process involves the BLA. For instance, Hatfield and her colleagues (1996) demonstrated the BLA is critical to developing second order conditioning. In this task, rats receive first-order light → food pairings, followed by second-order tone → light pairings in the absence of food. Rats with BLA lesions do acquire the first-order CRs to light, but fail to acquire second-order conditioning to the tone (Hatfield et al., 1996). Furthermore, if food is devalued by an injection of lithium chloride (a toxin causing gastric malaise) following first-order light → food conditioning, BLA-lesioned rats do develop aversion to the taste of food but *do not* display reduced CR to the light CS (Hatfield et al., 1996). These findings suggest that the BLA is critical in a process by which a CS gains access to a representation of the current incentive value of the US, updated by subsequent experience.

Everitt and his colleagues have demonstrated experimentally the capacity of a Pavlovian CS to support the development of new instrumental behaviour (Taylor and Robbins, 1986; Everitt et al., 1999). In their conditioned reinforcement paradigm, rats

learn to lever press for presentations of a Pavlovian CS that has already been endowed with the incentive salience of food. The ability of a Pavlovian CS to serve as a conditioned reinforcer depends on the transfer of information from the BLA to the NAc core. Accordingly, rats with bilateral lesions of the BLA or the NAc core cannot acquire this task (Burns et al., 1993; Whitelaw et al., 1996). Furthermore, the impact of conditioned reinforcers on conditioned responding can be enhanced by increases in DA transmission in the NAc. Lesion studies suggest that an intact CeN and DA transmission in the shell region of the NAc is necessary for the rate-enhancing effects of intra-NAc infusions of amphetamine on responding for conditioned reinforcers (Taylor and Robbins, 1986; Robledo et al., 1996; Parkinson et al., 1999).

The studies conducted by Everitt and his colleagues (Everitt et al., 1999; Everitt et al., 2000) described above indicate that modulation of DA transmission in the NAc by the CeN is important in both Pavlovian conditioning and conditioned reinforcement.

As mentioned at the outset of this thesis, the mesotelencephalic DA system has a primary and pervasive role in mediating incentive-motivated behaviours. Consequently, it is subject to modulation by numerous neural systems that influence behavioral output. There are data indicating that the amygdala is involved in certain aspects of the assessing the hedonic value of the sensory properties of food, but the amygdala may also provide modulatory influences on DA function, and thus, facilitate behavioural responses to incentive stimuli (Everitt et al., 1999; Parkinson et al., 2000c). In this regard, the amygdala may be considered to be an integral component of the three-step incentive motivation theory proposed by Berridge and Valenstein (1991). The amygdala appears to be related to the second step in which a hedonic experience is associated with sensory

stimuli to form an incentive. Thus, by interacting with the DA system, the amygdala may have the capacity to direct the attribution of incentive salience to the appropriate incentive stimulus in the environment. The remainder of this section introduces the concept sensory-specific satiety. This paradigm offers a unique opportunity to test the proposed relationship between the amygdala, DA transmission and the regulation of food intake by the sensory incentive properties of food.

Sensory-specific satiety

With few exceptions, most animals cannot obtain all the essential nutrients they require from one type of food. There exists, therefore, a natural tendency for animals and humans to prefer a varied diet. If a choice of several foods is available within a single meal (often referred to as a "cafeteria diet"), a considerably larger amount will be consumed than when only one type of food is offered (Rolls et al., 1981b; Treit et al., 1983; Louis-Sylvestre et al., 1984; Rogers and Blundell, 1984). Similarly, if an animal is given the opportunity to eat a different food in each of several meals, it will eat significantly more than if the same food is offered in every meal. Le Magnen (1956) demonstrated that varying just the flavour (i.e., the smell and taste) of one basic food was sufficient to increase food intake. He allowed rats to eat food with one flavour for 30 min and then continued to replace the dish with another flavour for 30 min, for a total of four flavours. He found that the rats ate two to three times more than when given a single flavour for all four meals. This effect can be obtained with foods that contain no nutritive value or can be based on foods that differ for odour, taste, or even colour alone (Rolls et al., 1981a; Rolls and Rolls, 1982; Rolls and Rolls, 1997). As sensory factors have a dominant effect over metabolic factors in these procedures, the effect is referred to as

sensory-specific satiety and is defined as the selective rejection of a food eaten to satiety and the acceptance and consumption of another (novel) food differing in sensory properties in a satiated state. In addition to rats (Le Magnen, 1956; Berridge, 1991; Balleine and Dickinson, 1998), this phenomenon has been described in other species including primates (Rolls et al., 1986) and humans (Rolls et al., 1983; Johnson and Vickers, 1992; Hetherington, 1996).

Sensory-specific satiety emphasizes the role of cephalic sensory signals in initiating food intake but it is also proposed to be a mechanism by which a meal can be terminated (Hetherington, 1996). In human subjects, ratings of the pleasantness of a food decrease as the food is eaten to satiety (Johnson and Vickers, 1992). In one study, subjects were asked to give a reason for ending the meal (Hetherington, 1996) and individuals who gave sensory fatigue ("I got tired of eating that food") as the answer consumed significantly fewer calories in the meal than those reporting gastric fullness ("I felt full"). Thus, the finding that sensory fatigue (or a decrease in hedonic "liking") is associated with decreased consumption of a given food suggests that the development of sensory-specific satiety may precede the development of gastric fullness and thereby serve as a more sensitive signal for ending a meal (Hetherington, 1996).

Mechanisms that underlie the expression of sensory-specific satiety are complex, involving the interaction of cephalic sensory and physiological satiety factors (Rolls et al., 1983; Johnson and Vickers, 1992; Vandewater and Vickers, 1996; Porrini et al., 1997). As Hetherington (1996) emphasizes, the "appraisal of the orosensory features of the food as positive (i.e., pleasant) is not an absolute property of the food, but rather depends upon energy status, previous experience of eating that food, and the hedonic

response to the orosensory features of the food as it is perceived by the organism". Thus, this phenomenon may also involve neural mechanisms of food reward (Berridge, 1991; Rolls, 1999), in which satiety modulates the incentive value of the sensory properties of food.

Neural substrates of sensory-specific satiety in the primate

Neural pathways involved in sensory-specific satiety have been studied extensively in primates by Rolls and his colleagues (Rolls et al., 1986; Rolls, 1999). Based on electrophysiological recordings of neurons in the taste pathway, as well as key cortical and subcortical regions involved in motivated behaviours, Rolls (1999) has argued that neurons critical for the computation of sensory-specific satiety in the primate are located in the orbitofrontal cortex. Accordingly, he argues that after many stages of information processing, the taste system interfaces with motivation systems in the orbitofrontal cortex. These neurons are not only finely tuned in their response to specific tastes, but are also affected by internal satiety signals, including gastric distention and glucose utilization. As a result of such modulation, these neurons decrease their firing rates in response to tasting a specific palatable food that has been eaten to satiety while retaining the capacity to increase their firing rates when the monkey consumes a different food (Critchley and Rolls, 1996). This phenomenon cannot be accounted for by adaptation of taste receptors or indeed of any neuron in the taste pathway, from the nucleus of the solitary tract (NST) to the primary taste cortex. Thus, Rolls concludes that neuronal activity, first in the primate orbitofrontal cortex and subsequently in the lateral hypothalamus, closely mirrors a decrease in the hedonic value of a food eaten to satiety, as well as the unchanged incentive value of a novel food (Rolls et al., 1986; Rolls, 1999).

He further suggests that efferent projections from these brain regions to the ventral striatum may convey information about the hedonic value of specific taste stimuli to behavioural control systems (Rolls, 1999).

Neural substrates of sensory-specific satiety in the rat

In the rat, sensory-specific satiety has been demonstrated in a variety of paradigms (Le Magnen, 1956; Berridge, 1991; Balleine and Dickinson, 1998; Ahn and Phillips, 1998). However, neural pathways mediating the basic phenomenon have not been examined as thoroughly as in the primate. Although the neural circuitry involved in “computing” sensory-specific satiety in rats undoubtedly differs from primates (Rolls, 1999), the convergence of external sensory information (olfactory and gustatory being the most important for the rat) and internal motivational states in the rat forebrain must be a critical determinant of sensory-specific satiety, as they are in the primate brain.

The initial convergence of cephalic sensory and internal satiety signals occurs at the level of the brainstem, in the NST. The rostral NST receives gustatory information from taste receptors via the facial, glossopharyngeal and vagus nerves (Carlson, 1991; Zeigler, 1994). The caudal NST receives satiety signals from the gastrointestinal system (e.g., gastric distention and gut peptides such as cholecystokinin) through the vagus nerve or by activation of peptide receptors in the area postrema just adjacent to the NST (Zeigler, 1994; Mei, 1994; Woods et al., 1998; Inui, 1999). The NST transfers the processing of gustatory and visceral information to the parabrachial pontine nucleus. From this point, sensory processing diverges into cortical and subcortical pathways. The cortical pathway continues through sensory thalamic areas to the primary gustatory (insular) cortex, whereas the subcortical pathway projects to reward-related regions in the

forebrain, such as the amygdala and the hypothalamus (Norgren, 1995). In addition to these inputs, the amygdala also receives olfactory information from the main and accessory olfactory systems (Shiple et al., 1995). The CeN is notable in that it not only receives the majority of the gustatory and visceral information directly from the brainstem, but additionally receives highly specific and processed taste information from the insular taste cortex (Norgren, 1995) and olfactory (or pheromonal/flavour) information from olfactory components of the amygdala (Swanson and Petrovich, 1998). Thus, the amygdala, and in particular the CeN, may play a pivotal role in "computing" sensory-specific satiety in the rat in a manner similar to the orbitofrontal cortex in the primate. On this point, it is important to note that Rolls (1999) has argued that many motivational functions of the amygdala have been subsumed by the primate orbitofrontal cortex during evolution, and that this evolutionary shift allows more flexible representation of changes in reward value.

An equally important aspect of the sensory-specific satiety mechanism that has received less attention involves the interface between the "computation" of sensory-specific satiety with incentive motivational systems. Given the proposed role of the mesotelencephalic DA system as a neural substrate of incentive motivation (Berridge, 1991; Blackburn et al., 1992; Robbins and Everitt, 1996; Salamone et al., 1997), a functional interaction between the amygdala and the DA system may be especially important in the expression of sensory-specific satiety.

Objectives of experiments

Experiments in this thesis utilize a feeding paradigm based on the sensory-specific satiety effect to examine the role of the mesotelencephalic DA system in the control of food intake. In this paradigm, rats are given an opportunity to develop satiety for a particular food during an initial meal and then are subsequently presented with the same food again or a different food during a second meal. Differences in the amount of intake of the same food and the different food during the second meal are assumed to be related to the sensory properties of the familiar and novel foods. *In vivo* microdialysis and high-pressure liquid chromatography are used to monitor changes in extracellular levels of DA efflux in two telencephalic DA terminals, the NAc and mPFC, during the anticipatory and consummatory phases of the two meals. Conducting microdialysis experiments during a two-meal test of sensory-specific satiety offers a unique opportunity to examine the role of DA in more complex and subtle aspects of the regulation of food intake that may not be revealed in a single meal test with only one type of food present.

Numerous *in vivo* monitoring studies have observed increases in DA efflux in the NAc and mPFC during the appetitive and consummatory phases of feeding behaviour (Wilson et al., 1995; Bassareo and Di Chiara, 1997; Ahn and Phillips, 1998). However, there is little data on the neurochemical correlates of satiety and reinitiation of food intake. Thus, the purpose of Experiment I is to describe the changes in DA efflux in the NAc and mPFC during the different phases of a two-meal sensory-specific satiety test. Given the proposal that the DA system mediates the attribution of salience to incentive stimuli (Berridge and Robinson, 1998), it is hypothesized that DA efflux in the NAc and mPFC will increase when food is perceived as having high incentive value (i.e., when the

animal is hungry or when the food is novel) and remain unchanged when food has low incentive value (i.e., when the animal is satiated and the food is familiar).

Studies utilizing lesion and pharmacological techniques suggest that the CeN and the BLA are functionally related to DA transmission in the NAc, during the acquisition and performance of appetitive conditioning tasks (Everitt et al., 1999). However, it has yet to be demonstrated whether the CeN and BLA actually modulate extracellular levels of DA in the NAc and mPFC, and if so, whether the two subregions provide differential influences to each terminal region. To address these unknowns, Experiment II compares the effect of transient inactivations of the CeN and BLA on DA efflux in the NAc and the mPFC. The lesions are produced by reverse-dialyzing lidocaine, a sodium channel blocker, into the amygdalar subregions. Given that the CeN sends GABAergic projections to midbrain DA neurons and the BLA sends glutamatergic projections to the NAc and mPFC, it is hypothesized that each amygdalar subregion will have differential influences on basal levels of DA efflux. Experiment II also examines whether the functional relationships of the CeN and BLA with the DA system, which has been shown to be important for different forms of appetitive associative learning (e.g., Pavlovian conditioning and conditioned reinforcement), may have dissociable effects on a simpler form of feeding behaviour, namely the approach and consumption of a single palatable food. The results of this experiment will serve to indicate the plausibility of investigating the functional relevance of amygdalar-DA transmission relationships in mediating sensory-specific satiety in a third experiment.

Thus, Experiment III examines the effect of transient CeN and BLA lesions on sensory-specific satiety and associated changes in DA efflux in the NAc and mPFC. The

CeN is proposed to be of particular importance in the expression of sensory-specific satiety, as neural processes within the CeN change external sensory stimulation and internal motivational status into neural activity that has the potential to guide behaviour. It is hypothesized that lesions of the CeN will not only disrupt sensory-specific satiety, but also dysregulate DA efflux correlated with the different phases of sensory-specific satiety (e.g., the initiation and reinitiation of food intake).

Food restriction has been shown to induce stress responses, including the release of glucocorticoid stress hormones (Broocks et al., 1990), as well as to increase food consumption in humans and animals under subsequent *ad libitum* conditions (Specker et al., 1994; Hetherington, 1996). In particular, repeated cycles of food restriction and binge feeding have been linked to intense hyperphagia in rats (Hagan and Moss, 1997). Experiment IV examines the effect of this feeding cycle on sensory-specific satiety. It is hypothesized that restriction-binge cycles may lead to stress-induced sensitized responses to food (i.e., hyperphagia), and thereby disrupt the regulation of food intake by sensory and satiety factors. The results of this experiment have implications for understanding how stress may alter behaviour motivated by food-related incentive stimuli through glucocorticoid sensitization of DA function.

GENERAL METHODS

All experimental protocols were approved by the Committee on Animal Care, University of British Columbia and conducted in compliance with guidelines provided by the Canadian Council of Animal Care. All efforts were made to minimize any stress or pain animals might endure in the experiments. Within-subject designs were employed to minimize the number of animals included in the experiments.

Subjects

Long-Evans male rats (Charles River Canada, St. Constant, Quebec), weighing 250-275 g upon arrival, were housed five to six per hanging wire cage in a colony room maintained at ~21 °C with a 12 hr/12 hr light-dark cycle (light on at 06:00 hr). Food (Rat Diet 5012; PMI Feeds Inc., Delta, B.C.) and water were available *ad libitum*.

Following surgery, rats were housed individually in plastic bins with corncob bedding and allowed a minimum of one week for recovery. Rats were then placed on a restricted feeding schedule, which maintained their body weight between 85-90% of free-feeding weight. Animals were weighed and then fed (20-25 g) daily with water available *ad libitum*. Novel objects (e.g., sterilized egg and milk cartons) were introduced into home bins weekly to promote exploratory and play behaviour. Training sessions and experiments took place from 08:00 to 14:00 hr. Figure 1 depicts the time-line along which surgery, training and testing occurred within sensory-specific satiety and microdialysis experiments.

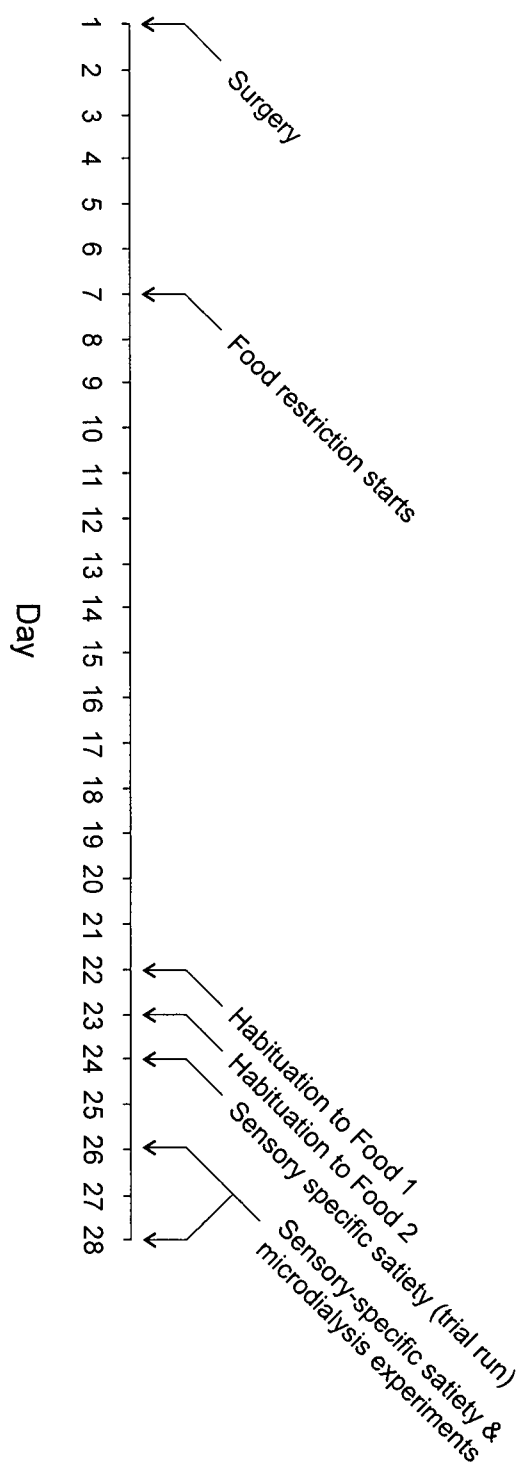


Figure 1. Time-line of events in sensory-specific satiety experiments.

Stereotaxic surgery

Surgery was performed on animals that weighed $\sim 300 \pm 20$ g. Animals were anaesthetized with xylazine (7 mg/kg, *intra peritoneal*) and ketamine hydrochloride (100 mg/kg, *intra peritoneal*) and placed in a stereotaxic frame in a flat-skull position. Nitric acid-passivated stainless steel guide cannulae (10 gauge, 15 mm), for inserting microdialysis probes to target structures, were implanted bilaterally as follows. Cannulae tips were lowered to 1 mm below dura, directly over the NAc (+1.7 mm AP and ± 1.1 mm ML from bregma, -8.0 mm DV from dura) or the mPFC (+3.0 mm AP and ± 0.6 mm ML from bregma, -4.5 mm DV from dura). Subjects in Experiments III and IV were implanted bilaterally with additional guide cannulae, for insertion of reverse-dialysis probes, over the CeN (-2.3 mm AP and ± 4.0 mm ML from bregma, -7.7 mm DV from dura) or BLA (-3.0 mm AP and ± 5.0 mm ML from bregma, -8.4 mm DV from dura).

Apparatus, materials and sensory-specific satiety protocol

A sensory-specific satiety pattern of consumption has been proposed to represent the interaction of physiological and sensory inputs. To demonstrate satiety based on the sensory properties of food, two meals consisting of two foods differing in sensory properties were presented in the different food condition or the same food for both meals were presented in the same food condition. Differences in the amount of intake during the second meal of each condition were assumed to be related to the sensory properties of the novel and familiar foods.

The paradigm was also designed to accommodate DA microdialysis studies and thus, included some specific features. First, a recess was imposed between the two meals

to allow sufficient time for DA efflux to return to baseline levels before presentation of a second meal. This was necessary to allow comparison of relative changes in DA levels during the two meals from an initial baseline control value determined at the start of the experiment. Second, each meal was divided into an appetitive (i.e., food presented behind a screen) and consummatory period to test hypotheses of DA function in the two periods.

All experiments took place in a plexiglass chamber divided by a removable perforated plexiglass screen and fitted on the top with a two-channel liquid swivel (Instech Laboratories Inc., Plymouth Meeting, PA). The liquid swivel allowed continuous perfusion of microdialysis and reverse-dialysis probes during behavioural experiments in freely moving animals. At the beginning of a sensory-specific satiety testing session, animals were placed into one compartment of the chamber (16 cm x 38 cm x 38 cm). Removing the screen allowed access into the adjacent compartment (26 cm x 38 cm x 38 cm) where the food bin was located (Figure 2).

Foods used in the sensory-specific satiety experiments were Froot Loops, a sweet fruit-flavoured cereal (Kellogg Canada Inc., London, Ontario) and Onion Rings, a salty-savoury onion-flavoured chip (Nong Shim Co. Ltd., Seoul, Korea). These foods were chosen based on a pilot study that showed that hungry rats, given a choice of a variety of foods, preferred to consume the Loops and Rings. The two foods were similar in caloric content (4 Cal/g of Froot Loops and 5 Cal/g of Onion Rings), but each was readily detectable and identifiable by its odour and taste properties. As such, they were readily distinguishable at a distance of 1-2 m by human observers and most certainly by rats at less than 30 cm. For each sensory-specific satiety experiment, Loops and Rings were

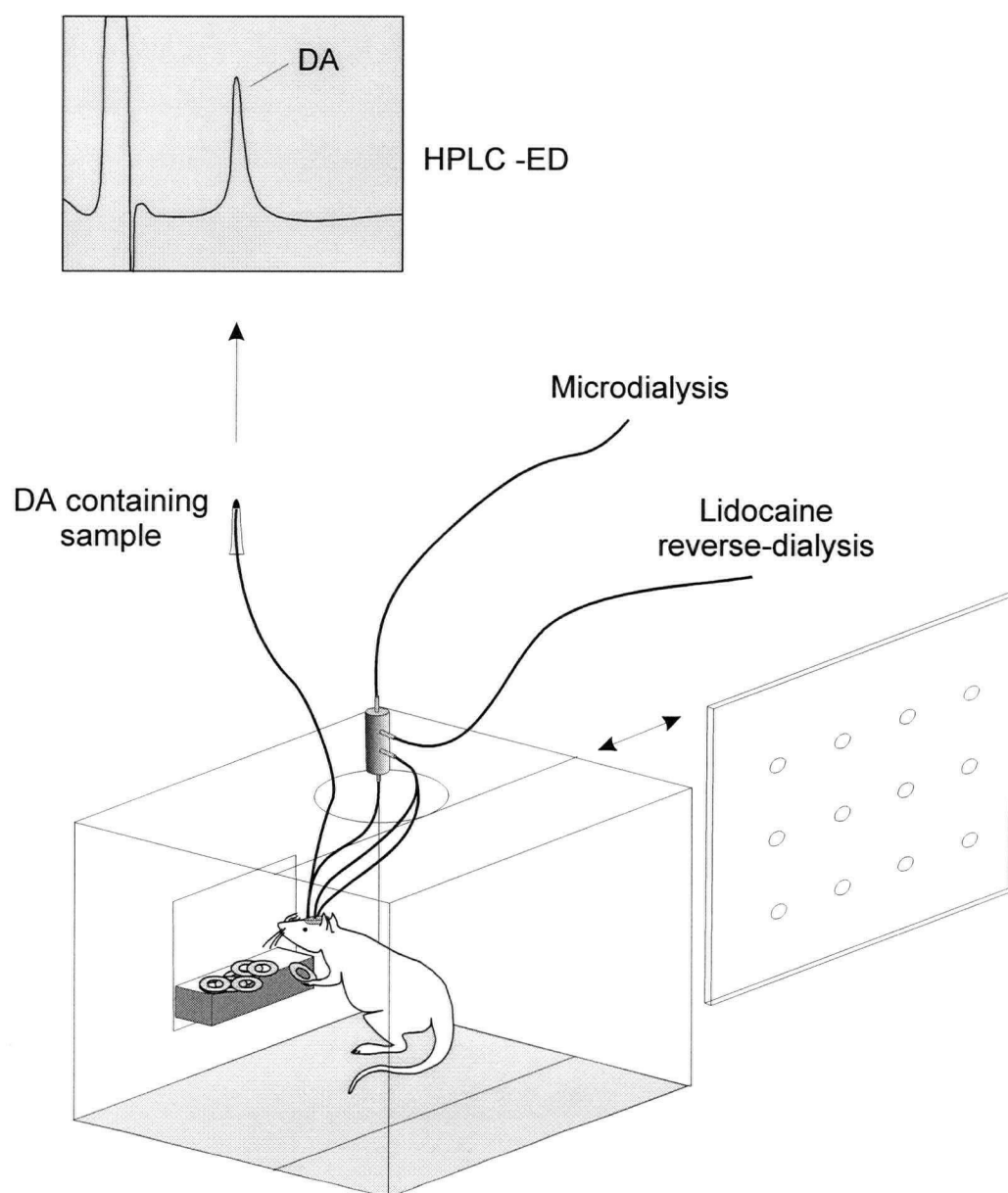


Figure 2. Schematic of materials and techniques used in experiments. The testing chamber is divided into two areas by a transparent removable screen. Sliding the screen out allows an animal to enter the side with the food bin. A dual-channel liquid swivel allows microdialysis and reverse-dialysis perfusion lines to be attached to freely-moving animals. HPLC-ED is used to analyze microdialysis samples.

designated as Foods 1 and 2, with the designations being counterbalanced between animals.

Two weeks into a restricted feeding schedule, rats were given an opportunity to habituate to the testing chamber and to overcome neophobia to the two foods. They were allowed 20 min in the testing chamber to eat Food 1 on Day 22 and Food 2 on Day 23 (Figure 1). On Day 24, rats were exposed to a trial run of a sensory-specific satiety procedure that consisted of two meals separated by a 40 min recess during which no food was available. At the start of the first meal, 3 g of Food 1 was presented in the food bin behind a perforated screen for 10 min (the appetitive period). The screen was then removed and animals had access to the food for 10 min, after which the remaining food, if any, was replaced by another 3 g of the same food every 10 min for a total of 40 min (the consummatory period). The recess was followed by presentation of a second meal, in the same manner, of Food 1 (same food condition) for half of the animals and Food 2 (different food condition) for the other half of the animals.

Experiments described in this thesis employed a sensory-specific satiety protocol that allowed simultaneous *in vivo* monitoring of correlated changes in DA efflux. In order to minimize individual differences in neurochemical responses, sensory-specific satiety was assessed using within-subject comparisons. As such, subjects were tested in the same food condition (Food 1 for both meals separated by a 40 min recess) and different food condition (Food 1 for the first meal and Food 2 for the second meal, separated by a 40 min recess) in the manner described for the trial run on Day 24 (Figure 1). Tests were conducted on Days 26 and 28, with the order of test conditions counterbalanced within each group of rats.

Microdialysis and high-pressure liquid chromatography (Experiments I, II and III)

The use of *in vivo* microdialysis and high-pressure liquid chromatography with electrochemical detection (HPLC-ED) allowed changes in extracellular concentrations of DA to be monitored during behavioural experiments. In principle, microdialysis mimics a capillary blood vessel (Ungerstedt and Hallstrom, 1987): a physiological solution is continuously perfused through fine tubing with a semi-permeable dialysis membrane tip (Figure 3); any molecule within the molecular weight cut-off range of the dialysis membrane is able to diffuse down the concentration gradient into the probe and is carried out by the flow within the tubing and collected for analysis. DA in each sample is then separated from other chemical species by high-pressure liquid chromatography (HPLC) and quantified by electrochemical detection (ED). This combined analytical approach provides better selectivity and sensitivity over other *in vivo* monitoring techniques such as *in vivo* electrochemical methods (i.e., any molecule that can cross the membrane can be separated and quantified but electrochemical methods require that the molecule be electroactive and molecules with similar oxidizing potentials cannot be distinguished by the signals they provide). However, microdialysis is limited in temporal and spatial resolution (i.e., microdialysis is limited by the time needed for collection of samples and its probes sample from a bigger area than electrochemical probes).

Microdialysis probes were concentric in design with silica inlet/outlet lines (Figure 3). The active surface consisted of a semi-permeable membrane 2mm in length (340 μm od, 65 000 MW cut-off, Filtral 12, Hospal). A stainless steel coil tethered to a liquid swivel mounted on top of the testing chambers acted as a protective sheath around the silica inlet/outlet. Probes were flushed continuously at 1 $\mu\text{l}/\text{min}$ with a modified

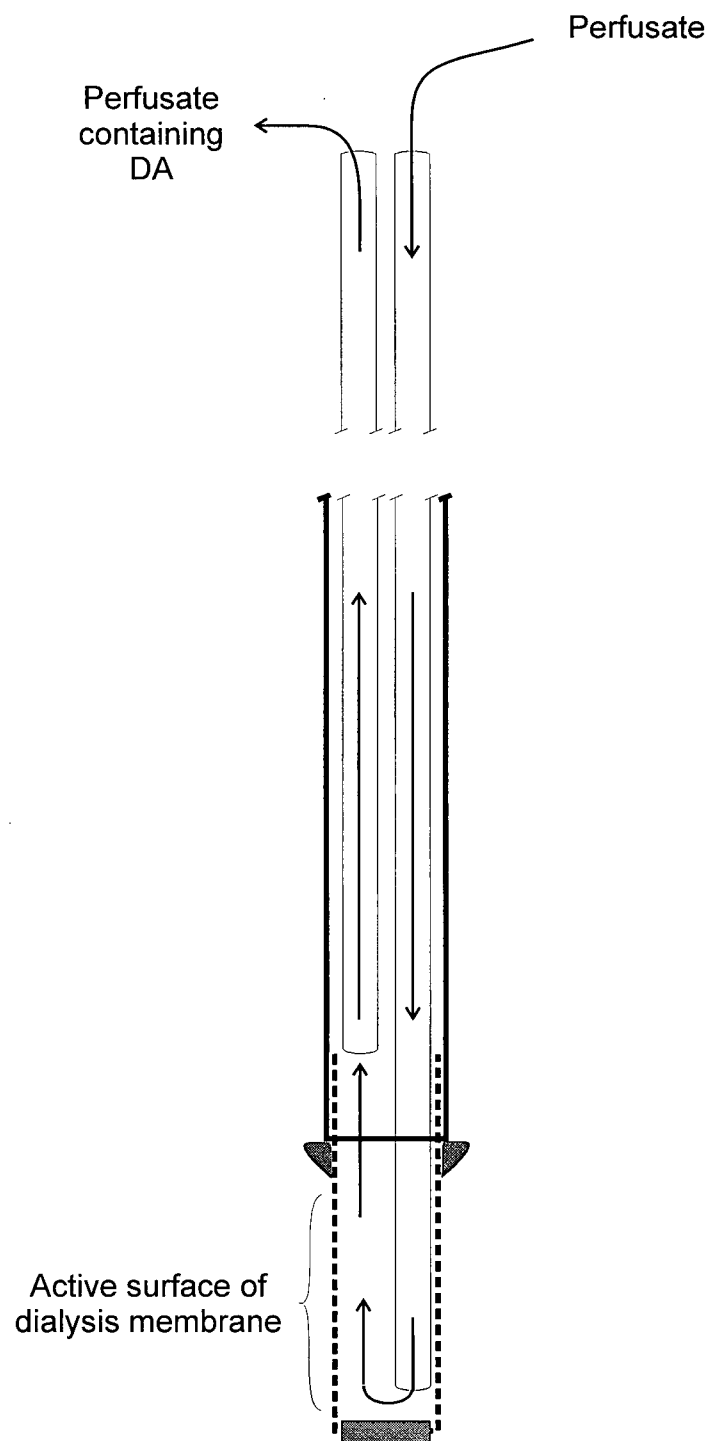


Figure 3. Diagram of a microdialysis probe indicating the direction of perfusate flow.

Ringer's solution (here on referred to as "perfusate"; 10 mM sodium phosphate, 1.2 mM CaCl_2 , 3.0 mM KCl, 1.0 mM MgCl_2 , 147.0 mM NaCl, pH 7.4) using a 2.5 ml gastight syringe (Hamilton, Reno, NV) and a syringe pump (model 22, Harvard Apparatus, South Natick, MA). Typical *in vitro* probe recoveries conducted at room temperature were $18 \pm 1\%$. Probes were implanted into target sites (mPFC or NAc) through the guide cannulae 14-16 hr prior to conducting experiments. Probe collars were used to secure the probes at the appropriate target depth. Implanted animals remained overnight in the test chamber with their daily food ration and free access to water. This allowed a sufficient period of time for the probe to come into equilibrium with the local extracellular environment and for subjects to habituate to being tethered to the liquid swivel. Dialysates were collected from the NAc and mPFC at 10 min intervals (10 μl).

Two different HPLC methods were employed to analyze microdialysis samples from the mPFC and NAc. As reported below in Experiments I, II and III, the observed concentration of DA in samples from the NAc is at least fifteen times higher than in samples from the mPFC (this ratio is based on concentration values from the NAc and mPFC uncorrected for the efficacy of the probe). This relative difference in concentration required an HPLC assay that was capable of detecting very small amounts of DA (i.e., in the picogram range) to analyze microdialysates from the mPFC. Each HPLC system was separately calibrated using standard DA solutions to convert chromatographic peak parameters (e.g., area under the curve, peak height) into concentration values of DA.

Analysis of DA content in mPFC dialysates involved separation by reverse-phase high-pressure liquid chromatography and quantification by electrochemical detection

(ED). The system consisted of a GBC LC1120 HPLC pump (Victoria, Australia), a Scientific Systems Inc pulse damper (316 Stainless Steel Model; State College, PA), a Rheodyne manual injector (20 μ l injection loop; Model 9125), a Princeton SPHER C₁₈ column (2 x 100 mm, 60A 3 μ m particles; Cranbury, NJ), an Antec Leyden Links system and an Intro EC detector with a VT-03 electrochemical flowcell ($V_{\text{applied}} = +0.7$ V; Leyden, The Netherlands). The mobile phase, a 67 mM sodium acetate buffer containing 2.15 mM sodium octyl sulfate and 0.07 mM EDTA (pH 3.5, 12% v/v methanol), flowed through the system at 0.2 ml/min. Waters Maxima software was used to collect and analyze chromatographic data.

Analysis of DA content in NAc dialysates involved separation by reverse phase HPLC and quantification by ED. The system consisted of an ESA HPLC pump (Model 582; Chelmsford, MA), a Scientific Systems Inc pulse damper (316 Stainless Steel Model; State College, PA), a Rheodyne manual injector (20 μ l injection loop; Model 9125; Rohnert Park, CA), a Beckman Ultrasphere column (46 x 150 mm, 5 μ m particles; Fullerton, CA) and an ESA Coulochem II EC detector (Electrode 1 $V_{\text{applied}} = +450$ mV, electrode 2 $V_{\text{applied}} = -300$ mV, guard cell $V_{\text{applied}} = -450$ mV). The mobile phase, a 73 mM sodium acetate buffer containing 0.65 mM sodium octyl sulfate and 0.03 mM EDTA (pH 3.5, 12% v/v methanol), flowed through the system at 1.2 ml/min. A dual channel Kipp and Zonen BD41 chart recorder was used to record the chromatographic data.

Reverse-dialysis (Experiments II and III)

Lidocaine is a sodium channel blocker often used in functional studies to block neuronal activity within a specified nucleus or region of the brain (Floresco et al., 1997; Carrive et al., 2000). Unlike other temporary lesion methods (such as tetrodotoxin,

another sodium channel blocker), lidocaine's anaesthetic effect is much shorter lasting, has a smaller radius of diffusion and produces less neuronal damage (Tehovnik and Sommer, 1997; Boehnke and Rasmusson, 2001). Experiments II and III used reverse-dialysis rather than microinfusion to administer lidocaine because of the length of the inactivation required to assess basal and food-evoked DA efflux. Moreover, this novel application of the reverse-dialysis method allowed for a much more reliable, accurate comparison of the effects of inactivation of the CeN versus BLA. It allowed 1) additional control over the radius of diffusion, thereby ensuring better localization of the inactivation within either the CeN or BLA; 2) less disturbance of the immediate environment due to mechanical pressure of the injection volume; and 3) active removal of lidocaine, facilitating faster recovery of function (for parametric studies see Boehnke and Rasmusson, 2001).

Reverse-dialysis probes were a modified version of the microdialysis probes described above (Figure 3). The length of the active membrane surface was adjusted to maximize the dorsoventral extent of each structure: 1.2 mm for the CeN and 1.8 mm for the BLA. These probes were flushed with perfusate at 1 μ l/min in the same manner as microdialysis probes. These probes were also implanted at the same time as microdialysis probes. For the period of lidocaine reverse-dialysis, probes were flushed at 1 μ l/min with perfusate containing dissolved lidocaine hydrochloride (20 mg/ml; Research Biochemicals International, Natick, MA).

Histology

Animals were anesthetized with chloral hydrate and perfused intracardiacally with 0.9% NaCl and then 3.7% paraformaldehyde. Brains were removed and stored in formalin with 20% w/v sucrose for a few days, sliced into 50 μ m coronal sections and then stained with cresyl violet. Placements were located using a light microscope and an atlas of the rat brain (Paxinos and Watson, 1997).

Data analyses

For the purposes of statistical analyses as well as graphical representation, neurochemical data were normalized to fluctuate around a baseline value. This value (calculated by averaging the concentration of DA in the three samples preceding the final baseline sample) is represented as 0% change from baseline on all figures showing neurochemical data.

All data were analyzed using repeated measures ANOVA followed, when appropriate, by tests of simple main effects. In the latter cases, the significance level for each test was adjusted according to the total number of tests conducted in order to maintain a family-wise rate of Type I error at $p < 0.05$. Neurochemical data were further analyzed using Dunnett's method of multiple comparisons, as this test allows comparison of a baseline value (control mean) against subsequent changes in DA efflux. Behavioural data (food intake and body weight) were further analyzed by the Dunn method of multiple comparisons, as this test uses family-wise error rates for simple and complex contrasts. The Huynh-Feldt correction for nonsphericity was applied to the degrees of

freedom for all within-subject analyses. Statistical analyses were performed using Systat or SPSS statistical packages.

EXPERIMENT I:

**DOPAMINERGIC CORRELATES OF SENSORY-SPECIFIC SATIETY IN
THE MEDIAL PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS**

(as published in the Journal of Neuroscience 19:RC29,1-6, 1999)

Introduction

Sensory-specific satiety is a critical factor in the selection of a varied diet by animals (Rolls et al., 1986; Berridge, 1991; Warwick and Weingarten, 1994) and humans (Rolls et al., 1983; Vandewater and Vickers, 1996; Rolls and Rolls, 1997). When hungry animals are allowed to satiate on one palatable food, they continue to display satiety when given that same food again, whereas those given a novel food with different sensory properties consume a second meal (Rolls et al., 1986; Berridge, 1991).

A neural mechanism underlying sensory-specific satiety for taste in primates has been proposed based on extensive data from electrophysiological recordings of neurons in the taste pathway as well as key cortical and subcortical regions involved in motivated behaviours (Rolls, 1984; Rolls et al., 1986; Rolls, 1989). Rolls (1999) argues that after many stages of information processing, the taste system interfaces with motivation systems in the orbitofrontal cortex. Neurons in the orbitofrontal cortex decrease their firing rates in response to tasting a specific palatable food that has been eaten to satiety but increase their firing rates when the monkey consumes a different food (Rolls et al., 1986). Furthermore, Rolls (1999) suggests that by communicating with the orbitofrontal cortex, regions involved in behavioural output like the ventral striatum can utilize the highly specific reward-related information to guide appropriate behavioural responses.

An equally important, but as yet undetermined, aspect of the sensory-specific satiety mechanism may involve the mesotelencephalic DA system. Its role as a neural substrate of incentive motivation is widely recognized (Blackburn et al., 1992; Kiyatkin, 1995; Robbins and Everitt, 1996; Salamone et al., 1997; Beninger and Miller, 1998; Di Chiara, 1998). In particular, DA activity is believed to mediate appetitive or approach behaviours triggered by incentive stimuli associated with rewards (Fibiger and Phillips, 1986; Berridge and Robinson, 1998). Berridge and Robinson (1998) maintain that phasic increases in DA activity endow immediate salience to biologically important stimuli in the environment ("wanting"), thus prompting animals to select and engage in appropriate patterns of behavioural responses. Consistent with this view, *in vivo* monitoring studies in rats have demonstrated that increases in DA efflux accompany both appetitive and consummatory phases of feeding in terminal regions of the DA system, such as the NAc (Phillips et al., 1993; Wilson et al., 1995) and mPFC (Cenci et al., 1992; Feenstra and Botterblom, 1996; Taber and Fibiger, 1997).

A recent report by Bassareo and Di Chiara (1997) emphasizes important differences in DA efflux in the NAc and mPFC in response to food reward and associated incentive stimuli. Specifically, when nondeprived rats eat a novel palatable food, there is a significant increase in DA efflux in both the NAc and mPFC. This effect is greatly attenuated in the NAc, when rats have had an opportunity to ingest the novel food a few hours earlier. Surprisingly, a similar attenuation effect on DA efflux was reported in rats that had consumed the novel food 48 hr earlier. Further differences in DA efflux have been reported within the NAc, in the core and shell regions (Bassareo and Di Chiara, 1999). In nondeprived rats, incentive stimuli increased DA efflux in the core but not in

the shell. Upon ingestion of the food, DA efflux was further increased in the core but no changes were observed in the shell. In contrast, incentive stimuli and consumption of food evoked a significant increase in DA efflux in the mPFC (Bassareo and Di Chiara, 1997). On the basis of these data, Di Chiara (1998) proposes that DA transmission in the NAc does not code for general motivational salience as hypothesized by Berridge and Robinson (1998), but instead reflects an associative learning process through which novel or motivationally-relevant stimuli (under food-deprivation conditions) gain the ability to elicit appetitive responses. Motivational salience is a property attributed to the mesocortical DA system.

This experiment sought to address three main issues by monitoring changes in dialysate DA levels from the rat mPFC and NAc during appetitive and consummatory phases of a sensory-specific satiety protocol. First, given that the mesotelencephalic DA system is involved in the initiation and maintenance of incentive-motivated behaviours (Blackburn et al., 1992) and that the NAc and mPFC are key sites for integration of information influencing behavioural responses, it was predicted that DA efflux in these regions would reflect changes in the incentive value of food as modulated by the deprivation state of the animal and the sensory properties of food. Second, specific features of this protocol addresses the question of whether prior opportunity to learn about the incentive value of food reward (i.e., incentive learning, Balleine and Dickinson, 1998) is a critical determinant of increased DA efflux in response to food-related incentive stimuli. Third, results may provide further information on the emerging differences and similarities of DA function in the mPFC and NAc during appetitive and consummatory phases of feeding.

Methods

Twenty-eight Long-Evans male rats were obtained, housed and cared for as described in the General Methods section. Procedures pertaining to surgery, microdialysis, sensory-specific satiety experiments as well as histological and statistical analyses were as described in the General Methods section.

Half of the rats were implanted bilaterally with guide cannulae over the mPFC and the other half over the NAc. Following surgery, rats were placed on a food restriction schedule. Two weeks later, rats were given three opportunities to habituate to the foods, the testing chamber and to being tethered by the steel coil.

Microdialysis experiments were conducted while rats were tested in the same food and different food sensory-specific satiety conditions (Figure 1). With respect to microdialysis, the left and right hemispheres were randomly assigned to the same food condition and the other hemisphere to the different food condition to equalize for any hemispheric differences. On each experiment day, dialysis samples were collected at 10 min intervals from the NAc or mPFC and immediately assayed for DA using HPLC-ED. Baseline conditions continued until four consecutive samples showed less than 5% fluctuation in DA content before the first meal was presented.

Following the last microdialysis session, brains were removed to verify placement of probes. Only data obtained from those animals with tracts in the prelimbic/infralimbic region of the mPFC or in the shell/core region of the NAc of both hemispheres were included in the statistical analyses.

Results

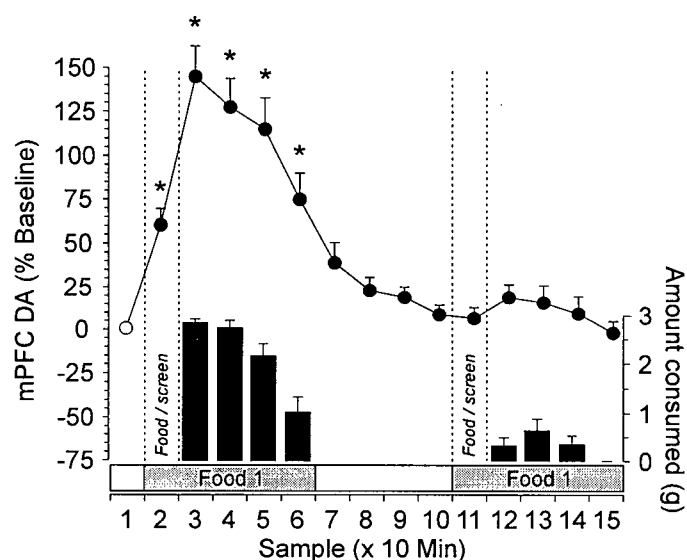
Sensory-specific satiety

A sensory-specific satiety pattern of feeding was observed in all subjects (Figures 4 and 5, bar graphs). Upon removal of the screen that prevented access to the food bin, subjects quickly approached and began eating the first meal (Food 1). The total amount consumed in this meal (7.4 ± 0.5 g in the same food condition, 7.9 ± 0.4 g in the different food condition) was not significantly affected by the type of food offered [$F_{(1,26)} = 1.154$; $p > 0.34$]. The gradual decrease in rate of consumption during the first meal indicated that animals were reaching satiety. During the second meal, animals consumed significantly more when Food 2 was offered than when Food 1 was offered ($p < 0.05$) with the maximal difference in intake occurring during the first 10 min (1.5 ± 0.2 g of Food 2 versus 0.2 ± 0.1 of Food 1).

Basal concentration of DA in microdialysates collected from the mPFC and NAc

The average concentration of DA (uncorrected for probe recovery) in the last three samples prior to presentation of food behind the screen was 0.18 ± 0.01 nM in the mPFC and 3.16 ± 0.19 nM in the NAc. These values likely reflect true basal concentrations of DA: first, animals were allowed an extended period of time (14-16 hr in the testing chamber following implantation of microdialysis probes) to habituate them to the testing chamber without the presence of food; and second, samples were collected for at least 60 min prior to presentation of food behind the screen during which there was a decreasing trend in DA levels which eventually stabilized prior to the three samples used to estimate basal values.

Same Food Condition



Different Food Condition

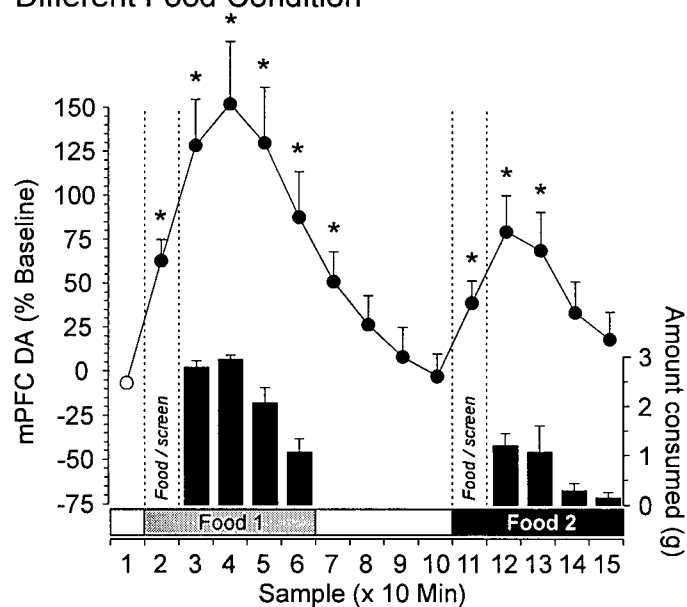
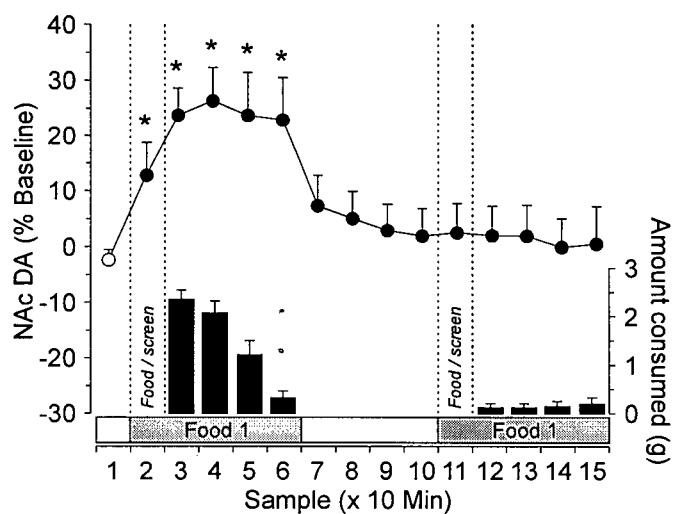


Figure 4. Dopaminergic correlates of sensory-specific satiety in the mPFC. Changes in DA efflux (line graph, left Y-axis) and amount of food consumed (bar graph, right Y-axis) are presented as mean+SEM per 10 min. Same food condition (n=14), the effect of presenting Food 1 during two consecutive meals. Different food condition (n=14), the effect of presenting Food 1 during the first meal and Food 2 during the second meal. Samples 2 and 11, highlighted by dashed lines, represent anticipatory periods during which access to food was prevented by a screen. Samples 3-6 and 12-15 represent consummatory periods during which rats had access to food. The last baseline value (Sample 1) was used as the control mean in Dunnett's method of multiple comparisons (* $p < 0.05$).

Same Food Condition



Different Food Condition

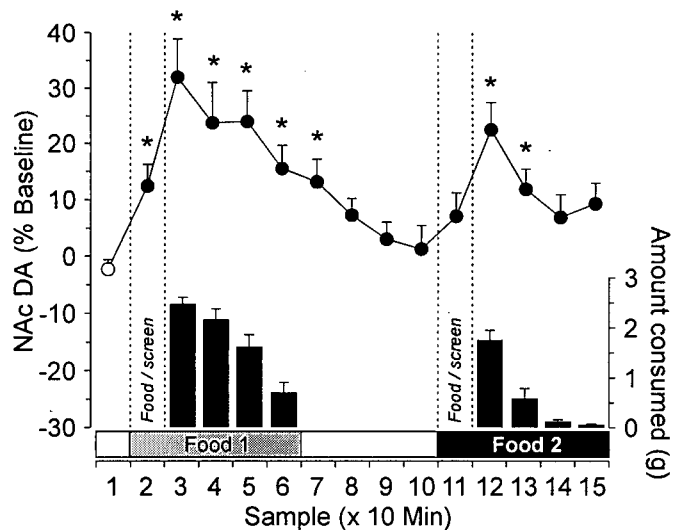


Figure 5. Dopaminergic correlates of sensory-specific satiety in the NAc. Shown are changes in DA efflux (line graph, left Y-axis) and amount of food consumed (bar graph, right Y-axis) per 10 min during the same (n=14) and different (n=14) food conditions. See Figure 4 legend for further explanation.

Changes in DA efflux in the mPFC

There was a significant main effect of time on DA efflux in the mPFC in the same [$F_{(14,182)}=22.656$; $p<0.001$] and different food [$F_{(14,182)}=13.808$; $p<0.001$] conditions. The pattern of changes in DA efflux closely modeled the sensory-specific satiety pattern of feeding. When the first meal consisting of Food 1 was presented behind the screen, animals began scratching at or sniffing through the screen as well as locomoting around the testing chamber. These appetitive behaviours were accompanied by a significant rise in DA efflux (+60% in the same food condition and +63% in the different food condition, $p<0.05$; Figure 4, line graphs). When the screen was removed and animals began to eat, there was a further enhancement in DA efflux in the mPFC, reaching values greater than double the basal level (+145% in the same food condition; +154% in the different food condition, $p<0.05$) within the first 20 min. Subsequently, as animals developed satiety for Food 1, a gradual decline in DA levels mirrored the decreasing rate of food consumption. Reinsertion of the screen into the chamber and removal of any remaining food marked the start of a 40 min recess. During this time animals settled down in a corner of the chamber and engaged in periodic bouts of grooming while DA efflux returned to baseline levels.

Presentation of Food 1 at the start of the second meal (same food condition) did not lead to significant levels of anticipatory activity; animals remained inactive as they were during the recess and DA efflux remained at baseline values (Figure 4, top panel, line graph). Following the removal of the screen, there was a delayed approach response and occasional sampling of the food but no significant change in DA efflux. In marked contrast, presentation of Food 2 behind the screen during the second meal (different food

condition) led to increased appetitive behavioural responses and a significant increase in DA efflux in the mPFC (+39%, $p < 0.05$; Figure 4, bottom panel, line graph). When animals ate the novel food, DA levels continued to climb to +80% ($p < 0.05$) before descending to baseline levels again, as animals reached satiety for Food 2.

Changes in DA efflux in the NAc

There was a significant main effect of time on DA efflux in the NAc in the same [$F_{(14,182)}=2.601$; $p=0.002$] and different food [$F_{(14,182)}=9.193$; $p < 0.001$] conditions. The pattern of DA efflux in the NAc closely mirrored the pattern observed in the mPFC, with two key exceptions. Similar to the DA response observed in the mPFC, presentation of Food 1 during the first meal resulted in a significant increase in DA efflux during the appetitive phase (+13% in the same food condition and +12% in the different food condition, $p < 0.05$; Figure 5, line graphs) which continued to rise during the consummatory phase (+26% in the same food condition and +32% in the different food condition within the first 20 min, $p < 0.05$). As in the mPFC, presentation of Food 1 during the second meal was not accompanied by any changes in NAc DA efflux (Figure 5, top panel, line graph). However, in contrast to the large DA efflux observed in the mPFC during the appetitive period of the second meal, presentation of a different palatable food behind the screen did not elicit a similar robust anticipatory DA response in the NAc (Figure 5, bottom panel, line graph). DA efflux in the NAc showed an insignificant increase during the appetitive phase (+7%) and a small but significant increase during the consummatory phase (+23%, $p < 0.05$). It was noted that although the baseline concentration of DA in the NAc was approximately eighteen times higher in the

NAc than in the mPFC (see above), the magnitude of increase in DA efflux in the NAc, in comparison to that observed in the mPFC, was approximately five times smaller.

Histology

As shown in Figure 6, tracts left by microdialysis probes were located in the prelimbic/infralimbic region of the mPFC (+3.2 to +2.7 mm AP from bregma) or the shell/core region of the NAc (+2.2 to +1.6 mm AP from bregma).

Discussion

Similarities in DA efflux in the mPFC and NAc during consummatory phases of sensory-specific satiety

The present findings confirm previous reports that when rats are food-deprived, DA efflux in both the mPFC and NAc is increased in response to the presence of a novel food behind a screen and during ingestion of the meal (Bassareo and Di Chiara, 1997). When rats display sensory-specific satiety, and sample only small quantity of the original food during a second meal, DA efflux in both terminal regions remains unchanged. Another important finding is the second increase in DA efflux in both the mPFC and NAc, when rats that are no longer food-deprived consume a different palatable meal. Together, these results point to major similarities in increases in DA efflux in the NAc and mPFC during the different phases of sensory-specific satiety.

The patterns of DA efflux in the NAc and mPFC are consistent with the incentive salience hypothesis (Berridge and Robinson, 1998), which predicts that changes in incentive value of different food items through the different phases of sensory-specific

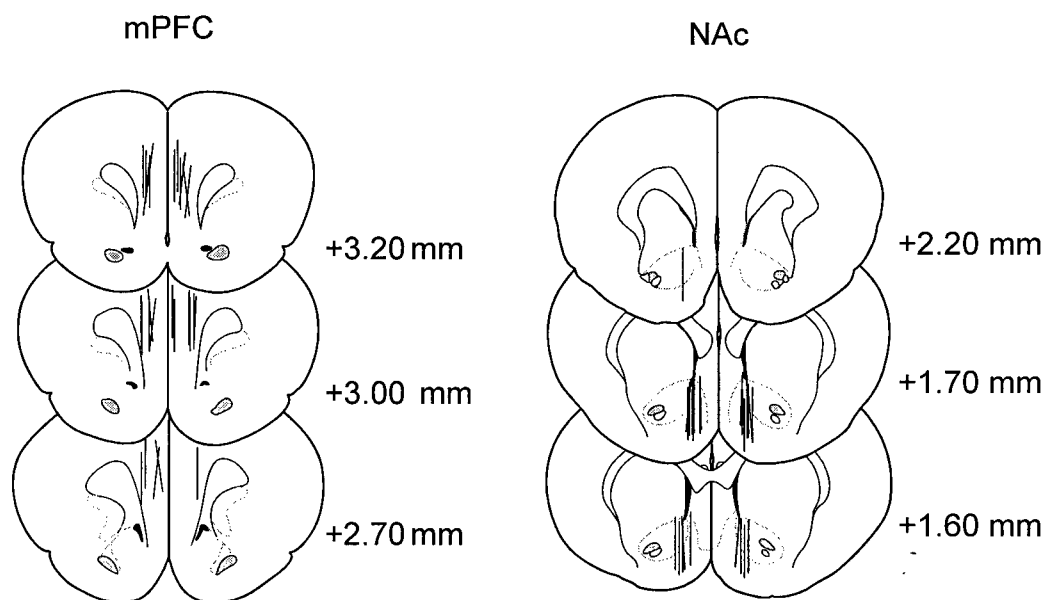


Figure 6. Location of probes in Experiment I. Vertical lines represent the 2mm dialyzing lengths of microdialysis probes in the mPFC and NAc. Drawings of coronal sections were adapted from Paxinos and Watson (1997). Distance from bregma is indicated.

satiety, should be accompanied by corresponding changes in mesotelencephalic DA activity. In the sensory-specific satiety protocol employed here, the relative incentive values of the two palatable foods is altered by allowing hungry rats to feed to satiety on one of the foods. The results of a recent study suggest that as a food is consumed to satiety, its incentive value is gradually devalued through consummatory contact (Balleine and Dickinson, 1998). In the present study, a similar devaluation process may have influenced DA efflux in the mPFC and NAc, as a gradual decrease in food intake was paralleled by a reduction in the magnitude of DA efflux during both meals, in both brain regions. It is suggested that as a consequence of this food-specific devaluation, presentation of the same food as a second meal was not accompanied by a change in DA efflux and only a small quantity of the food was consumed. Furthermore, the food-specific devaluation is likely to have persisted into the second meal, producing an inflation (or relative enhancement) of the incentive properties of a novel food in comparison to the same food through "positive incentive contrast". This is an effect that arises when a stimulus of greater incentive value follows one of lesser value (Kentridge and Aggleton, 1993; Balleine and Dickinson, 1998). Thus, the relatively enhanced incentive value of the novel food may account for the rise in DA efflux and robust behavioural response during the second meal. If correct, this account implies that devaluation of a familiar food requires an "opportunity for incentive learning" (Balleine and Dickinson, 1998), whereas attribution of incentive salience to a food, entirely or relatively, novel does not require previous experience.

Differences in DA efflux in the mPFC and NAc during sensory-specific satiety

The deprivation state of an animal is an important determinant of whether DA efflux in the NAc is elevated during feeding behaviour, particularly during the appetitive phase (Wilson et al., 1995). In food-deprived animals, presentation of a palatable food in a perforated box or behind a screen (Wilson et al., 1995; Bassareo and Di Chiara, 1997) or even a cue associated with food (Phillips et al., 1993) is associated with increased DA efflux in the NAc. In nondeprived rats, however, no anticipatory changes in DA efflux in the NAc are observed prior to the consumption of a palatable food or fluid. Furthermore, a prior opportunity to eat the food earlier greatly attenuates DA efflux during a subsequent presentation (Bassareo and Di Chiara, 1997). All these effects were also observed in the present experiment. These findings suggest that, in the NAc, increases in DA efflux during appetitive and consummatory phases of feeding are closely tied to the motivational state of the organism and prior experience with the incentive sensory qualities of the food. The magnitude of DA activity may determine the general level of motivation and effort expended by an animal in seeking a reward (Salamone et al., 1997).

Recently, Bassareo and Di Chiara (1999) observed in nondeprived rats that DA efflux in the core, in comparison to the shell, region of the NAc is preferentially increased upon exposure to a food-filled box, suggesting a possible difference in the role that DA may serve in the two regions. However, in the present study, these intra-accumbens differences in DA function are difficult to evaluate as most of the microdialysis probes were located across the shell/core boundaries (Figure 6). Nevertheless, the fact that DA efflux in the NAc increased in the appetitive phase prior to the first meal indicates that our probe placements were located in a region of the NAc that responds to appetitive

stimuli. In light of the findings of Bassareo and Di Chiara (1999), this increase in DA efflux may be attributed to an increase in the core portion of the NAc.

There appears to be a subtle but critical difference in the conditions leading to increased DA efflux in the mPFC and NAc. In contrast to their findings in the NAc, Bassareo and Di Chiara (1997) observed a significant elevation of DA efflux in the mPFC of nondeprived rats presented with food in a perforated box. In the present study, a similar difference between the two brain regions was highlighted by the selective increase in DA efflux in the mPFC, but not in the NAc, of *sated* rats when a different palatable food was presented behind a screen as the second meal. The results of both studies suggest that DA efflux in the mPFC is influenced solely by the incentive salience of rewards or by environmental stimuli associated with them and, unlike the NAc, is not modulated by the deprivation state of the animal. This feature of DA activity may be linked to the finding that neurons in the primate dorsolateral prefrontal cortex show differential activity in response to different types of food, suggesting that these neurons can discriminate between preferred and less preferred foods (Watanabe, 1996). Therefore, it is possible that DA input to the mPFC reflects the assignment of incentive salience to behaviourally important stimuli in the environment, thereby mediating the initial approach behaviours leading to consumption of a second meal.

Mechanisms of sensory-specific satiety in the rat

Using an information processing approach, Rolls and his colleagues have determined that, in the primate, the orbitofrontal cortex is a key neural substrate involved in the computation of sensory-specific satiety. Firing rates of neurons in the orbitofrontal cortex closely mirrors the rejection of a food eaten to satiety (i.e., baseline or spontaneous

rates of firing) and the acceptance of a different food (i.e., increased rate of firing from baseline) (Rolls et al., 1986). Rolls (1999) has proposed that efferent projections from this brain region to the ventral striatum may convey information about specific taste stimuli devalued by satiety to behavioural output systems. The present experiment showed that, in rats, changes in DA efflux in the mPFC and NAc also mirrors the patterns of food intake in sensory-specific satiety. It is possible that areas of the brain involved in the computation of sensory-specific satiety communicates with the DA system to modulate the attribution of incentive salience, thus selectively facilitating appetitive behaviours towards foods with high incentive value.

EXPERIMENT II:

**EFFECT OF TRANSIENT INACTIVATION OF THE CENTRAL AND
BASOLATERAL NUCLEI OF THE AMYGDALA ON BASAL AND FOOD-
EVOKED DA EFFLUX IN THE NUCLEUS ACCUMBENS AND MEDIAL
PREFRONTAL CORTEX**

Introduction

Presentation of food reward or a cue predictive of the reward elicits a phasic increase in extracellular concentrations of DA in the striatum and prefrontal cortex (Cenci et al., 1992; Phillips et al., 1993; Wilson et al., 1995; Feenstra and Botterblom, 1996; Bassareo and Di Chiara, 1997; Ahn and Phillips, 1998). Recent analysis of circuits in the rat brain indicate that limbic structures, such as the hippocampus and amygdala, provide excitatory inputs to the NAc and mPFC and thus, have the potential to modulate DA efflux (Lipska et al., 1992; Blaha et al., 1997; Floresco et al., 1998; Legault et al., 2000; Jackson and Moghaddam, 2001; Floresco et al., 2001).

In a series of important studies, Everitt and his colleagues (Robledo et al., 1996; Parkinson et al., 1999; Everitt et al., 1999; Parkinson et al., 2000c) provide evidence to suggest that the CeN and BLA subregions of the amygdala may engage DA transmission in telencephalic terminal regions to mediate dissociable aspects of associative learning. Functional differences arising from amygdalar modulation of the DA system may depend on the afferent target and the neurochemical mode of transmission within those targets. The BLA sends glutamatergic afferents to the NAc that terminate in close apposition to

tyrosine hydroxylase-containing varicosities (Johnson et al., 1994) and may provide a potential site at which glutamatergic input from the BLA can increase DA efflux in the NAc (Floresco et al., 1998). The BLA also sends projections to areas of the mPFC (McDonald, 1991a) that are innervated by DA terminals (Fallon and Moore, 1978; Björklund and Lindvall, 1984). However, an ultrastructural relationship between glutamate and DA terminals, similar to that observed in the NAc, has yet to be reported. Recent studies show that electrical stimulation of the BLA elicits phasic increases in DA efflux in the NAc and mPFC (Floresco et al., 1998; Jackson and Moghaddam, 2001) and the mechanism(s) by which these effects occur is currently under debate. There is evidence for at least two possible mechanisms: (i) a direct presynaptic modulation of DA terminals by BLA afferents which elicits an impulse-independent release of DA (Floresco et al., 1998) and (ii) an indirect polysynaptic BLA → prefrontal cortex → DA cell bodies, and thus, an impulse-dependent modulation of DA in the NAc and mPFC (Jackson and Moghaddam, 2001).

CeN-modulation of DA activity in the NAc and mPFC has yet to be demonstrated. However, disconnection studies of the CeN and striatum (which interrupt the flow of information between the two structures but allow information to be processed within each structure unilaterally) suggest that the two structures are functionally interconnected (i.e., information is relayed from one structure to the other) (Han et al., 1997). It is notable that the CeN is distinguished from the BLA by its direct GABA-containing projections to midbrain DA cell bodies (Phillipson, 1979; Wallace et al., 1992). Given that presentation of food reward is associated with increased firing rates in CeN neurons (Uwano et al., 1995) and midbrain DA neurons (Freeman et al., 1985;

Strecker and Jacobs, 1987; Ljungberg et al., 1991; Schultz et al., 1993), the CeN may indirectly modulate DA transmission via its projections to the midbrain.

Previous studies have used electrical stimulation of the amygdala to assess mechanisms of DA release in the NAc and mPFC (Floresco et al., 1998; Jackson and Moghaddam, 2001). The electrical nature of the stimulus has the capacity to activate all (direct and indirect) pathways leading to DA terminals in the brain. Therefore, these same studies have employed pharmacological block of glutamatergic and DA receptors to eliminate and deduce critical circuits mediating the stimulation-evoked DA activity. An alternative method for examining the relationship between the amygdala and terminal DA activity is to use chemical inactivation to produce transient lesions of the amygdala. In contrast to the phasic responses of the DA system following electrical stimulation, inactivation methods assess tonic influences that may influence the levels of DA concentration in the extracellular fluid. For the purposes of this experiment, reverse-dialysis of lidocaine was used to provide discrete reversible lesions of the CeN and BLA while microdialysis was used to monitor changes in DA efflux in the NAc and mPFC.

Experiment II addressed two objectives. First, it assessed whether the CeN and/or the BLA can exert tonic and possibly, differential, effects on DA efflux in the NAc and mPFC. Given the behavioural evidence mentioned above (in particular Everitt et al., 1999), as well as the anatomical circuitry and neurotransmitters involved, it was hypothesized that inactivation of the CeN and BLA would differentially influence DA efflux in the NAc and mPFC. Given that the CeN sends projections to the vicinity of DA neurons in the midbrain and that the BLA sends afferents to regions innervated by DA terminals, it is predicted that the CeN and BLA may provide impulse-dependent and

presynaptic (impulse-independent) modulation of DA transmission, respectively.

Experiment II also investigated the possibility that the changes in food-related behaviour associated with amygdalar damage are mediated, in part, by the DA system. It is unknown whether specific regions of the amygdala differentially or preferentially mediate food intake. It was of particular interest to determine if inactivation of the CeN and BLA subregion(s) within the amygdala would alter food intake and the associated changes in DA efflux.

Methods

Twenty-six Long-Evans male rats were housed and cared for as described in the General Methods. Procedures pertaining to surgery, microdialysis, reverse-dialysis as well as histological and statistical analyses were as described in the General Methods section.

Subjects were assigned randomly to one of four reverse-dialysis/dialysis groups (CeN/NAc, CeN/mPFC, BLA/NAc, BLA/mPFC) and implanted accordingly with guide cannulae. Following surgery, rats were placed on a food restriction schedule. Two weeks later, rats were given three opportunities to habituate to the foods, the testing chamber and to being tethered by the steel coil (Figure 1).

Each animal served as a subject in two experiments conducted over two days. One session involved bilateral reverse-dialysis of lidocaine in the CeN or BLA (lidocaine condition) and the other session involved continuous flushing with perfusate only (control condition). For each animal, microdialysis probes were implanted into the NAc or mPFC unilaterally and reverse-dialysis probes into the CeN or BLA bilaterally. With respect to

microdialysis, the left and right hemisphere was randomly assigned to the lidocaine and control conditions to equalize for any hemispheric differences. Dialysis samples were collected at 10 min intervals from the NAc or mPFC and immediately assayed for DA using HPLC-ED.

On each experiment day, baseline conditions continued until four consecutive samples showed less than 5% fluctuation in DA content before lidocaine or the vehicle (i.e., perfusate) was reverse-dialyzed into the CeN or BLA for 50 min. Palatable food (3 g Froot Loops) was presented to the rats on a plexiglass tray on two occasions, once when the lidocaine had taken full effect (30 min into the reverse-dialysis) and again, after the effect had dissipated (30 min following the end of the reverse-dialysis). In the Control Condition, probes in the CeN and BLA were continuously flushed with perfusate only and food was presented at the same interval as in the Lidocaine Condition.

Following the last microdialysis session, brains were removed for verification of probe placements. Only those animals with tracts in the 1) prelimbic/infralimbic region of the mPFC or shell/core region of the NAc and 2) CeN in the rostral amygdala or BLA in the caudal amygdala of both hemispheres were included in the statistical analyses.

Results

Basal concentration of DA in microdialysates collected from the mPFC and NAc

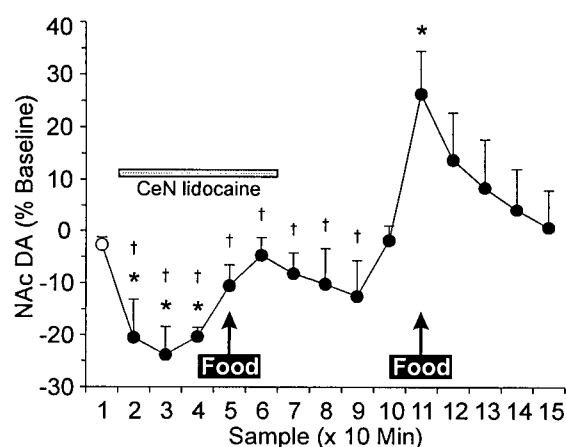
The average concentration of DA (uncorrected for probe recovery) in the last three samples prior to administration of lidocaine by reverse dialysis in the CeN and BLA was 0.13 ± 0.06 nM in the mPFC and 2.78 ± 0.32 nM in the NAc.

Effects of transient inactivation of the CeN on food intake and DA activity

Within minutes of administering lidocaine into the CeN, a majority of the animals in the CeN/NAc and CeN/mPFC groups showed increased behavioural activity. Typically, animals awoke from sleeping, began grooming and/or exploring the chamber. When palatable food was presented immediately in front of them, animals were varied in their latency to initiate contact with the food, but all eventually did so within the 10 min meal. Although animals handled and placed all morsels of food in their mouths, animals ingested less than 50% of the food and emitted the remaining amount. Once the lidocaine effects had dissipated presentation of a second meal lead to the food being consumed immediately into entirety. When the same animals were tested in the control condition on another day, both meals were consumed immediately.

In CeN/NAc animals, there was a significant interaction of Condition x Time on DA efflux in the NAc [$F_{(14,70)}=3.356$; $p<0.001$]. Simple main effects analyses of Groups revealed that the interaction was due to decreased levels of DA efflux which lasted for the duration of the CeN inactivation [Figure 7, top panel, samples 2-6; $F_{(1,70)}=6.986$; $p<0.01$]. There was also a simple main effect of Time in each of the lidocaine [$F_{(14,70)}=9.08$; $p<0.001$] and control [$F_{(14,70)}=6.09$; $p<0.001$] conditions. Reverse-dialysis of lidocaine into the CeN significantly decreased basal DA efflux in the NAc by 20-24% below baseline ($p<0.05$; Figure 7, top panel). Despite a small rise in DA during the first meal (+9% in the first 10 min), maximal food-evoked changes (+15% in the second 10 min) were below baseline. After the reverse-dialysis probes were cleared of lidocaine-containing perfusate, DA levels returned to pre-lidocaine values within 30 min, and consumption of a second meal was accompanied by a significant increase

Lidocaine condition



Control condition

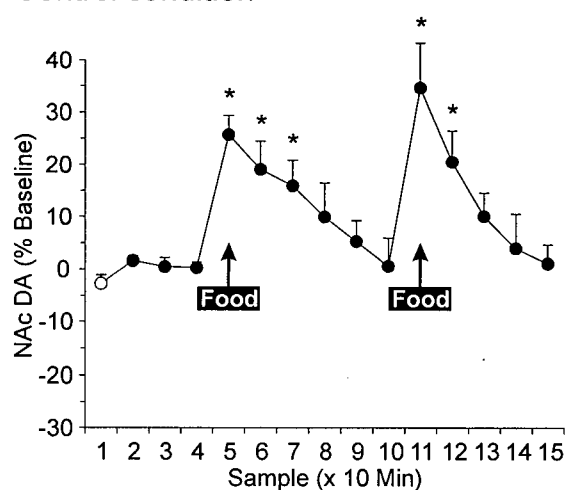


Figure 7. Effect of transient inactivation of the CeN on basal and food-evoked DA efflux in the NAc. Lidocaine condition ($n=6$), reverse-dialysis of lidocaine in the CeN bilaterally during samples 2-6 and normal perfusate during other samples. Control condition ($n=6$), continuous dialysis of the CeN with normal perfusate during all samples. Samples 5 and 11 represent periods during which palatable food was presented to the rats. Data are represented as mean+SEM. The baseline value (white circle) was used as the control mean in Dunnett's method of multiple comparisons ($*p<0.05$). Dunn's test was used for comparisons between the lidocaine and control conditions ($^{\dagger}p<0.05$).

in DA efflux (+26%; $p < 0.05$). When the same rats were tested without lidocaine, consumption of the meals elicited a significant increase in DA efflux, with a maximal mean increase of 26% ($p < 0.05$) during the first meal and 35% ($p < 0.05$) during the second meal (Figure 7, bottom panel).

In CeN/mPFC animals, there was a significant interaction of Condition x Time on DA efflux in the mPFC [$F_{(14,70)} = 3.275$; $p < 0.001$]. A simple main effects analysis revealed that the source of the interaction was a smaller increase in DA efflux correlated with the consumption of food in the lidocaine condition than in the control condition in samples 5 [$F_{(1,70)} = 42.319$; $p < 0.0001$] and 12 [$F_{(14,70)} = 4.461$; $p < 0.05$] of Figure 8. Additionally, there was a simple main effect of Time in each of the lidocaine [$F_{(14,70)} = 2.082$; $p < 0.05$] and control [$F_{(14,70)} = 10.396$; $p < 0.001$] conditions. Unlike the NAc, basal DA efflux in the mPFC was not altered by CeN inactivation and consumption of the first meal was accompanied by a small insignificant increase in DA efflux (+17%). Consumption of the second meal was accompanied by a small but significant increase in DA efflux (+46%, $p < 0.05$). In control animals (Figure 8, bottom panel), consumption of the first and second meals was associated with large significant increases in DA efflux (+106% and +66%, respectively; $p < 0.05$).

Effects of transient inactivation of the BLA on food intake and DA activity

In BLA/NAc and BLA/mPFC animals, there were no observable changes in baseline behaviours or responses towards the presented food following infusion of lidocaine. Animals immediately approached the food and ate both meals.

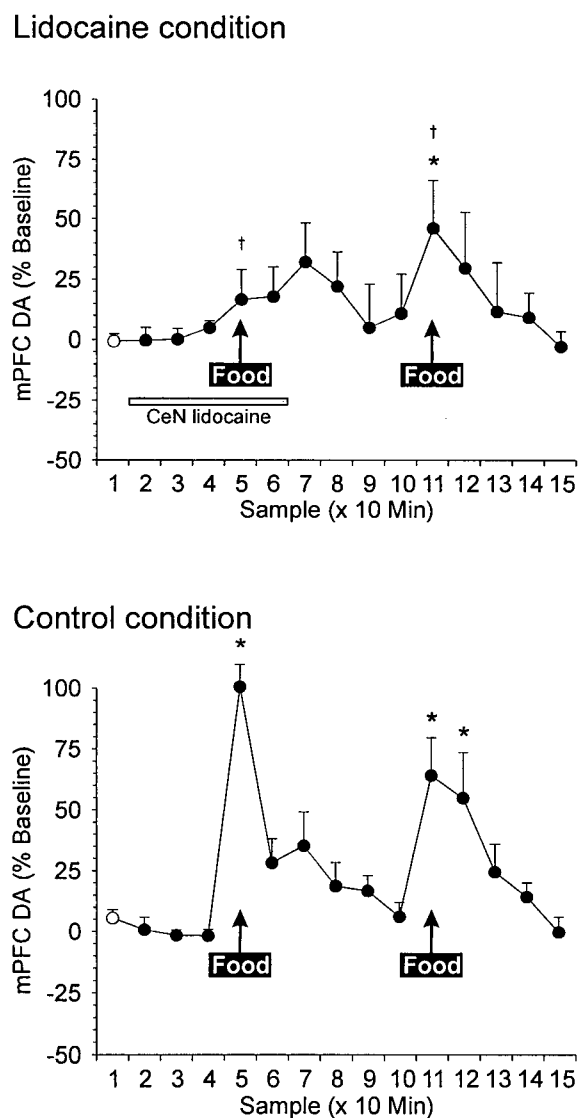


Figure 8. Effect of transient inactivation of the CeN on basal and food-evoked DA efflux in the mPFC. Shown are the lidocaine (n=6) and control (n=6) conditions. See Figure 7 legend for further explanation.

In BLA/NAc animals, there were no statistical differences in DA efflux between the lidocaine and control conditions; that is, administration of lidocaine into the BLA altered neither basal levels nor food-evoked changes in DA efflux in the NAc. There was a significant main effect of Time in each of the lidocaine [$F_{(14,70)}=8.026$; $p<0.001$] and control [$F_{(14,70)}=4.246$; $p<0.001$] conditions. As such, presentation and consumption of a palatable food was associated with a significant increase in DA efflux in both the lidocaine (+34% during the first and second meals; $p<0.05$) and control (+29% during first meal and +34% during the second meal; $p<0.05$) conditions (Figure 9).

In BLA/mPFC animals, administration of lidocaine into the BLA triggered large oscillations in DA efflux above and below (–100% to +150%) baseline levels in the mPFC (Figure 10). In each of the subjects (individual data are shown as separate panels in Figure 10), oscillations lasted well beyond the anaesthetic effects of lidocaine in the BLA. Furthermore, although subjects consumed all of the offered food, food-related changes in DA efflux were not detectable above the noise of the oscillating basal DA levels. The variability in periodicity and magnitude of DA efflux oscillations between animals precluded any statistical analyses of these data. When the same rats were tested in the control condition, presentation of food was associated with significant increases in DA efflux in the mPFC during the first (+71%, $p<0.05$) and second (+86%, $p<0.05$) presentation of food (Figure 11). To confirm that the oscillations in DA levels were triggered only by lidocaine in the BLA, and not by the combination of lidocaine and food, a second group of BLA/mPFC animals were tested without presentation of food.

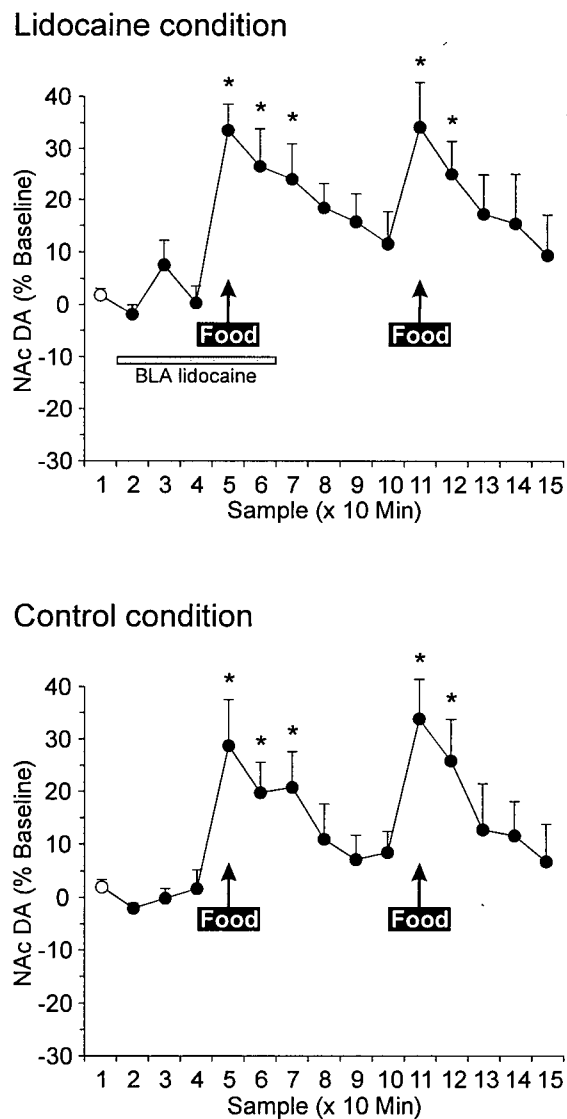


Figure 9. Effect of transient inactivation of the BLA on basal and food-evoked DA efflux in the NAc. Shown are the lidocaine (n=6) and control (n=6) conditions. See Figure 7 legend for further explanation.

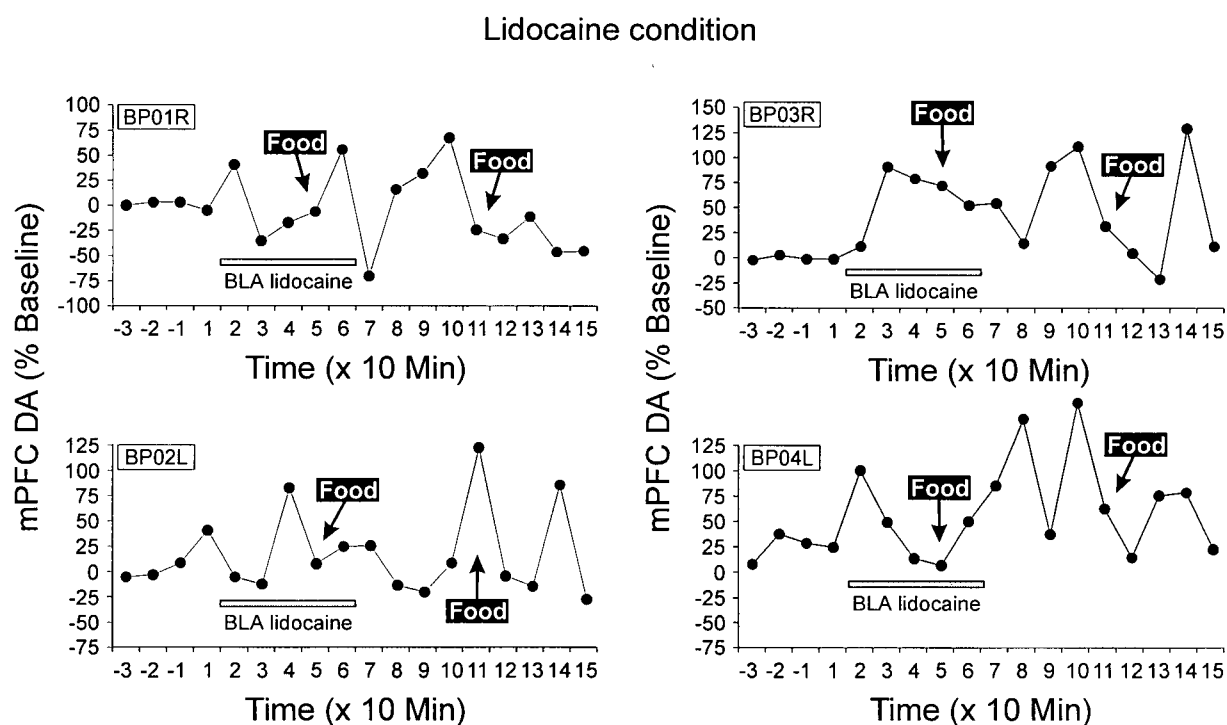


Figure 10. Effect of transient inactivation of the BLA on DA efflux in the mPFC with presentation of food. Lidocaine condition, reverse-dialysis of lidocaine in the BLA bilaterally during samples 2-6 and normal perfusate during other samples. Samples 5 and 11 represent periods during which palatable food was presented to the rats. Data from four individual subjects are shown. The Y-axis values have been adjusted to include all data points from each subject while maintaining an equivalent range for all panels.

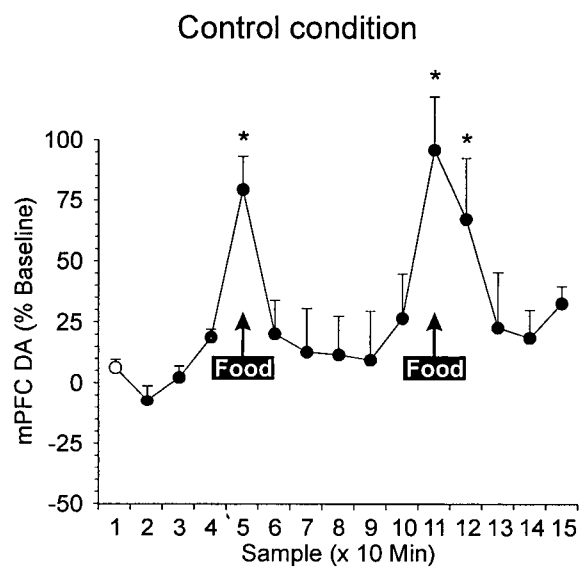


Figure 11. Effect of reverse-dialysis of normal perfusate in the BLA bilaterally on DA efflux in the mPFC. Control condition for Figure 10 ($n=5$). Samples 5 and 11 represent periods during which palatable food was presented to the rats. The data are presented as mean+SEM. The baseline value (white circle) was used as the control mean in Dunnett's method of multiple comparisons (* $p<0.05$).

A similar oscillating pattern in DA efflux was observed. Data from four individual subjects are presented in Figure 12.

Histology

As shown in Figures 13 and 14, tracts left by microdialysis probes were located in the prelimbic/infralimbic region of the mPFC (+3.2 to +2.7 mm AP) or straddled the shell/core region of the NAc (+2.2 to +1.6 mm AP). Tracts left by reverse-dialysis probes were located mainly in the CeN and but included some surrounding regions such as the intercalated masses, and substantia innominata (−1.8 to −2.6 mm AP). BLA tracts were seen in the basal and lateral nuclei, and in some animals, in the lateral aspect of the CeN (−2.8 to −3.3 mm AP).

Discussion

Specificity of CeN versus BLA inactivation using reverse-dialysis

In the present study, reverse-dialysis was chosen over the conventional microinfusion method to administer lidocaine as it provided the necessary control over the physical and temporal parameters required for inactivating the CeN and the BLA for an extended period of time (i.e., 50 min). Restriction of inactivation to the lesion site is best evidenced by the differential effects of lidocaine in the CeN and the BLA on behavioural and DA activity. Moreover, active removal of lidocaine from the target areas by reverse-dialysis of normal perfusate was accompanied by recovery of function. This is demonstrated in the CeN/NAc group in which cessation of lidocaine administration lead to rapid restoration of DA concentrations to pre-lidocaine values and normal magnitudes of food-evoked DA increases (Figure 7, top panel).

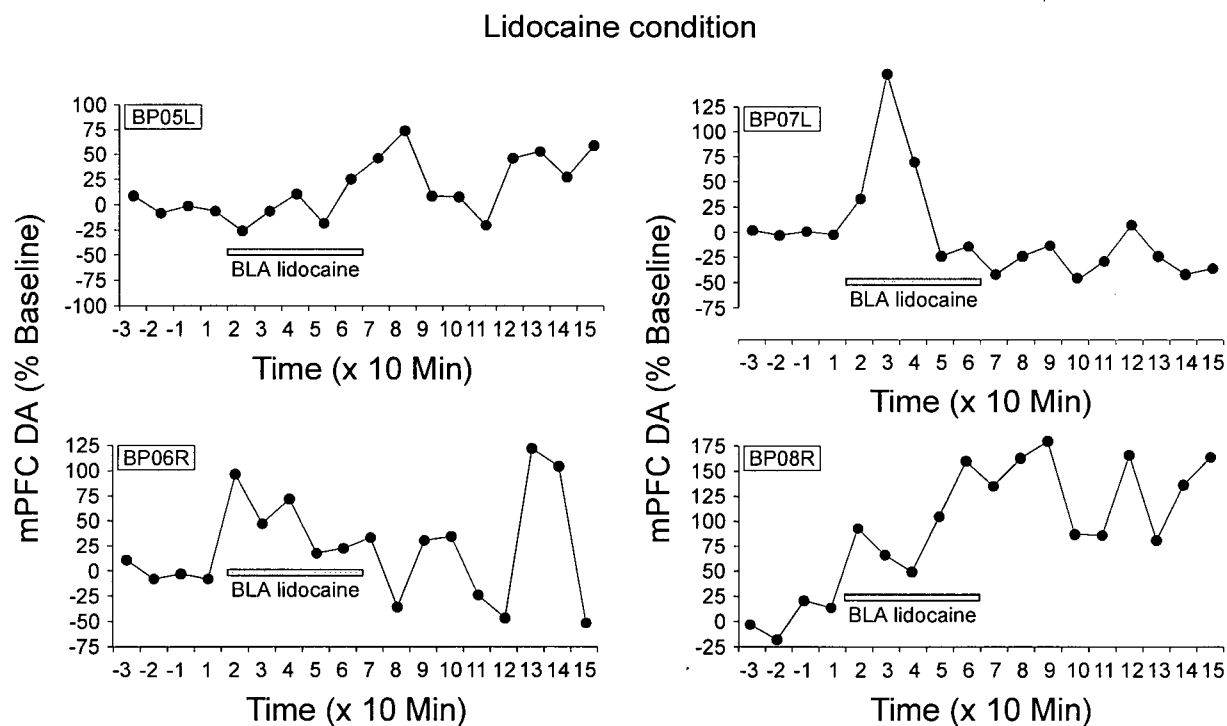


Figure 12. Effect of transient inactivation of the BLA on DA efflux in the mPFC in the absence of food. Lidocaine condition, reverse-dialysis of lidocaine in the BLA bilaterally during samples 2-6 and normal perfusate during other samples. Data from four individual subjects are shown. The Y-axis values have been adjusted to include all data points from each subject while maintaining an equivalent range for all panels.

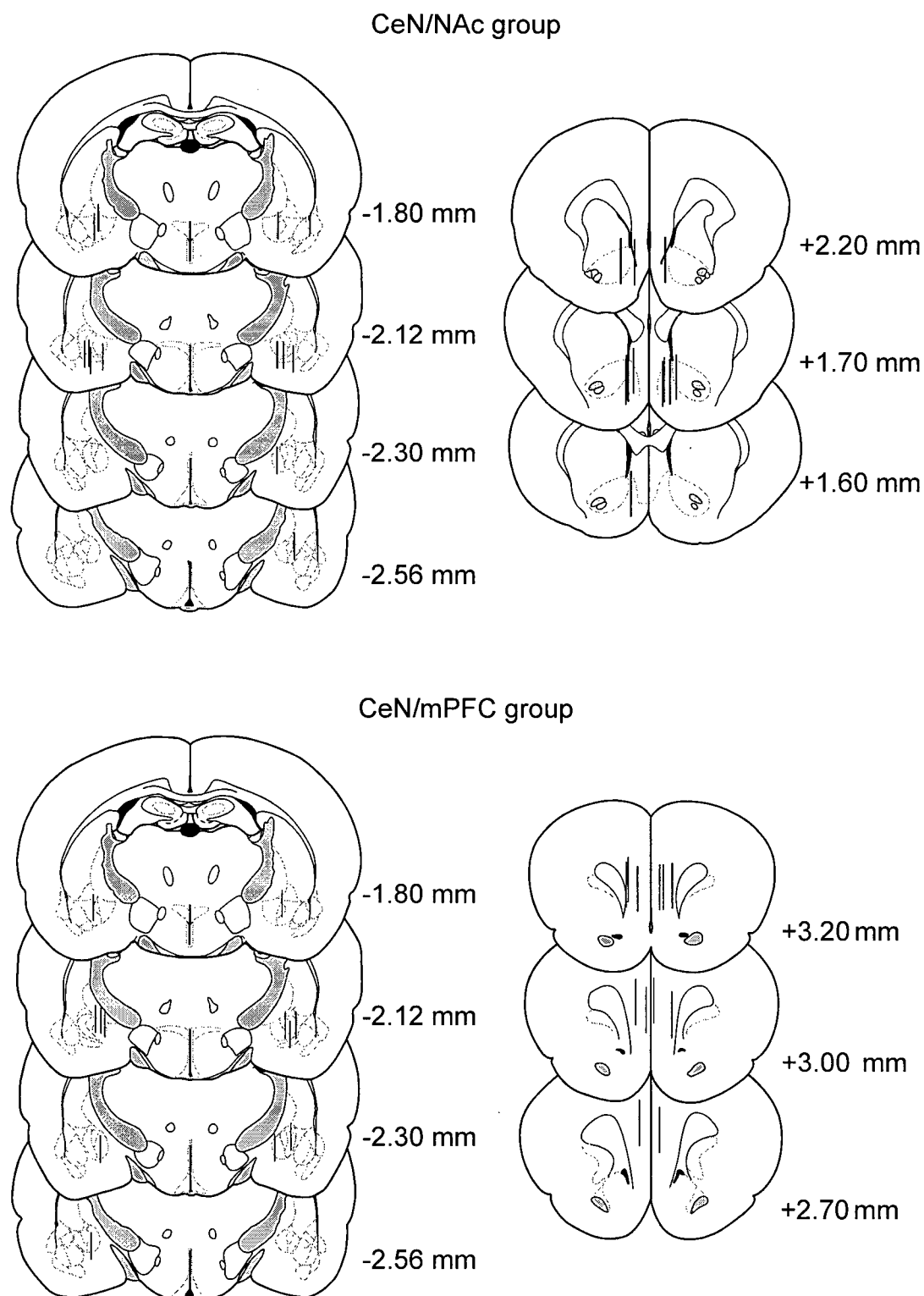


Figure 13. Location of probes in Experiment II (CeN groups). Vertical lines represent the dialyzing lengths of microdialysis probes (2 mm) in the NAc and mPFC and reverse-dialysis probes (1.2 mm) in the CeN. Drawings of coronal sections were adapted from Paxinos and Watson (1997). Distance from bregma is indicated.

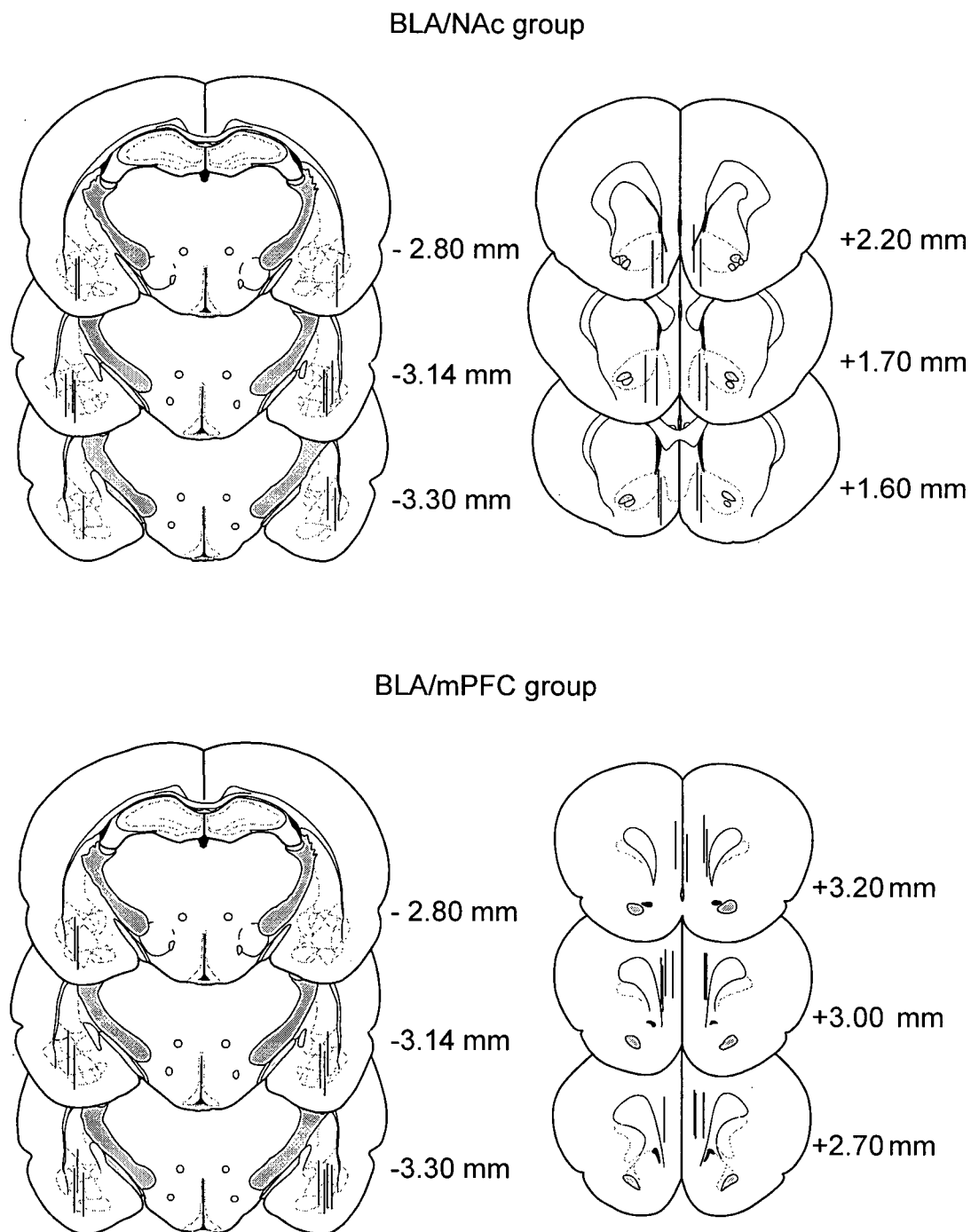


Figure 14. Location of probes in Experiment II (BLA groups). Vertical lines represent the dialyzing lengths of microdialysis probes (2 mm) in the NAc and mPFC and reverse-dialysis probes (1.8 mm) in the BLA. Drawings of coronal sections were adapted from Paxinos and Watson (1997). Distance from bregma is indicated.

Role of CeN and BLA in food intake

Lidocaine inactivation of the BLA did not have any observable effects on feeding related behaviours. Following BLA inactivation, rats approached and immediately consumed all the food. In contrast, rats with CeN-inactivation placed all morsels of the offered food into their mouths but did not ingest all of the food. This finding is not surprising given that the CeN has connections to brainstem nuclei, such as the NST and parabrachial nucleus, which are the initial central sites involved in the control of food intake (Zeigler, 1994; Mei, 1994; Woods et al., 1998). The unique nature of this deficit may be related to impairment of a specific function associated with the amygdala, namely the integration of sensory and rewarding properties of food (Mishkin and Aggleton, 1981; Uwano et al., 1995). As originally described Klüver and Bucy (1939), monkeys with amygdalar-lesions are unable to show appropriate behavioural responses to external sensory stimuli, and thus consume a food that is normally avoided and orally examine non-food items (Aggleton and Passingham, 1982; Uwano et al., 1995; Murray et al., 1996). In the present study, rats with CeN inactivations could have been trying (unsuccessfully) to evaluate the rewarding properties of the food by tasting every available morsel. Thus, in the rat, Klüver-Bucy symptoms appear to be associated with damage confined to the CeN, and possibly with DA interactions as discussed below.

Mechanisms underlying differential modulation of DA efflux in the NAc and mPFC by the CeN

Reverse-dialysis of lidocaine into the CeN resulted in a gradual decrease in basal levels of DA efflux in the NAc (Figure 7). This effect may involve inhibition of VTA

DA neurons because blocking DA cell firing, for example by intra-VTA infusions of lidocaine (Floresco et al., 1998) or GABA (Suaud-Chagny et al., 1992), leads to a similar decrease in extracellular concentrations of DA in the NAc. Anatomical data supporting a direct modulatory influence on the activity of DA cells include a light to moderate GABAergic projection from the CeN to DA neurons in the VTA (Phillipson, 1979; Wallace et al., 1992; Sun and Cassell, 1993). However, such a circuit cannot account for the decreased basal DA efflux in the NAc during CeN inactivation. If inactivations of the CeN decrease a direct inhibitory influence on postsynaptic DA neurons (i.e., disinhibition), this should result in an increase in spontaneous firing of DA neurons and a corresponding increase in DA transmission. The converse was observed. This decrease in basal DA efflux can be explained if CeN GABAergic projections contact inhibitory interneurons, rather than DA neurons in the VTA (see Figure 15). In agreement with this proposed circuit, CeN neurons have been shown to target non-DA (possibly inhibitory) neurons in the VTA (Wallace et al., 1992), some of which contain GABA receptors (Suaud-Chagny et al., 1992). In this circuit, removal of inhibitory inputs from the CeN would lead to disinhibition of these interneurons resulting in increased inhibition of DA neurons. This mechanism could account for the present findings of decreased basal levels of DA efflux in the NAc following inactivation of the CeN. Alternatively, CeN projections also project to DA cells in the retrorubral field, which in turn send collaterals to VTA DA neurons and to the NAc (Deutch et al., 1988). In this case, retrorubral DA cells could be disinhibited by CeN lesions and thereby increase somatodendritic release of DA in the VTA, which would in turn activate somatodendritic autoreceptors to effectively decrease DA release from terminals.

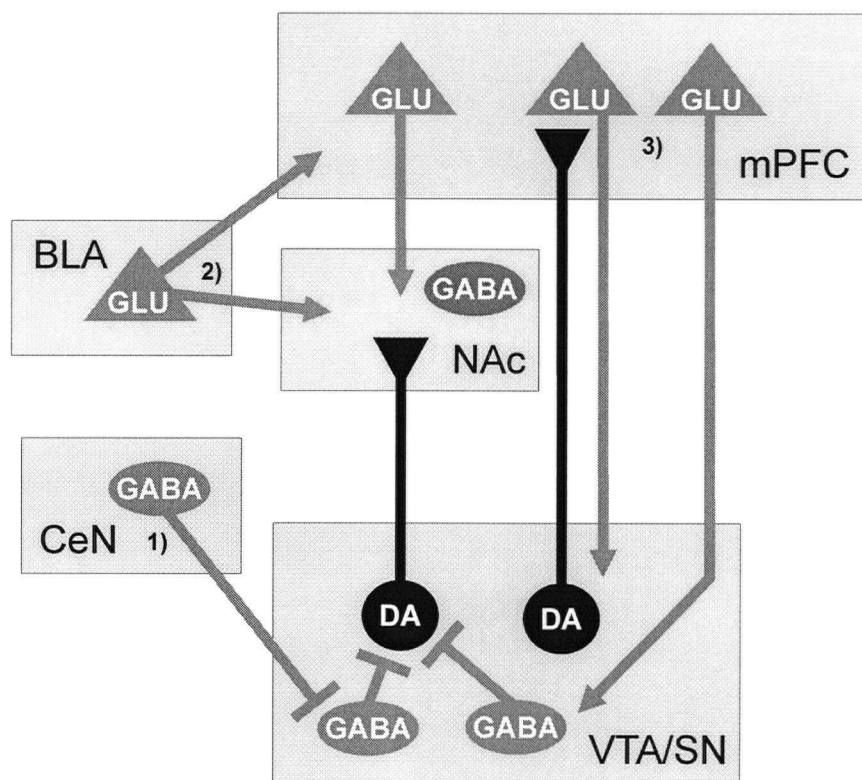


Figure 15. Schematic representation of proposed mechanisms by which the CeN and BLA could modulate DA efflux in the NAc and mPFC. “T”-shaped afferent endings indicate inhibitory projections whereas “↑”-shaped endings indicate excitatory projections. DA terminals are shown as “↑”. 1) The CeN sends inhibitory GABAergic projections to GABAergic interneurons in the VTA/SN, which in turn have inhibitory influences on DA neurons. Through this pathway, it is proposed that the CeN provides a tonic influence on spontaneous firing rates of DA neurons, and contribute to maintaining a basal level of DA efflux in the NAc. Removal of inhibitory inputs from the CeN would increase the inhibitory influence on DA neurons, causing hyperpolarization of these cells, and a decrease in DA efflux in the NAc. 2) The BLA sends glutamatergic afferents to both the NAc and mPFC. In the NAc, these inputs may presynaptically modulate the release of DA from terminals. In the mPFC, BLA afferents contact prefrontal neurons that are innervated by DA neurons; this suggests that DA efflux in the mPFC may occur in an impulse-dependent manner. 3) The prefrontal cortex provides glutamatergic inputs to the VTA/SN. These inputs synapse on mesoprefrontal DA neurons and indirectly contact mesoaccumbens DA neurons via inhibitory interneurons. Input from prefrontal neurons would selectively increase DA efflux in the mPFC but not the NAc. Together, these anatomical details provide insight into mechanisms for independent and differential modulation of DA efflux in the NAc and mPFC by the CeN and BLA.

CeN inactivation did not affect basal levels of DA efflux in the mPFC (Figure 8). The differential influence on DA efflux in the mPFC and NAc could be related to the subpopulation of DA neurons contacted by CeN efferents to the midbrain: the CeN projects more heavily to DA neurons in the retrorubral field and the SN and less so to neurons in the VTA (Wallace et al., 1992). This could explain why the CeN had a relatively greater effect on DA efflux in the NAc than the mPFC, since the NAc receives DA terminals that arise from the retrorubral field and SN in comparison to the mPFC, which is mainly innervated by DA projections from the VTA (Björklund and Lindvall, 1984).

In contrast to the differential effects of CeN inactivation on basal levels of DA efflux in the NAc and mPFC, food-evoked DA efflux was significantly attenuated, in time-course and magnitude of effect, in both terminal regions (Figures 7 and 8). This suggests that at least a part of the drive mediating food-evoked phasic increases in DA activity in both terminal regions originates from a source other than the CeN. A potential candidate may be neurons in the prefrontal cortex that send glutamatergic projections to DA neurons in the midbrain (Carr and Sesack, 2000) (see Figure 15). These projections could carry food reward-related information, since prefrontal neurons have been shown to increase their firing rates in response to presentation of salient food rewards (Watanabe, 1996; Schultz et al., 1998).

The smaller rise in DA efflux in the NAc and mPFC during the first meal in the CeN lidocaine condition compared to the control condition (Figures 7 and 8) is difficult to explain. The attenuation may be related to a difference in total food intake, as CeN-lesioned animals ate less food in the lidocaine condition than in the control condition.

However, this is unlikely to be the primary explanation as there is evidence to indicate that the amount of ingested food does not always (or necessarily) correlate with the magnitude of DA efflux (Ahn et al., 1997; Ahn et al., 2000). Alternatively, if CeN lesions effectively hyperpolarized DA neurons, prefrontal excitatory input would be less likely to trigger burst firing patterns from DA neurons. Lastly, the increase in DA efflux could be viewed as a residual contribution from a small population of DA neurons that were not affected by CeN inactivation.

In contrast to the first meal, presentation of the second palatable meal following recovery of DA levels (in the NAc) to pre-lidocaine values resulted in an interesting dissociation of DA activity in the NAc and mPFC (Figures 7 and 8). Food-evoked DA efflux in the NAc was similar to that in the control condition whereas food-evoked DA efflux in the mPFC was significantly attenuated. It is possible that CeN inactivation may have longer-lasting effects on prefrontal projecting DA neurons than those projecting to the NAc, but a mechanism by which this may occur (most certainly involving additional modulatory influences on the DA system) is unclear at present and requires further exploration.

Mechanisms underlying differential modulation of DA efflux in the NAc and mPFC by BLA

Jackson and Moghaddam (2001) observed that electrical stimulation of the BLA resulted in a significant increase in DA and glutamate efflux in the mPFC and NAc. However, the increase in NAc DA efflux did not occur until stimulation of the BLA ended, suggesting that BLA activity may provide a time-locked facilitation of DA efflux in the mPFC and concurrent inhibition of DA release in the NAc. In the present study,

lidocaine inactivation of the BLA did not have any observable effects on basal and food-evoked increases in DA efflux in the NAc (Figure 9). In the mPFC, however, BLA inactivation triggered dramatic oscillation of DA efflux that lasted well beyond the effects of lidocaine (Figures 10 and 12). Together these data suggest that excitatory inputs from the BLA to the NAc and mPFC (Kelley et al., 1982; McDonald, 1991b) provide important phasic and tonic influences on DA efflux in these terminal regions. Whereas electrical (Floresco et al., 1998; Jackson and Moghaddam, 2001) stimulation of the BLA elicit phasic increases in DA efflux in the NAc and mPFC, the effect of BLA inactivation on mPFC DA efflux demonstrates a role for the BLA in providing a stabilizing tonic influence on DA activity in the mPFC.

Both tonic and phasic influences on DA efflux in the NAc and mPFC likely involve BLA glutamatergic afferents to each of the two terminal regions (Kelley et al., 1982; McDonald, 1991a; McDonald, 1991b) rather than to DA neurons in the midbrain (Phillipson, 1979) (Figure 15). Studies that have utilized stimulation in conjunction with pharmacological techniques indicate that the BLA can modulate phasic DA efflux in the NAc and mPFC via presynaptic (Floresco et al., 1998) or polysynaptic pathways (Jackson and Moghaddam, 2001). A presynaptic mechanism of modulation is supported by the fact that the BLA sends direct projections to regions of the NAc and mPFC which also receive DA innervation (McDonald, 1991a; Johnson et al., 1994). This arrangement provides a means for independent modulation of DA efflux in the two terminal areas. Further differentiation between DA efflux in the NAc and mPFC may be subserved cortical regulation of subcortical DA. That is, glutamatergic projections from the mPFC to the NAc have been proposed to modulate DA efflux in the NAc (Sesack et al., 1989;

Wilkinson, 1997). Another mechanism that accounts for the differential effects of BLA inactivation on DA efflux in the NAc and mPFC (i.e., the oscillation effect in the mPFC and the absence of effect in the NAc) may involve different glutamate receptor subtypes in stimulating DA release in the two terminals. Both NMDA and AMPA/kainate receptors have a role in modulating DA release in the mPFC and NAc, but AMPA/kainate receptors may be preferentially involved in modulating DA release in the mPFC (Jedema and Moghaddam, 1996; Moghaddam et al., 1997).

An alternate explanation for these different effects of BLA inactivation may be related to recent finding that mPFC neurons project both directly and indirectly to DA neurons in the midbrain (Carr and Sesack, 2000)(see Figure 15). DA neurons that receive direct projections from the mPFC send dopaminergic projections back to the mPFC whereas those DA neurons that receive mPFC inputs via an interneuron provide dopaminergic input to the NAc (Carr and Sesack, 2000). If BLA neurons preferentially interact with prefrontal neurons that contact mPFC projecting DA neurons in the VTA, then interference with a tonic influence of the BLA on the mPFC could in turn destabilize a tonic excitatory influence of mPFC on DA neurons and thus on DA efflux in the mPFC.

In summary, recent studies, including the present experiment, suggest that the CeN and BLA have both phasic and tonic influences on terminal DA release in the NAc and mPFC. Specifically, the CeN exerts a tonic influence on DA activity in the NAc, and modulates stimulated phasic increases in DA in both the NAc and mPFC. The BLA provides a significant stabilizing influence on basal levels of DA in the mPFC. Additionally, the disruptive effects of CeN inactivation on food intake and the associated changes in DA efflux raises the possibility that CeN-modulated changes in DA activation

may be an important mechanism by which the sensory properties of food influence feeding behaviour.

EXPERIMENT III:

**EFFECT OF TRANSIENT INACTIVATION OF THE CENTRAL AND
BASOLATERAL NUCLEI OF THE AMYGDALA ON DOPAMINERGIC
CORRELATES OF SENSORY-SPECIFIC SATIETY**

Introduction

The approach to and consumption of food (i.e., food reward) can be explained by two dissociable psychological processes, the appetite or decision to eat food ("wanting") and the hedonic assessment of the sensory properties of food ("liking"), which in turn are mediated by different neural substrates (Berridge and Robinson, 1998). Evidence from *in vivo* monitoring studies suggests that the "wanting" of food is mediated by the mesotelencephalic DA system, as increased activity of DA neurons and terminal DA release is associated with both intrinsic (e.g., olfactory) and extrinsic (conditioned stimulus) cues associated with the food (Schultz et al., 1993; Bassareo and Di Chiara, 1997; Ahn and Phillips, 1998). Berridge and Robinson (1998) propose that activation of the mesotelencephalic DA system is required for the attribution of salience to sensory stimuli, which gain the capacity to act as incentives (i.e., to elicit behaviour). Functional disruption of the DA system, by lesions of DA terminals or blockade of DA receptors, leads to decreased food intake without affecting affective taste reactions which is a measure of the "liking" the food (Berridge and Schulkin, 1989; Pecina et al., 1997). Lesion and pharmacological manipulations which alter taste reactivity suggest that the "liking" of food involves benzodiazepine circuits in the brainstem (Berridge and Treit, 1986) and GABAergic, glutamatergic and opioid circuits in the forebrain (Bechara and

van der Kooy, 1992; Bakshi and Kelley, 1994; Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997).

Although there is substantial evidence for the dissociation of neural substrates of “liking” and “wanting” food reward, little has been done to address the neural mechanisms by which the “liking” of food may influence the “wanting” of food. In other words, how does the hedonic value of food stimuli modulate the incentive motivating activity of the mesotelencephalic DA system? One possibility may involve a forebrain target of the neural circuit for “liking”: namely, the amygdala. The amygdala appears to play a role in assigning motivational or emotional (positive and negative) significance to specific sensory stimuli. Animals lacking an intact amygdala are unable to associate sensory properties of food with its reward or aversive value (Aggleton and Passingham, 1982; LeDoux, 2000). It appears, then, that processes within the amygdala may be especially important in the modulation of the incentive value of information related to external stimuli, such as the sensory qualities of food, by internal motivational signals, such as hunger and satiety. In Berridge and Robinson’s terms (1998), the amygdala may relay the hedonic “liking” of food to the mesotelencephalic DA system to produce a motivational state of “wanting” the food.

The main objective of this experiment was to test the hypothesis that amygdalar modulation of DA activity is a critical neural substrate of sensory-specific satiety. Specifically, I propose that the amygdala is required for the attribution of incentive salience to food in accordance with the sensory properties of food under the current motivational state. There are several reasons to suggest that the CeN, rather than the BLA, may have a greater role in sensory-specific satiety. The CeN appears to mediate a

simpler form of associative learning (i.e., Pavlovian conditioning) (Everitt et al., 1999) which is relevant to specific information about deprivation states (Galaverna et al., 1993; Benoit et al., 1999). For example, rats with CeN lesions fail to show increased appetitive responding to a CS predictive of sodium chloride following administration of furosemide, a sodium-depleting agent (Galaverna et al., 1993). As such, the CeN may convey changes in the hedonic or reward value of food to the DA system, thereby modulating the differential attribution of incentive salience to food. The BLA, which has been implicated in mediating more complex forms of instrumental behaviour (Everitt et al., 1999), may have a less important role in modulating sensory-specific satiety.

To address these issues, Experiment III investigated the effect of CeN and BLA lesions, produced by reverse-dialysis of lidocaine, on sensory-specific satiety and associated changes in DA efflux in the NAc and mPFC. Only the "same food" condition was tested, with the first meal being presented while the CeN or BLA was reverse-dialyzed with lidocaine and the second meal being presented when the anaesthetic effects of the lidocaine had worn off. The discovery in Experiment II that inactivation of the BLA triggers oscillatory changes in DA efflux in the mPFC, precluded the assessment of food-evoked changes in DA activity. Thus, in this experiment the effects of lidocaine in the BLA on dopaminergic correlates of sensory-specific satiety were examined only in the NAc.

Methods

Twenty-six Long-Evans male rats were housed and cared for as described in the General Methods. Procedures pertaining to surgery, microdialysis, reverse-dialysis,

sensory-specific satiety experiments as well as histological and statistical analyses were as described in the General Methods section.

Subjects were assigned randomly to one of three reverse-dialysis/dialysis groups (CeN/NAc, CeN/mPFC, BLA/NAc) and implanted accordingly with guide cannulae. Following surgery, rats were placed on food restriction. Two weeks later, rats were given three opportunities to habituate to the foods, the testing chamber and to being tethered by the steel coil (Figure 1).

Each animal served as a subject in sensory-specific satiety tests conducted over two days. On each day, only Froot Loops were offered during both meals (i.e., the same food conditions). One session involved bilateral reverse-dialysis of lidocaine in the CeN or BLA (lidocaine condition) and in the other session the probe was flushed continuously with normal perfusate (control condition). For each animal, microdialysis probes were implanted into the NAc or mPFC unilaterally and reverse-dialysis probes into the CeN or BLA bilaterally. With respect to microdialysis, the left and right hemispheres were randomly assigned to the lidocaine condition and the other hemisphere to the control condition to equalize for any hemispheric differences. Dialysis samples were collected at 10 min intervals from the NAc and mPFC and immediately assayed for DA using HPLC-ED.

On each experiment day, baseline conditions continued until four consecutive samples showed less than 5% fluctuation in DA content before lidocaine or vehicle (perfusate) was reverse-dialyzed into the CeN or BLA for a total of 70 min. Following 20 min of perfusion with lidocaine the first meal was presented behind the screen. The second meal was presented 40 min following the end of lidocaine administration by

reverse-dialysis (i.e., when DA efflux had returned to baseline levels). In the control condition, probes in the CeN and BLA were continuously flushed with perfusate only and the two meals were presented at the same interval as in the lidocaine condition.

Following the final microdialysis session, brains were removed for histological verification of probe placements. Only those animals with tracts in the 1) prelimbic/infralimbic region of the mPFC or shell/core region of the NAc and 2) CeN in the rostral amygdala or BLA in the caudal amygdala of both hemispheres were included in the statistical analyses.

Results

Basal concentration of DA in microdialysates collected from the mPFC and NAc

The average concentration of DA (uncorrected for probe recovery) in the last three samples prior to administration of lidocaine by reverse dialysis in the CeN and BLA was 0.14 ± 0.19 nM in the mPFC and 3.03 ± 0.92 nM in the NAc.

Effects of transient inactivation of the CeN on sensory-specific satiety and correlated DA activity

Administration of lidocaine into the CeN lead to behavioural responses that were similar to those observed in CeN/NAc and CeN/mPFC rats in Experiment II. Generally, animals awoke from sleep and explored the chamber. However, when the first meal was presented in a food bin behind a screen, these rats did not make the normal anticipatory or orienting responses towards the food bin. In fact, several animals returned to a corner of the chamber and resumed sleeping or just sat still. When the screen was removed, both

CeN/NAc and CeN/mPFC rats took slightly longer than normal rats to approach the food bin (>1 min versus <1 min, respectively).

The effect of CeN inactivation on sensory-specific satiety was examined in a lidocaine and control condition utilizing only the “same food” protocol. There were no significant differences in the pattern of food consumed between the CeN/NAc and CeN/mPFC groups and, as such, behavioural data from the two groups were collapsed for subsequent statistical analyses. Rats infused with lidocaine into the CeN during the first meal did not show sensory-specific satiety (Figure 16, top panel, bar graph). There was a significant interaction of Condition \times Time on Food 1 consumed per 10 min [$F_{(7,112)}=4.616$; $p<0.001$]. Further analyses showed that, although the total quantity of food consumed during the first meal was not statistically different between the lidocaine and control conditions [$F_{(1,16)}=0.001$; $p<0.996$], there was a notable qualitative difference in the manner and rate of intake. In the lidocaine condition, rats handled and placed all morsels of the food in their mouths. However, over the 40 min meal, they ingested just over 50% of the food offered to them and dropped the rest. Furthermore, these rats did not show the typical decrease in amount consumed per 10 min associated with the development of satiety; their rate of intake remained relatively stable throughout the first meal. Following the 40 min recess (during which the CeN had been flushing with normal perfusate), animals were presented with a second meal of Food 1 and all CeN rats immediately approached the food bin and began to eat. During the first 10 min, rats consumed an amount that was similar to that consumed in the first 10 min of the first meal (1.8 ± 0.6 g and 1.6 ± 0.3 g, respectively). The decreased rate of food intake during the second meal was consistent with the development of satiety. When

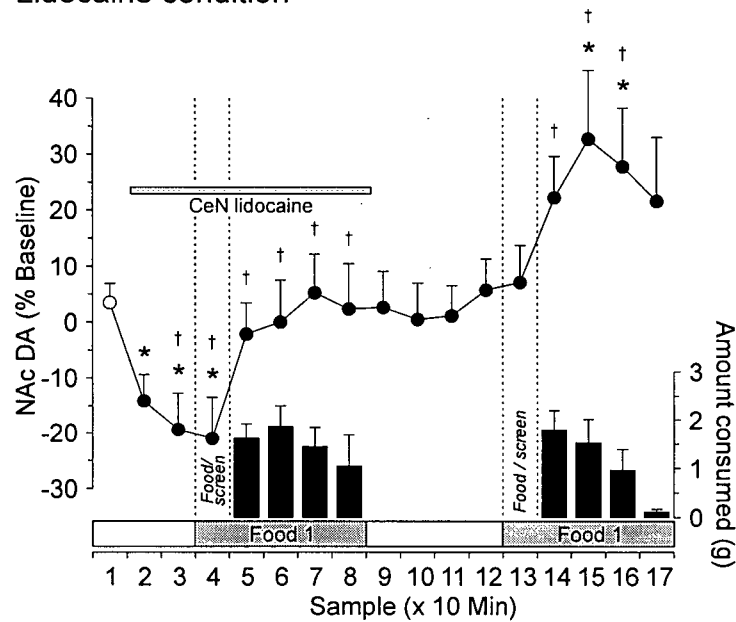


Figure 16. Effect of transient inactivation of the CeN on dopaminergic correlates of sensory-specific satiety in the NAc. Changes in DA efflux (line graph, left Y-axis) and amount of food consumed (bar graph, right Y-axis) per 10 min are presented as mean+SEM. Lidocaine condition (n=8), reverse-dialysis of lidocaine into the CeN bilaterally during samples 2-8 and normal perfusate during all other samples. Control condition (n=8), continuous dialysis of CeN with normal perfusate during all samples. Samples 4 and 13, highlighted by dashed lines, represent anticipatory periods during which access to food was prevented by a screen. Samples 5-8 and 14-17 represent consummatory periods during which rats had access to food. The baseline value (white circle) was used as the control mean in Dunnett's method of multiple comparisons (* $p < 0.05$). Dunn's test was used for comparisons between the lidocaine and control conditions († $p < 0.05$).

these same rats were tested in the control condition, they displayed normal sensory-specific satiety patterns of food intake over the two meals (Figure 16, bottom panel, bar graph).

A two-way repeated measure ANOVA showed that there was a significant Condition x Time interaction on DA efflux in the NAc [$F_{(16,112)}=7.602$; $p<0.001$]. Further analyses revealed a simple main effect of Time in the lidocaine [$F_{(16,112)}=6.089$; $p<0.001$] and control [$F_{(16,112)}=6.607$; $p<0.001$] conditions. Reverse-dialysis of lidocaine into the CeN significantly decreased basal DA efflux in the NAc by 19-21% below baseline ($p<0.05$; Figure 16, top panel, line graph). When the first meal was presented behind the screen, DA efflux in the NAc did not change; that is, there were no anticipatory changes in DA efflux. Consumption of the first meal of Food 1 was accompanied by increased DA efflux (+19%) that was significantly higher than the preceding (decreased) basal levels of DA ($p<0.05$). Although DA efflux remained elevated through the first meal, levels did not go beyond pre-lidocaine values. Presentation of Food 1 again as a second meal did not elicit anticipatory changes in DA efflux (sample 13); however upon consumption of this meal, there was a significant increase in DA efflux, reaching a maximal value of 33% ($p<0.05$), which then gradually returned to baseline. When the same rats were tested in the control condition, DA efflux mirrored the sensory-specific satiety pattern of food intake (Figure 16, bottom panel, line graph). The first meal elicited significant anticipatory (17%, $p<0.05$) and consummatory increases (upon to 42%, $p<0.05$). During the 40 min recess, DA efflux descended to baseline levels and remained there throughout the second meal.

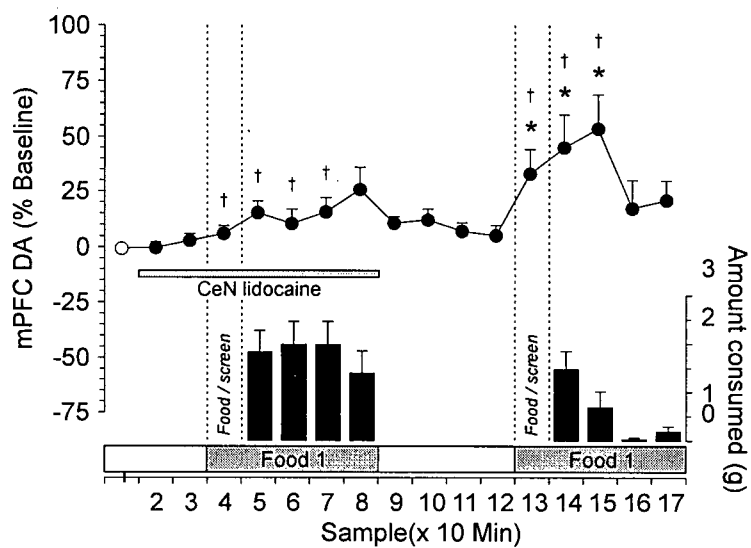
A two-way repeated measure ANOVA showed that there was a significant Condition x Time interaction on DA efflux in the mPFC [$F_{(16,128)}=8.331$; $p<0.001$]. Further analysis revealed a simple main effect of Time in the lidocaine [$F_{(16,128)}=4.561$; $p<0.001$] and control [$F_{(16,128)}=11.708$; $p<0.001$] conditions. Administration of lidocaine into the CeN did not affect basal DA efflux in the mPFC (Figure 17, top panel, line graph). However, there were no significant changes in DA efflux associated with the anticipatory and consummatory phases of the first meal despite normal consumption of Food 1. Presentation of the second meal elicited significant increases in DA efflux associated with the anticipatory and consummatory behaviours displayed during this meal (+43% and +58%, respectively; $p<0.05$).

When the same rats were tested in the control condition, changes in DA efflux mirrored the sensory-specific satiety pattern of food intake by the rats (Figure 17, bottom panel, line graph). Presentation of the first meal elicited significant increases in DA efflux during the anticipatory (+43%) and consummatory phases (+92%). Presentation of the second meal lead only to sampling small amounts of food and no significant changes in DA efflux were observed.

Effects of transient inactivation of the BLA on sensory-specific satiety and correlated DA activity

In contrast to CeN-inactivated rats, rats with BLA-inactivation did not show behavioural activation coincident with lidocaine administration. A similar observation was made in Experiment II. No statistical differences in the pattern of food intake were observed between the lidocaine and control conditions [$F_{(1,8)}=2.918$; $p<0.126$]. Thus, sensory-specific satiety was observed in both conditions, with rats consuming

Lidocaine condition



Control condition

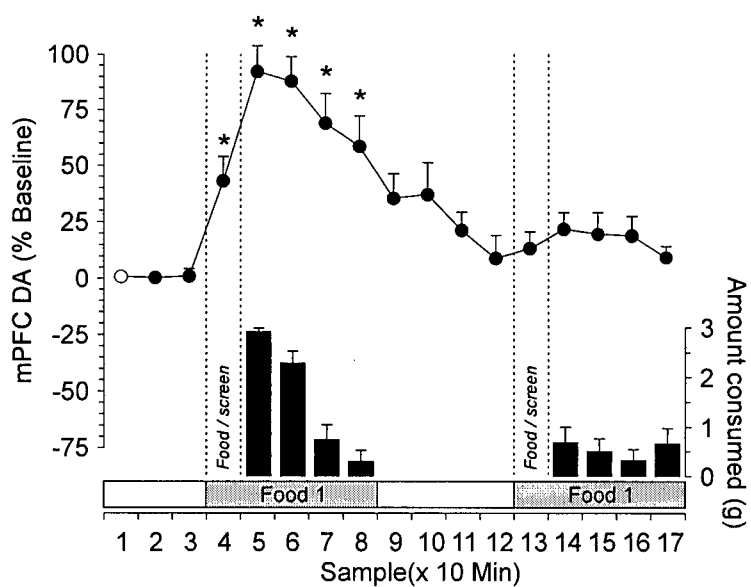


Figure 17. Effect of transient inactivation of the CeN on dopaminergic correlates of sensory-specific satiety in the mPFC. Shown are the lidocaine (n=9) and control (n=9) conditions. See Figure 16 legend for further explanation.

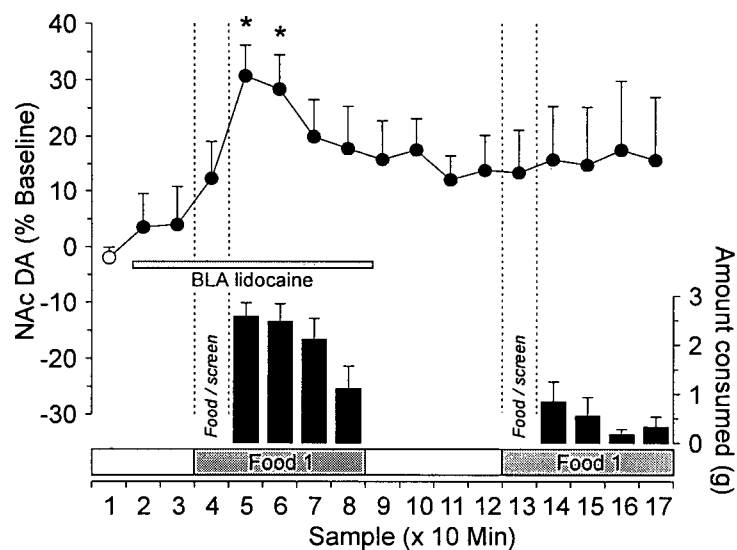
significantly less food during the second meal in the lidocaine (8.3 ± 1.3 g versus 1.9 ± 0.9 g; $p < 0.05$) and control (6.1 ± 1.1 g versus 1.1 ± 0.4 g; $p < 0.05$) conditions (Figure 18, bar graphs).

There were no statistically significant differences between the lidocaine and control conditions [$F_{(1,8)} = 0.848$; $p < 0.384$] but there was a significant main effect of Time in the lidocaine [$F_{(16,128)} = 1.745$; $p < 0.05$] and control [$F_{(16,128)} = 5.729$; $p < 0.001$] conditions. Changes in DA efflux mirrored the sensory-specific satiety pattern of food intake by the rats (Figure 18, line graphs). Presentation of the first meal behind the screen elicited anticipatory increases in DA efflux which were significant in the control condition (+16%, $p < 0.05$) but not in the lidocaine condition (+12%). Consumption of the meal was associated with significant increases in DA efflux, with a maximal increase of +30% and +31% in the lidocaine and control conditions, respectively ($p < 0.05$). The second presentation of the same meal did not elicit significant changes in DA efflux in either condition.

Histology

As depicted in Figures 19 and 20, tracts left by microdialysis probes were located in the prelimbic/infralimbic region of the mPFC (+3.2 to +2.7 mm AP) or straddled the shell/core region of the NAc (+2.2 to +1.6 mm AP). Tracts left by reverse-dialysis probes were located mainly in the CeN and but included some surrounding regions such as the intercalated masses, and substantia innominata (−1.8 to −2.6 mm AP). BLA tracts were seen in the basal and lateral nuclei, and in some animals, in the lateral aspect of the CeN (−2.8 to −3.3 mm AP).

Lidocaine condition



Control condition

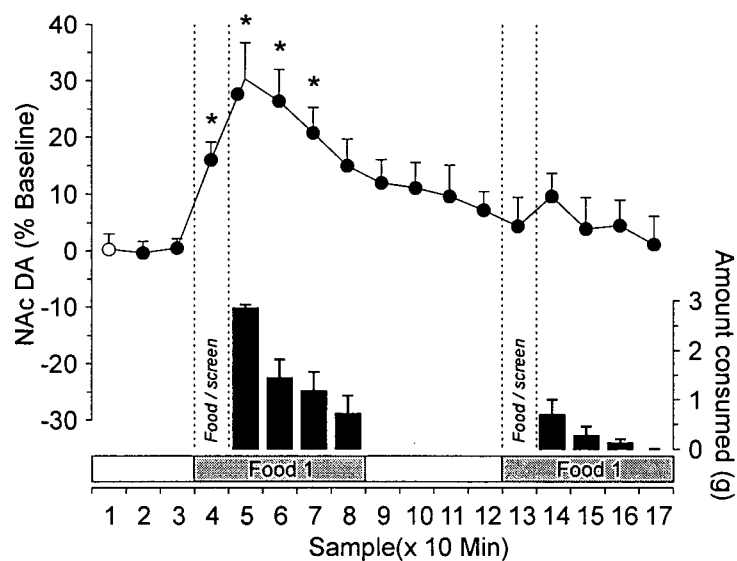


Figure 18. Effect of transient inactivation of the BLA on dopaminergic correlates of sensory-specific satiety in the NAc. Shown are the lidocaine (n=9) and control (n=9) conditions. See Figure 16 legend for further explanation.

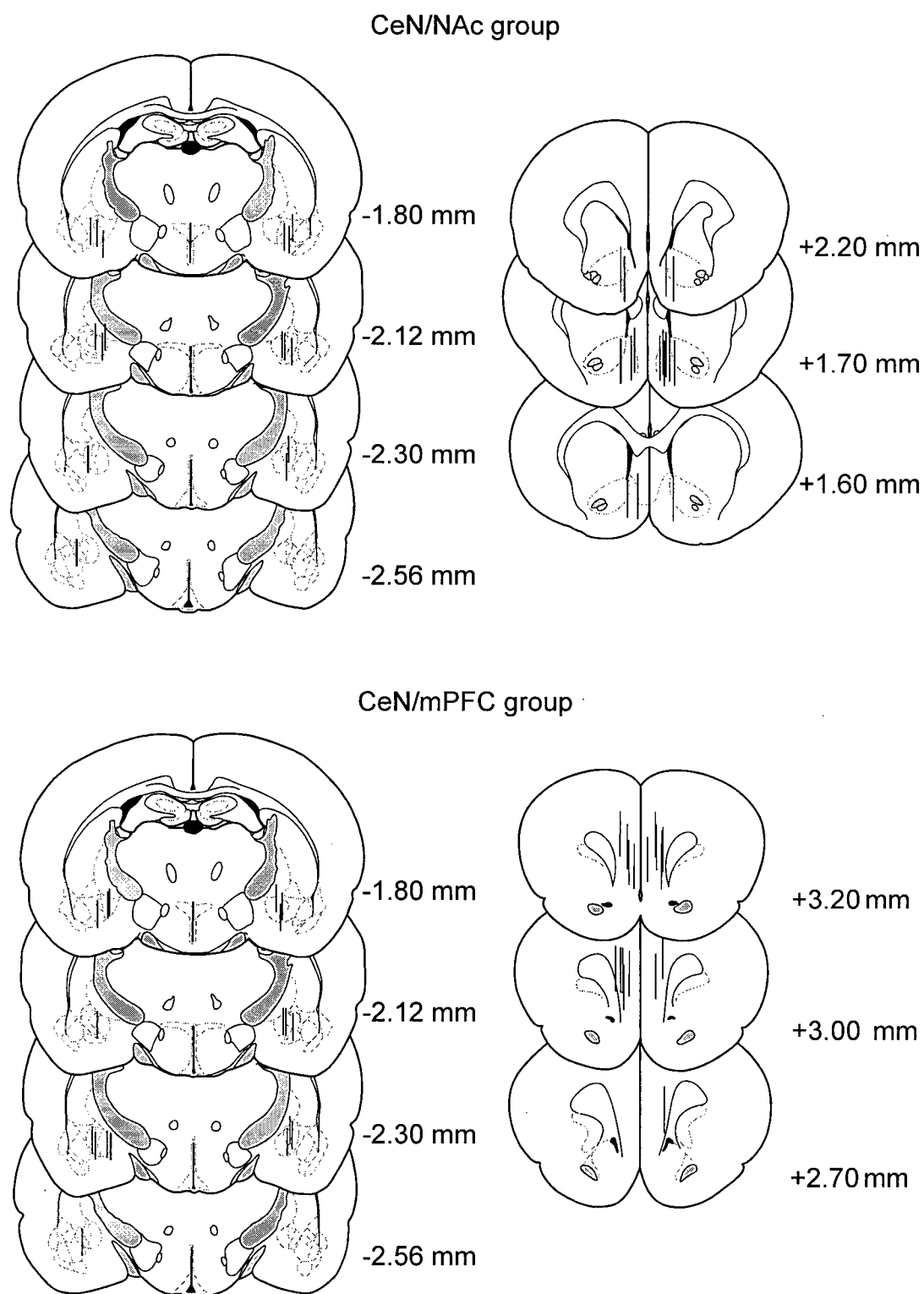


Figure 19. Location of probes Experiment III (CeN groups). Vertical lines represent the dialyzing lengths of microdialysis probes (2 mm) in the NAc and mPFC and reverse-dialysis probes (1.2 mm) in the CeN. Drawings of coronal sections were adapted from Paxinos and Watson (1997). Distance from bregma is indicated.

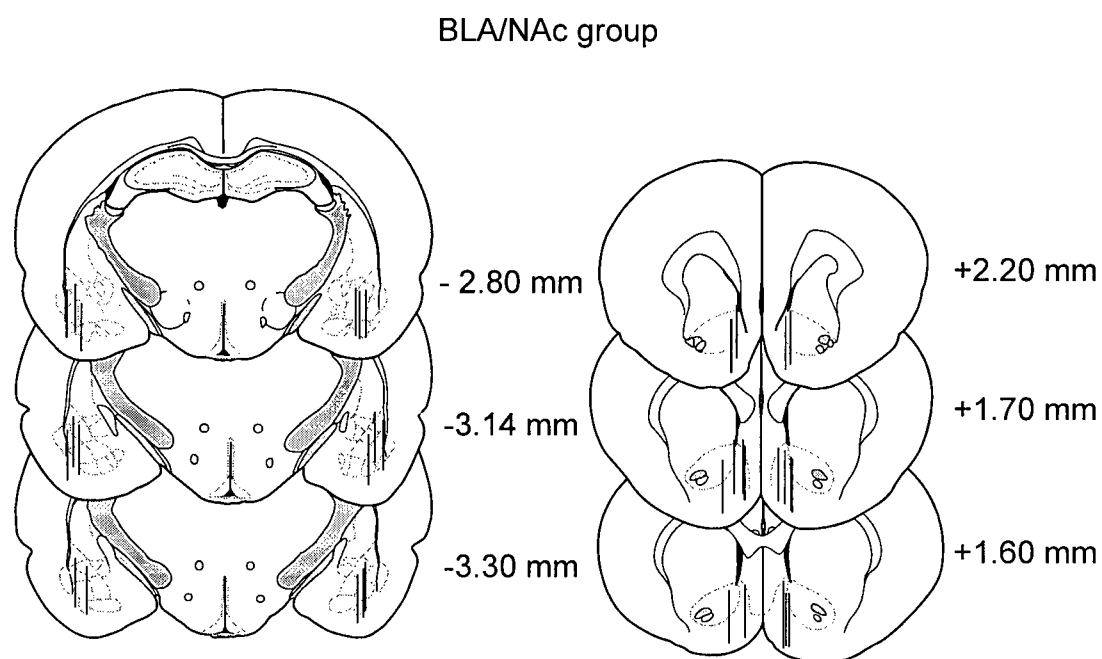


Figure 20. Location of probes in Experiment III (BLA group). Vertical lines represent the dialyzing lengths of microdialysis probes (2 mm) in the NAc and reverse-dialysis probes (1.8 mm) in the BLA. Drawings of coronal sections were adapted from Paxinos and Watson (1997). Distance from bregma is indicated.

Discussion

Role of CeN versus BLA in sensory-specific satiety

Lidocaine-inactivation of the CeN, but not the BLA, impaired sensory-specific satiety and the associated changes in DA efflux in the NAc and mPFC (Figures 16, 17 and 18). These results are in concordance with recent reports that the CeN and BLA are involved in different aspects of appetitively motivated learning (Everitt et al., 1999; Holland and Gallagher, 1999).

Everitt and his colleagues (Everitt et al., 1999; Everitt et al., 2000) have provided data to suggest that the CeN mediates conditioned reflexive responses towards stimuli predictive of food whereas the BLA is required for voluntary learned responses instrumental in obtaining food reward. This indicates that the functional involvement of the CeN and BLA in behavioural expression is dependent on task-specific demands. The paradigm used in the present study may be considered a "Pavlovian approach" version of sensory-specific satiety, in that the olfactory and visual stimuli (i.e., CS) associated with a particular food (i.e., US) serve as incentive sensory cues to exert control over approach and consummatory behaviour. The effects of CeN on sensory-specific satiety and mesotelencephalic DA transmission are consistent with this idea. Since BLA lesions do not have an effect on Pavlovian tasks, but do impair conditioned reinforcement tasks, there is a possibility that a conditioned reinforcement version of sensory-specific satiety may be impaired by inactivation of the BLA, in which learned behavioural responses are performed based on the predictive value of a cue. A recently reported instrumental version of sensory-specific satiety may be of relevance to this issue. Balleine and Dickinson (1998) trained animals to perform two instrumental actions for two differently

flavoured rewards. Animals were then prefed to satiety on one flavour (i.e., an incentive devaluation procedure) and then tested in extinction for their ability to adjust the relative rates of performance on the two actions based on their recall of the relatively devalued and inflated incentive value of the two rewards. The authors showed that animals perform less of the action associated with the prefed reward than the other action. In this context, inactivation of the BLA may lead to nondifferential rates of performance on both actions, paralleling the pattern of impairments produced by CeN-lesions in the Pavlovian version of sensory-specific satiety.

The behavioural effects of decreased DA levels in the NAc following inactivation of the CeN is different from the effects due to 6-OHDA-induced depletion of DA in the NAc. In the latter group, rats have the ability to generate motor movements to approach and consume the food (Berridge and Robinson, 1998), but just do not. In the former group, rats do approach and consume the food, but are impaired in a more subtle aspect of the regulation of intake as indicated by the present results. The absence of sensory-specific satiety in CeN rats tested in the lidocaine condition (Figures 16 and 17) can be considered to be the cumulative result of two effects of CeN-inactivation: an effect of inactivation during the first meal and the consequence of this inactivation on the second meal. First, during the period of CeN-inactivation, rats did not show anticipatory behaviours towards a palatable food presented behind the screen (despite being mildly food-deprived) nor satiety for the food (despite having consumed some portion of the food) at the end of the 40 min meal (Figures 16 and 17, bar graphs). Instead, these rats exhibited a disturbed manner of food intake reminiscent of Klüver-Bucy symptoms as observed in Experiment II. Animals maintained a vigorous rate of "sampling", possibly

reflecting their futile effort to assess the hedonic value of the sensory properties of the food or to establish an association of the sensory properties of the food with the hedonic "liking" of the food. Second, when the anaesthetic effects of the lidocaine had dissipated and the CeN rats were presented with a second meal of the same palatable food, these animals consumed a large proportion of the second meal. The decreases in the amount and rate of food intake during the course of this meal were similar to that observed during the first meal under normal conditions. One explanation for this effect may be that without the CeN, and thus without the ability to assess the hedonic value of the olfactory and gustatory properties of the food, sensory-specific satiety can not be expressed.

The present results suggest that without the CeN, the first meal is not able to influence the second meal. This may be related to the idea of "oral metering" or memory for what and how much has been eaten (Rozin et al., 1998). In some fortuitous case studies, researchers have described the eating behaviour of densely amnesic but otherwise normal patients, including patient H.M. (Hebben et al., 1985; Rozin et al., 1998). All had suffered damage to the mediotemporal lobe, including the hippocampus and amygdala. These individuals would consume a fairly large meal and, 10 to 20 min later not be able to recall having had the meal. Furthermore, they would willingly accept and consume a second and sometimes, a third identical meal (Rozin et al., 1998). In one instance, one subject was heard to say that his "stomach felt a little tight" prior to refusing a subsequent meal (Rozin et al., 1998). Thus, within the physiological limits of satiety (or physical fullness), memory for what has recently been consumed is important in the initiation and termination of food intake. It is possible that in the rat, the CeN may have a central role in integrating sensory and satiety signals, in effect, forming a memory for what has been

eaten recently. As observed in this experiment, inactivation of the CeN lead subsequently to the consumption of a second meal of the same food – as if the subjects had no memory of the first encounter with the food 40 min earlier.

CeN modulation of incentive salience attribution

The present results suggest that memory of what has been eaten may reflect the incentive value of that particular food. If, as suggested above, the CeN is an important part of the circuit which transforms “liking” into “wanting” of food, then without this nucleus, the incentive salience attribution process may be disrupted. Support for this idea comes from the finding that during the period of CeN-inactivation, there was no change in DA efflux during the anticipatory period and a significant attenuation of DA efflux during the consummatory phase (Figures 16 and 17, line graphs). It is important to note that DA efflux was *attenuated* rather than *abolished* during the latter phase, as this indicates the possibility that the food may have had some residual incentive salience for the animals. Furthermore, Galaverna and his colleagues (1993) have shown that CeN-lesioned rats display increased hedonic taste reactivity to a salt solution when they are sodium-depleted, indicating that the “liking” aspect of reward is unaffected by CeN-lesions. Berridge (1996) suggests that the CeN may be important in directing the attribution of salience to external stimuli relevant to an animal’s internal need or deficit (i.e., deficit-specific salience). Similarly, the CeN may be important in directing the attribution of incentive salience to the olfactory or gustatory properties of food modulated by satiety (i.e., sensory-specific salience). In the rat, the CeN receives gustatory and olfactory information as well as feedback regarding the motivational state of the animal. Furthermore, anatomical and electrophysiological evidence suggests that GABAergic

projections from the CeN contact, either directly or indirectly, DA neurons in the midbrain.

EXPERIMENT IV:
EFFECT OF REPEATED FOOD RESTRICTION AND BINGE FEEDING
CYCLES ON SENSORY-SPECIFIC SATIETY

Introduction

The causal relationship between previous experience with food restriction and subsequent change in food-related behaviours, such as choice and meal size, is well documented in the animal literature (Specker et al., 1994; Del Prete et al., 1994; Hagan and Moss, 1997). In humans, food restriction – whether voluntary such as when dieting or compelled during starvation as a consequence of famine or war (Polivy et al., 1994) or infamous Minnesota semi-starvation experiment (Franklin et al., 1948) – has been shown to lead to compulsive overeating or binge eating that can last for years after the experience.

A recent study by Hagan and Moss (1997) confirm that persistent patterns of binge eating may be triggered by brief episodes of compensatory overeating (i.e., binge-eating) during prolonged food restriction. In their study, rats were placed on an 80-day feeding regimen that consisted of mild food deprivation periodically interrupted by opportunities to consume unlimited amounts of food. When given a feeding test 30 days later (during which time food was freely available), the rats engaged in “exaggerated bouts of feeding” that lasted for over 7 hr. Consumption of a highly preferred food (i.e., cookies) during the binge periods further intensified the hyperphagic effects.

In addition to the hyperphagia, restriction-binge patterns of food intake may lead to difficulty in utilizing physiological satiety cues to end a meal. In normal individuals, sensory-specific satiety, which precedes the development of gastric fullness, is thought to be an important control mechanism in the termination of a meal, in both humans and animals (Hetherington, 1996; Hetherington and Rolls, 1996; Rolls, 1999). Individuals who do not maintain a regular diet fail to display sensory-specific satiety. Hetherington and Rolls (1989) examined several groups of individuals and reported that anorectic patients and nondieters showed normal sensory-specific satiety whereas bulimic patients and dieters failed to do so. The authors proposed that the absence of sensory-specific satiety may explain why the latter group of individuals eat beyond the point of satiety during binge episodes (Hetherington and Rolls, 1989).

In mimicking the binge-eating patterns of bulimic patients, Hagan and Moss (1997) demonstrated that repeated alternations in food restriction and binge opportunities can alter normal limits of food intake in rats. It is possible that this hyperphagia is also related to impairment in the control of food intake by sensory-specific satiety. Thus, the objective of this experiment was to determine whether repeated cycles of food restriction and binge feeding opportunities, as compared to sustained mild food deprivation, can lead to diminished sensory-specific satiety in rats. As binge feeding on highly palatable food was reported to exacerbate the hyperphagic effects, the present study also included a condition in which rats had unlimited access to cookies during binge periods.

A particularly noteworthy aspect of the restriction-binge cycles of food intake is the periodic occurrence of binge opportunities which is similar to the "repeated, intermittent" administration of amphetamine in behavioural sensitization protocols

(Kalivas and Stewart, 1991; Fiorino and Phillips, 1999b). In addition, food restriction also triggers physiological stress responses (Broocks et al., 1990), which in turn activate the mesotelencephalic DA system (Imperato et al., 1989; Piazza et al., 1996), an important neural substrate of amphetamine- and cocaine-induced sensitization and cross-sensitization effects (Robinson and Berridge, 1993; Prasad et al., 1995; Sorg and Prasad, 1997; Fiorino and Phillips, 1999a). It is hypothesized that rats exposed to repeated food restriction-binge cycles will display decreased sensory-specific satiety, a factor that may account for subsequent hyperphagia when placed on a free-feeding schedule. This pattern of results would have important implications for stress-induced behavioural sensitization to food (i.e., stress-induced hyperphagia) and the involvement of DA in mediating this effect through altered attribution of incentive salience.

Methods

Thirty-six Long-Evans male rats were housed and cared for as described in the General Methods. Sensory-specific satiety procedures and statistical analyses were as described in the General Methods section.

Subjects were divided into nine tetrads matched for weight. Each rat within a tetrad was randomly assigned to one of four feeding conditions (Figure 21). In all instances of food restriction, rats were allowed enough chow (Rat Diet 5012; PMI Feeds Inc., Delta, B. C.) to maintain their body weights at 85-90% free-feeding weight.

Two groups were allowed four days of restricted access to chow followed by two days of unlimited food. One group of animals had unlimited access to Nilla vanilla

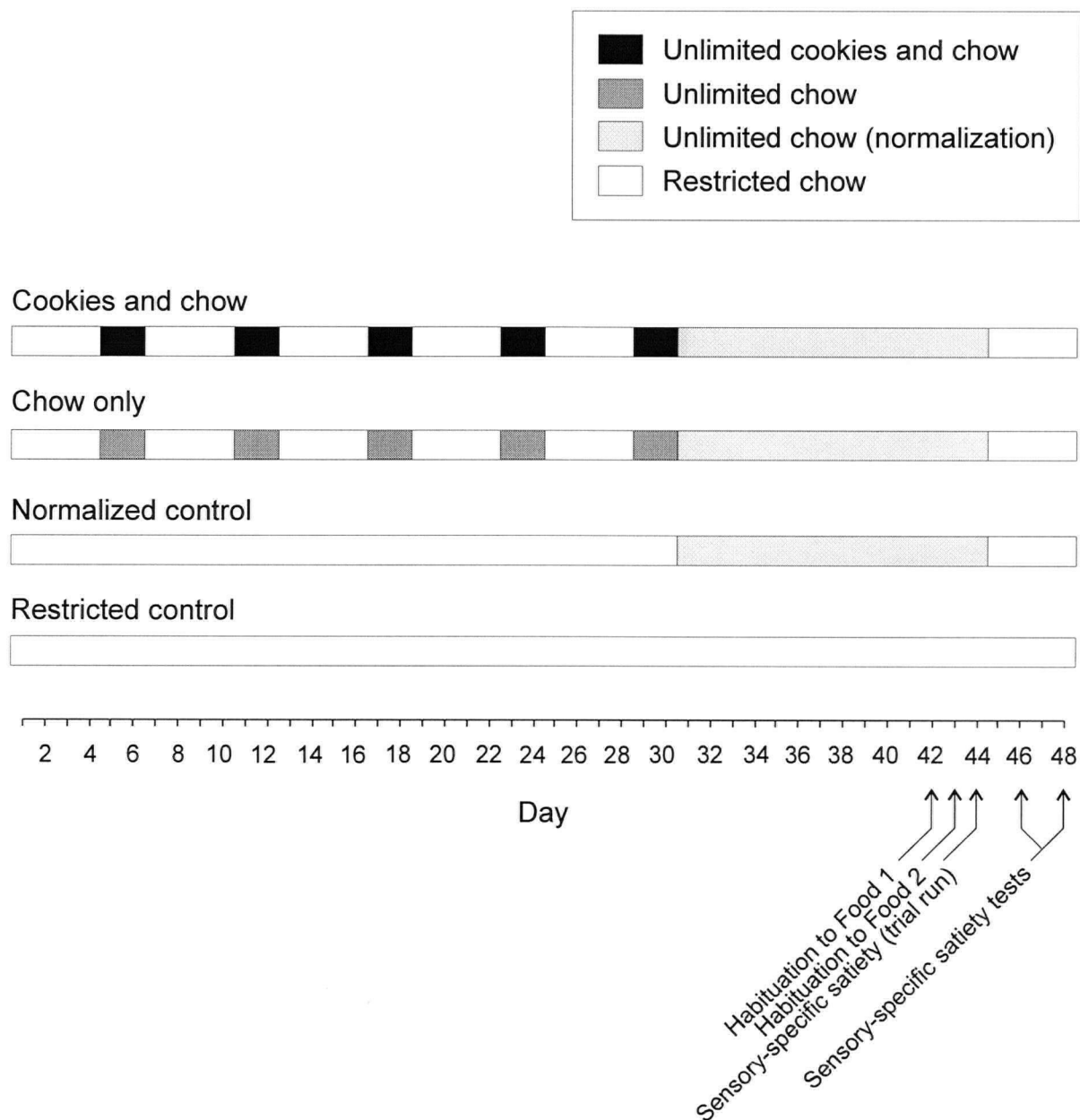


Figure 21. Schematic of repeated restriction-binge cycles used in Experiment IV.

flavoured cookies (4.75 cal/g; Christie Brown and Co., Division of Nabisco Ltd., Etobiko, Ontario) and maintenance chow (i.e., the “cookies and chow” group) and the other group had unlimited access to chow only (i.e., the “chow only” group). This restricted-unlimited pattern of food availability was repeated for five cycles (i.e., 30 days). Rats in both feeding conditions were then given 14 days of free access to chow to recover from the cycles of restriction and to provide an opportunity for food intake and body weights to normalize. A third “normalized” control group was placed on food-restriction for 30 days and then normalized for 14 days. A fourth “restricted” control group was maintained on a food restriction schedule for the entire 44 day period of the experiment. Body weights of all subjects were recorded at the same time daily, prior to testing or feeding. Habituation sessions for all animals took place during the last three days of the 44 day treatment period. On the 45th day, all groups were food-restricted before being assessed for sensory-specific satiety on the 46th and 48th day.

Due to the large amount of body weight data, statistical analyses were performed on blocked data. Blocking involved calculating an averaged body weight per designated period: one baseline period, five restriction periods, five free-feeding periods and one normalization period. In this way, the numbers of between group comparisons (i.e., from 44 to 12 comparisons) as well as Type I error rates were substantially reduced.

Results

Effect of restriction-binge cycles on rate of weight gain

At the start of the food restriction schedule, there was a slight decrease in body weight of all groups that lasted about 10-12 days (Figure 22). With the start of the third

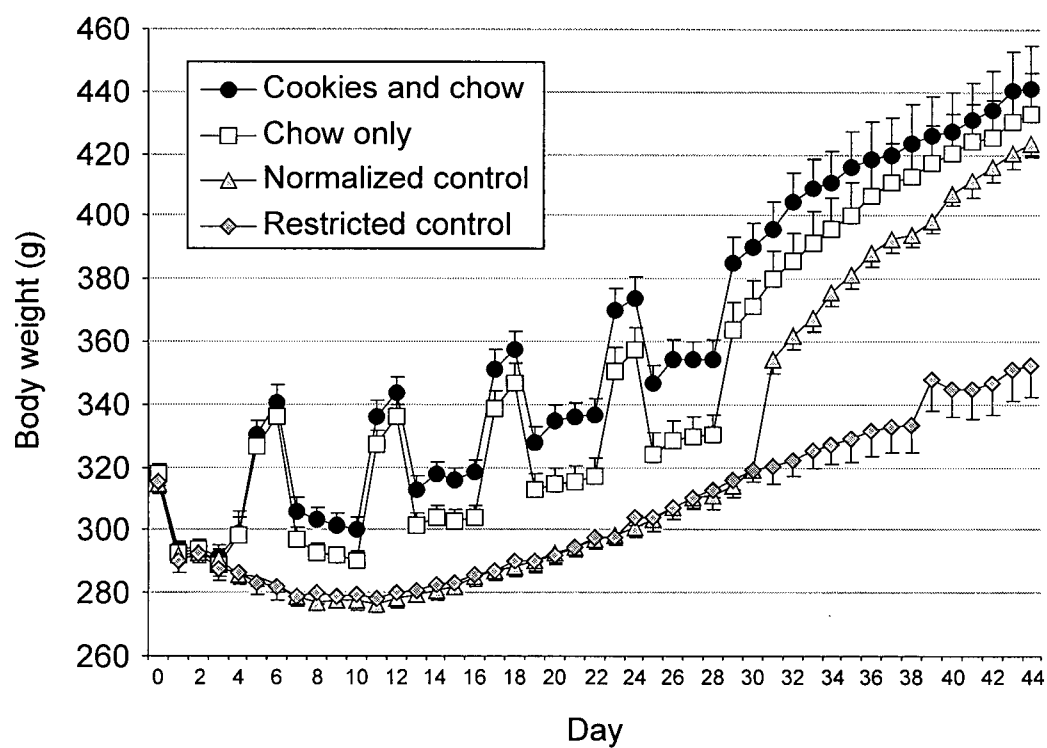


Figure 22. Effect of repeated restriction-binge cycles on body weight. Data are shown as mean \pm SEM.

cycle, the body weights of all animals began to increase gradually, even during the restriction phase in cycled animals. A two-way ANOVA showed that there was a significant Group x Day interaction on body weight [$F_{(30,320)}=25.549$; $p<0.001$]. Body weights of the “cookies and chow” and “chow only” cycled groups were significantly higher than the “normalized” and “restricted” control groups ($p<0.05$), but the two cycled groups were not statistically different from each other nor were the two control groups. Whereas the two control groups showed a gradual rate of weight gain, both cycled groups showed fluctuations in weight that closely mirrored the feeding cycles. The free-feeding phases of all cycles were accompanied by increases in body weight in both cycled groups (mean increases of 33 ± 3 g in the “cookies and chow” group and 32 ± 3 g in the “chow only” group), with the “cookies and chow” rats having a tendency for slightly bigger changes with each cycle than the “chow only” rats. Weights recorded during the normalization period showed that three normalized groups were not statistically different from each other but significantly different from the “restricted” control group ($p<0.05$).

Effect of restriction-binge cycles on sensory-specific satiety

There was a significant interaction of Group x Test Condition (same or different) on measures of sensory-specific satiety ($F_{(3,32)}=13.898$; $p<0.001$). Whereas the uncycled “restricted” and “normalized” control groups showed sensory-specific satiety, the cycled “cookies and chow” and “chow only” groups did not (Figure 23). There were no statistical differences in the amount consumed between the two cycled groups [$F_{(1,16)}=0.235$, $p<0.634$] or between the two control groups [$F_{(1,16)}=0.908$, $p<0.355$].

Control groups. When a screen preventing access to the food bin was removed, all rats quickly approached the food bin and began eating the first meal (Food 1). In the

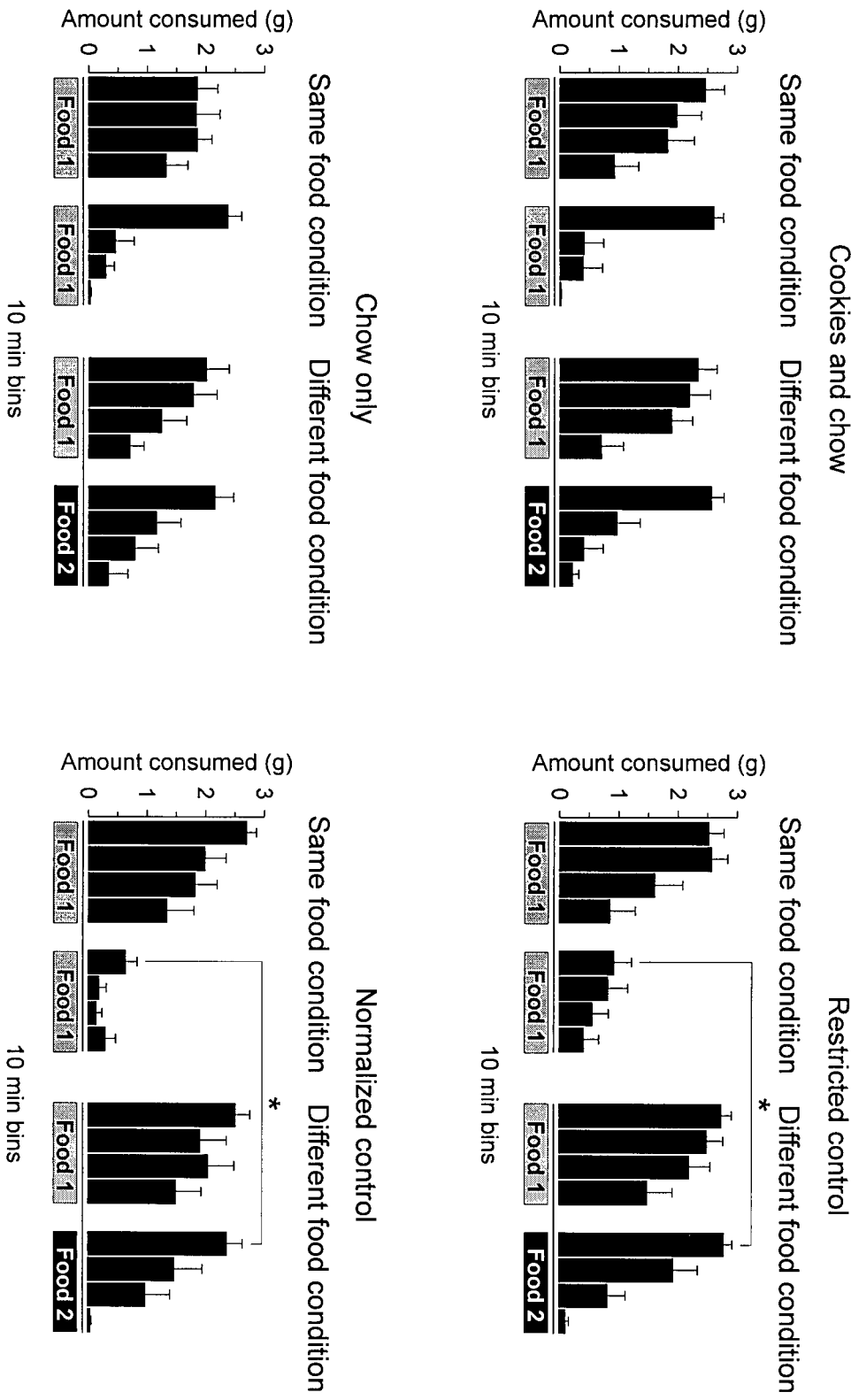


Figure 23. Effect of repeated restriction-binge cycles on sensory-specific satiety. Shown is the amount of food consumed per 10 min (mean+SEM) during the same and different food conditions of the “cookies and chow” (n=9), “chow only” (n=9), restricted control (n=9) and normalized control (n=9) groups. Dunn’s test was used to compare the amount of food consumed in the first 10 min of the second meal in the same and different food conditions (*p<0.05).

control groups, the total amount of food consumed during the first meal was similar in the two conditions (7.1 ± 0.8 g in the same food condition, 7.1 ± 0.8 g in the different food condition) and did not depend on whether Loops or Rings were offered [$F_{(1, 7)} = 0.153$; $p < 0.707$]. The gradual decrease in rate of consumption during the first meal indicated that animals were achieving satiety (Figure 23, right side, top and bottom panels). If Food 1 was offered again during a second meal (same food condition), animals took much longer to approach the food and occasionally sampled and consumed a much smaller amount than during the first meal (0.8 ± 0.3 g during the first 10 min). However, when rats were offered a novel palatable food (Food 2, different food condition), they approached the food promptly and ate significantly more (2.6 ± 0.5 g in the first 10 min) than when presented with Food 1 a second time ($p < 0.05$).

Cycled groups. Similar to the control groups, animals in the cycled groups quickly approached and ate the first meal (8.1 ± 0.8 g in the same food condition, 8.4 ± 0.8 g in the different food condition) and this effect did not depend on whether Loops or Rings were offered [$F_{(1, 7)} = 0.097$; $p < 0.656$]. The decreased rate of consumption over the 40 min meal indicated the development of satiety for Food 1 (Figure 23, left side top and bottom panels). When the cycled groups were presented with the second meal, the amount of Food 1 consumed was not significantly different from that of Food 2 (2.5 ± 0.3 g versus 2.5 ± 0.5 g, respectively, within the first 10 min).

Discussion

Changes in weight gain

Rats in the restriction-binge cycle groups had a higher rate of weight gain than the control group rats, with those given access to unlimited cookies during binge periods maintaining a higher body weight during the 44 day feeding regimen. This pattern of weight gain parallels the tendency for bulimics, who alternate between restriction and bingeing, to be moderately over weight (Diagnostic and Statistical Manual of Mental Disorders, 4th edition, American Psychological Association, 1994). The progressively bigger difference in weight between the "cookies and chow" and "chow only" groups is likely due to increased fat storage as a result of consuming large amounts of cookies, a highly caloric food. Variety in the type of foods available (i.e., a "cafeteria diet") is also associated with increased food intake and weight gain (Treit et al., 1983; Louis-Sylvestre et al., 1984). Thus, access to two types of foods, rather than any one food, may have contributed to the higher body weight of the "cookies and chow" group. However, the restriction and binge pattern itself may also promote weight gain. Support for this comes from a study that reported unusually high rates of lipogenesis in rats following multiple cycles of starvation-refeeding (Kochan et al., 1997).

Sensory-specific satiety is attenuated following restriction-binge cycles

A test for sensory-specific satiety was employed to investigate whether restriction-binge cycles had a significant influence on the regulation of food intake. Under normal circumstances, sensory-specific satiety is displayed by the development of satiety to a familiar food, whereas a food with different novel sensory qualities can elicit

further intake in sated animals and humans (Rolls, 1984; Hetherington and Rolls, 1996). The present experiment showed that rats that experienced repeated restriction-binge cycles followed by a two-week recovery period did not show sensory-specific satiety (Figure 23). Interestingly, the availability of cookies during binge episodes did not potentiate the disruptive effects of the restriction-binge cycles on sensory-specific satiety, although rats in the “cookies and chow” group did maintain a higher rate of weight gain than the “chow only” group throughout the experiment. In contrast to the cycled groups, rats that were mildly food-deprived throughout the 44 day feeding regimen or those allowed to normalize their weights during a 14 day period of *ad libitum* consumption showed normal sensory-specific satiety. These data suggest that the critical aspect of the restriction-binge cycles leading to disrupted sensory-specific satiety is the repeated switches from food restriction to unlimited access to food, and not to food deprivation *per se*. In fact, maintaining a mild level of deprivation, as in the “restricted” control group, likely facilitated sensory-specific satiety, as the general motivation to eat was increased in these animals and along with an increased tendency to consume a novel-tasting food during the second meal.

The feeding regimen used in this experiment was significantly abbreviated in length from that used by Hagan and Moss (1997) (44 days versus 110 days, respectively). Our results suggest that relatively few exposures to binge eating conditions during prolonged food restriction are sufficient to produce significant changes in the regulation of food intake. Specifically, the absence of sensory-specific satiety suggests that changes in the regulatory mechanism may include reduced sensitivity to cephalic satiety factors, which are believed to be important in the termination of a meal (Hetherington, 1996).

Hagan and Moss' study (1997) indicates that continued occurrence of binge episodes can lead to more severe dysregulation of food intake, as evidenced by the "exaggerated bouts of feeding" lasting over 7 hr, and possibly to the development and perpetuation of binge-eating disorders.

A stress-induced behavioural sensitization hypothesis: DA as a common denominator of sensitization

The present results have important implications for the role of DA in the behavioural sensitization effects of stress. Extreme stress is associated with inhibition of food intake (Harris et al., 2000), but mild stress, such as tail-pinch, short period of restraint or brief handling of an animal, can stimulate food intake (Levine and Morley, 1982; Morley et al., 1983; Badiani et al., 1996). Food restriction is also considered to be a mild form of stress in animals (Harris et al., 2000) leading to increased food intake (Specker et al., 1994; Del Prete et al., 1994; Hagan and Moss, 1997).

In contrast to acute or chronic stress, repeated, intermittent exposure to brief restraint or tail pinch can lead to several forms of stress-induced behavioural sensitization, including hyperphagia (Heinrichs et al., 1992; Badiani et al., 1996). The repeated, intermittent pattern of administration of stress or other sensitizing stimuli (e.g., psychostimulants like cocaine and amphetamine) is widely recognized to be an important factor for inducing behavioural sensitization (Kalivas and Stewart, 1991; Prasad et al., 1995; Sorg and Prasad, 1997; Cador et al., 1999; Fiorino and Phillips, 1999b). The restriction-binge cycles employed in the present experiment is reminiscent of these sensitization protocols and thus, raises the possibility that these cycles may trigger a sensitization process. That is, if each cycle of food restriction and binge episode is

experienced by subjects as a physiological stressor, then it is possible that repetition of such cycles may be sufficient to induce sensitized responses to food (i.e., hyperphagia). The absence of sensory-specific satiety in restriction-binge cycled rats raises the further possibility that stress-induced hyperphagia may also involve dysregulation of sensory factors that normally control food intake via sensory-specific satiety.

Behavioural sensitization phenomena appear to depend on functional changes in the mesotelencephalic DA system (Kalivas and Stewart, 1991; Robinson and Berridge, 1993). Specifically, the induction and expression of sensitization is dependent on altered DA function in the VTA and NAc (Robinson and Berridge, 1993; Cador et al., 1999; Fiorino and Phillips, 1999a). Cross-sensitization effects also support the hypothesis that the mesotelencephalic DA system is a common neural substrate in the expression of behavioural sensitization (Robinson and Berridge, 1993; Sorg and Prasad, 1997; Fiorino and Phillips, 1999a). As such, enhanced DA efflux in the NAc has been observed to occur during amphetamine-induced locomotion in foot-shock sensitized rats (Prasad et al., 1995). Similarly, enhanced DA efflux in the NAc is correlated with facilitation of sexual behaviour in rats after amphetamine-induced sensitization (Fiorino and Phillips, 1999a). Food restriction also has been effective in modulating food-evoked changes in DA efflux in the NAc (Pothos et al., 1995). This is consistent with the hypothesis that the hyperphagia and absence of sensory-specific satiety in food restriction cycled rats may also be attributed to changes in the mesotelencephalic DA system.

The critical link between stress-induced behavioural sensitization (as proposed here) and enhanced DA responses may involve the hypothalamic-pituitary-adrenal (HPA) axis. Stress activates the hypothalamus-pituitary-adrenal axis, which ultimately controls

the release of glucocorticoids. It is suggested that glucocorticoids can act as "endogenous psychostimulants" (Piazza et al., 1996) and similarly increase the activity of mesencephalic DA neurons. In support of this, DA neurons have been shown to express receptors for corticosteroids, the major glucocorticoid in the rat (Häfstrand et al., 1986). Corticosterone, administered at a dose similar to stress-induced plasma concentrations, inhibits DA reuptake (Gilad et al., 1987), reduces DA catabolism (Rothschild et al., 1985), and leads to increased basal levels of DA efflux in the NAc (Imperato et al., 1989) as well as augmented increase in DA efflux during feeding behaviour (Piazza et al., 1996).

Functionally, the mesotelencephalic DA system has been implicated in mediating appetitive responses related to the incentive value of food. Each restriction-binge cycle may lead to increased levels of plasma glucocorticoids that activate the DA system. In turn, repeated glucocorticoid activation of the DA system may alter its function, such that it becomes unresponsive to normal modulatory influences of sensory and satiety signals. Thus, the absence of sensory-specific satiety in stress-sensitized animals may be explained functionally as the inappropriate attribution of incentive salience to food by the DA system.

If, indeed, the present results are due to stress-induced sensitization involving the mesotelencephalic DA system, several interesting predictions follow. For instance, this may explain the effects of anxiogenic and anxiolytic drugs on food intake. If anxiogenic drugs were administered in place of the restriction-binge cycles, they may mimic the effects of the restriction-binge cycles and diminish sensory-specific satiety. In contrast, co-administration of anxiolytic drugs during restriction-binge cycles may attenuate the

stressful effects of the cycles, and normalize sensory-specific satiety. Additionally, enhancement or attenuation of corticosterone function may similarly interact with the restriction-binge cycles. Finally, direct sensitization of the DA system by repeated treatment with amphetamine may alter sensory-specific satiety, but in this case, it is predicted that sensory-specific satiety may be enhanced, as incentive properties of the novel food would be selectively endowed with enhanced salience while the familiar satiated food would remain devalued.

In summary, the results of this experiment suggested the possibility that the experience of stress, as a consequence of restriction-binge cycles, may have a dysregulatory influence on DA function thereby resulting in inappropriate attribution of incentive salience to food. This in turn would result in hyperphagia due to the dysregulation of cephalic factors known to play a major role in the regulation of feeding behaviour.

GENERAL DISCUSSION

Summary of experimental results

Experiments in this thesis utilized *in vivo* microdialysis and reverse-dialysis techniques to investigate whether DA transmission and its modulation by the CeN and BLA may be potential neural substrates of sensory-specific satiety in the rat. In each experiment, rats were given an opportunity to consume a meal of a palatable food to the point of satiety. In a subsequent test meal, animals were presented with the same food fed to satiety or a different food. Under normal circumstances, rats sampled very little of (or ignored) the familiar food but ingested significantly greater amounts of the novel food.

Experiment I demonstrated that changes in DA efflux in the NAc and mPFC closely mirrored the different phases of sensory-specific satiety. The consumption of an initial meal of a palatable food elicited significant increases in DA efflux in the mPFC and NAc. However, during the second meal, increased DA efflux in both terminals was observed only during consumption of a different palatable food but not the food eaten to satiety. During the anticipatory phases, when rats only had olfactory and visual information about the food placed behind a screen, food that was perceived as being high in incentive value (i.e., when the animals were hungry or when the palatable food had novel sensory properties) was associated with robust increases in DA efflux in the NAc and mPFC. Food with little incentive value (because it had recently been eaten to satiety) failed to elicit anticipatory changes in DA efflux in either terminal region. These results are consistent with the incentive salience hypothesis of DA (Berridge and Robinson,

1998) which predicts that salience should be attributed to food with high incentive value. Generally, no significant differences in the profile of changes in DA efflux were observed in the NAc and mPFC during sensory-specific satiety. However, there was one notable exception: when a novel food was presented behind the screen at the beginning of the second meal, there was an anticipatory increase in DA efflux in the mPFC but not in the NAc. The food-specific increases in DA efflux suggests that activation of the DA system is modulated by the incentive value of food. Specifically, DA efflux in the NAc may be more strongly influenced by the motivational state of the animal whereas DA efflux in the mPFC is related to the incentive sensory properties of food.

The results of Experiments II revealed for the first time that distinct subregions within the amygdala differentially affect basal and food-evoked DA efflux in the NAc and mPFC. Reverse-dialysis of lidocaine into the CeN lead to decreases in basal levels of DA efflux in the NAc, but not in the mPFC, that were time-locked to the duration of the sodium channel blocking effects of lidocaine. Furthermore, presentation of food during the period of inactivation evoked increases in DA efflux in both the NAc and mPFC that were significantly attenuated. In contrast, inactivation of the BLA affected neither food intake nor DA efflux in the NAc, but triggered long-lasting oscillating increases and decreases in DA efflux in the mPFC (irrespective of whether food was presented or not). These results suggested that modulation of DA transmission by the amygdala, and in particular by the CeN, may be a mechanism by which sensory-reward associations can direct behavioural responses.

In Experiment III, inactivation of the CeN, but not the BLA, impaired sensory-specific satiety. When the CeN was inactivated and the first meal was presented, rats

showed an altered pattern of food intake, which was not consistent with the development of satiety. These rats continuously examined the food orally (i.e., tasted the food) but did not consume all of the offered food. There were no anticipatory behavioural responses to the food when it was initially presented behind the screen, and accordingly, no anticipatory changes in DA efflux were observed. In the consummatory phase, there was an attenuated phasic increase in DA efflux in both the NAc and mPFC. Presentation of the same food in a second meal elicited both anticipatory and consummatory responses as well as associated increases in DA efflux in the NAc and mPFC, similar to that evoked by a novel food. However, inactivation of the CeN appeared to have a longer-lasting effect on DA efflux in the mPFC, as DA efflux in the mPFC was still attenuated during the second meal. The nature of the deficits in feeding behaviour and the disrupted changes in DA efflux were consistent with the hypothesis that the absence of CeN input to the mesotelencephalic DA system results in a decrease in "DA tone", which not only attenuates an animal's response to novel stimuli, but also impairs behavioural responses determined by the emotional or motivational value of the stimulus. In this regard, the CeN may be considered as an integral component of the three-step incentive motivation theory proposed by Berridge and Valenstein (1991), in which it subserves the formation of an incentive (i.e., association of a hedonic experience with sensory stimuli). Thus, in the subsequent third critical step, by interacting with the DA system, the CeN may have the capacity to direct the attribution of incentive salience to the appropriate incentive stimulus in the environment.

The results of Experiments I, II and III suggest that an important neurochemical substrate of sensory-specific satiety involves the mesotelencephalic DA system and

furthermore that modulation of DA transmission by the CeN is particularly important in the expression of sensory-specific satiety.

Given the relationship between the DA system and stress-induced behavioural sensitization (Marinelli et al., 1996), Experiment IV took the opportunity to investigate whether stress caused by repeated fluctuations in the availability of food may lead to disrupted regulation of food intake. Rats were exposed to repeated cycles of food restriction and binge feeding opportunities before being assessed for sensory-specific satiety. In contrast to rats that were maintained on a mild food deprivation schedule, rats that experienced restriction-binge cycles did not show sensory-specific satiety. In the first meal, there was a gradual decrease in the amount of food intake over a 40 min meal. In the second meal however, rats responded to the satiated food in the same manner as to a novel food (i.e., showed similar latencies to approach the food and consumed a similar amount of food). On the basis of the behavioural sensitization literature, it is suggested that sensitization of the DA system may underlie the hyperphagic effects of the restriction-binge cycles. Specifically, sensitization of the DA system may lead to excessive and inappropriate attribution of salience to food-related incentive stimuli.

Role of the mesotelencephalic DA system in incentive motivation

Role of DA in anticipatory versus consummatory behaviours

Activation of the mesotelencephalic DA system is proposed to mediate a process whereby incentive stimuli in the environment, such as the sight and smell of food, may engage the attentional and motor systems of an animal to fulfill a biological need (i.e., hunger) (Blackburn et al., 1992). Consistent with this hypothesis, increased DA efflux

occurs prior to consummation of the food (Wilson et al., 1995; Bassareo and Di Chiara, 1997; Ahn and Phillips, 1998), indicating that DA activity is an important factor in approach behaviour that brings an animal into contact with food (i.e., in mediating appetitive behaviours). However, robust increases in DA efflux also occur during the ingestion of food (Cenci et al., 1992; Pothos et al., 1995; Taber and Fibiger, 1997), suggesting that continued DA efflux may play a role in maintaining feeding behaviour. Consummatory changes in DA efflux are influenced by the degree and extent of food deprivation of the animal (Wilson et al., 1995). Indeed, it has been argued that DA efflux is more relevant for consummatory rather than appetitive or anticipatory acts (Wilson et al., 1995).

Experiments in this thesis provide further support for the involvement of DA transmission in the NAc during anticipatory and consummatory phases of feeding, and extend these findings to DA activity in the mPFC. During the first meal of a two-meal sensory-specific satiety test, presentation of a palatable food behind a perforated plexiglass screen was correlated with increased DA efflux in the mPFC and NAc. DA efflux continued to rise upon consummatory contact with the food and returned towards baseline in a pattern that mirrored the development of satiety. In both terminal regions, the magnitude of increase in DA efflux during the anticipation and intake of a second meal of a novel food was smaller than that observed during an initial meal of a novel palatable food. This difference suggests that the development of sensory-specific and (partial) gastric satiety during the first meal (since animals were still able to ingest substantial amounts of a second meal) had a mitigating effect on anticipatory and consummatory increases in DA efflux during the second meal.

Reports of increases in DA efflux in the NAc and mPFC during the appetitive period prior to the consummatory contact with food are often cited as evidence for the involvement of DA in mediating behaviours elicited by incentive stimuli. However, as observed in previous studies (Wilson et al., 1995; Bassareo and Di Chiara, 1997) and in the present experiments, the initial rapid increase from and subsequent gradual return to baseline levels of DA efflux associated with the consumption of food may subserve a process equal in importance to appetitive or anticipatory increases in incentive-motivated behaviour. Berridge and Robinson (1998) hypothesize that incentive salience must be “re-attributed” upon each encounter with the food (or in the present case, continued exposure to the food) thereby allowing for adjustments in behaviour to be made according to changes in the incentive value of food.

On a more general level, DA has been suggested to be a “common neural currency” by which various incentive stimuli may come to elicit appetitive behaviours (Berridge and Robinson, 1998). Experiment I showed that in sensory-specific satiety, differences in the incentive value of food are reflected in DA efflux as differing magnitudes of increase. As such, it may be expected that changes in the incentive value of motivationally or biologically relevant stimuli should elicit similar behavioural and neurochemical responses across many behavioural contexts. This prediction may be particularly evident in behavioural tests similar to sensory-specific satiety, where the incentive value of one stimulus may be devalued by consummatory contact while the incentive value of novel stimuli are inflated (i.e., a positive incentive contrast effect) (Balleine and Dickinson, 1998). Indeed, a previous study employing a sensory-specific satiety version of sexual behaviour in male rats, referred to as the “Coolidge effect” (so

named after a former president of the United States), reported a similar pattern of changes in DA efflux in the NAc (Fiorino et al., 1997). Anticipatory behaviours of male rats towards receptive females separated by a screen were accompanied by increased DA efflux, which then continued to increase upon consummatory contact with the females. When male rats became satiated with one female, there were no changes in DA efflux or increased appetitive behaviors towards that female. However, presentation of a different female with novel sensory properties elicited a second bout of sexual behaviour accompanied by increased DA efflux. Both sensory-specific satiety and the "Coolidge effect" reveal a fundamental relationship between DA activation and the (re)valuation of incentive stimuli, and the importance of this relationship in the (re)initiation of incentive-motivated behaviour.

Role of DA in mPFC versus NAc

As noted above, the overall profile of changes in DA efflux during sensory-specific satiety did not differ between the mPFC and the NAc. However, there was one exception: an anticipatory increase in DA efflux occurred prior to the second meal in the mPFC, but not in the NAc. This differential DA response indicates a subtle but important regional difference for the role of the mPFC and the NAc in food reward.

Within the mPFC, DA efflux may be more indicative of the salience of the incentive reward, and less so regarding the motivational status of the animal, as sensory stimuli with high incentive value elicited anticipatory increases in DA efflux even when animals were not hungry (Figure 4). The activity of prefrontal neurons in primates has been reported to encode a similar selective reward-related information (Watanabe, 1996; Schultz et al., 1998; Watanabe, 1998). When a cue predictive of a specific reward or

when the subject gains the sight of food itself, neurons in the primate dorsolateral prefrontal cortex [which is homologous with the rat mPFC (Pandya and Yeterian, 1990)] show reward-specific anticipatory activity (Watanabe, 1996). The neurochemical and neuronal activity data, considered together, support the idea that the prefrontal cortex is involved in “retrieving, retaining and/or anticipating the motivational value and visual, gustatory and/or olfactory images of the specific reward” (Watanabe, 1996) (i.e., *expectation of specific reward*).

In contrast to the mPFC, DA efflux in the NAc was strongly influenced by the motivational state of the animal, as anticipatory increases in DA efflux were observed only in food-deprived (hungry) rats, irrespective of whether the food was novel and palatable and whether the food was ingested (Figure 5). Neurons in the ventral striatum also have been reported to show similar reward-related activity (Apicella et al., 1991; Rolls, 1999). However, in contrast to the prefrontal cortex, striatal neurons do not display differential activity to various food rewards (Rolls, 1999). Thus, activity in the ventral striatum may reflect expectation of a preferred food, but depending on the motivational state of the animal, the food may be perceived as being less attractive (i.e., *the presence or absence of reward*).

Increases in DA efflux associated with specific phases of the two-meal sensory-specific satiety paradigm may be explained by a neural circuit that “links” reward-related activity the prefrontal cortex and the striatum. Thus, the following account of sensory-specific satiety is proposed. During an initial meal, when the motivation to eat is high, the detection of incentive stimuli of incentive sensory properties (and *expectation of reward*) may elicit increased neuronal firing in prefrontal neurons. In turn, prefrontal

neurons may provide increased excitatory input to DA neurons in the VTA thereby increasing DA efflux in both the mPFC and NAc. This account is consistent with the proposed “executive functions” of the prefrontal cortex (Goldman-Rakic, 1995; Fuster, 1997), including the planning and selection of appropriate behavioural action. In the present context, prefrontal control over the DA system, by means of reciprocal connections with DA neurons in the VTA (Carr and Sesack, 2000), may constitute a part of a functional circuit that determines the direction and intensity of appetitive behaviour toward incentive sensory stimuli. During a second meal, when the animal is satiated, only the detection of novel, different sensory properties associated with high incentive value (i.e., *expectation of a specific reward*) may elicit reward-related activity in the prefrontal cortex and, similarly, incentive-dependent increases in DA efflux in the mPFC and the NAc. In the NAc, however, DA efflux may be additionally “gated” by afferents from the hypothalamus or amygdala, which provide inputs regarding the motivational state of the animal (Rolls, 1999). As suggested above, motivational state may determine the *absence or presence of reward*, and in case of sensory-specific satiety, changes in motivational state may determine the *degree of reward*. This motivational contingency appears to be reflected as dynamic changes in DA efflux in the NAc.

Functional interactions between CeN versus BLA with the mesotelencephalic DA system

Distinct subregions within the amygdala mediate complementary but dissociable processes involved in the expression of emotional behaviour (Holland et al., 2000; LeDoux, 2000; Everitt et al., 2000). Fear conditioning models propose that a *serial flow of information* from the lateral nucleus, subserving the association of a CS and a US (e.g.,

an auditory tone and a food-shock, respectively), to the CeN, the main output to hypothalamic and brainstem areas, is critical for establishing conditioned reflexive responses to aversive or fearful stimuli (e.g., freezing and fear-potentiated startle) (Pascoe and Kapp, 1985; Sananes and Davis, 1992; Amorapanth et al., 2000; LeDoux, 2000).

There is growing support for an alternative functional organization of the amygdala, in which different subregions play a key role in *separate parallel neural pathways* that support a more diverse range of both aversively and appetitively motivated behaviours (Killcross et al., 1997; Holland and Gallagher, 1999; Everitt et al., 2000). According to this view, the BLA (which includes the lateral nucleus) and the CeN are not simply sensory input and motor output nuclei. Rather, each subregion can support the formation of CS-US associations and independently mediate different forms of behavioural expression that are dependent on these CS-US associations (Killcross et al., 1997; Everitt et al., 1999). In support of this conjecture, most of the reward-related activity (i.e., responses to food reward and cues associated with the reward) observed in the amygdala occurs in the CeN and BLA (Uwano et al., 1995). Killcross and his co-authors propose that “the basolateral amygdala [may be regarded] as part of a system responsible for voluntary or instrumental choice behaviour based on emotional events, whereas the central nucleus of the amygdala is involved more closely in the reflexive, automatic Pavlovian conditioned responses evoked by motivationally salient stimuli” (Killcross et al., 1997). Therefore, the CeN and the BLA appear to be integral components of a widely distributed neural system that mediates the influence of emotionally and motivationally salient stimuli on behavioural responses (Mogenson et al., 1993).

The CeN subregion of the amygdala

The acquisition and expression of Pavlovian approach behaviour and conditioned orienting responses have been proposed to depend on a neural circuit linking the CeN to the NAc (the medial aspect of the ventral striatum) and the dorsal striatum via pathways in the mesotelencephalic DA system (Han et al., 1997; Everitt et al., 1999). However, there is a paucity of data in support of the interaction between the CeN and the DA system. The present experiments provide the first demonstration of this relationship. Experiments II and III showed that inactivation of the CeN is associated with an immediate reduction (~20% below baseline) in basal levels of DA efflux in the NAc (Figure 7). As there are no direct projections from the CeN to the NAc that could mediate this effect, it would appear that the CeN influences DA function through afferent projections to the VTA and SN (Wallace et al., 1992) (see Figure 15). Modulation of "DA tone" by this mechanism is consistent with the notion that the CeN influences the general level of arousal or activational state of an animal (i.e., "energizing processes") via the mesoaccumbens DA system implicated in incentive motivation (Blackburn et al., 1992; Bassareo and Di Chiara, 1997; Ahn et al., 1997; Berridge and Robinson, 1998). A similar CeN projection may modulate the nigrostriatal DA system thereby controlling orienting responses (Holland et al., 2000). This proposed relationship between the CeN and the nigrostriatal pathway may also account for certain observations made during Experiments II and III. When lidocaine was first administered into the CeN, rats initially showed increased locomotion and exploratory behaviour, but upon presentation of a palatable food, failed to orient or sniff in the direction of the food behind the screen, which are typical components of anticipatory behaviour.

Attention and arousal processes mediated by the mesotelencephalic DA system are likely to be most critical during early stages of learning a new behavioural task. Accordingly, Schultz and his colleagues have demonstrated, using a simple Pavlovian conditioning task, that burst firing of DA neurons is strongly correlated with the presentation of a food reward and that this increased activity shifts forwards in time to be coincident with a cue that predicts the imminent delivery of food reward (Ljungberg et al., 1992; Schultz et al., 1993). The acquisition of Pavlovian conditioned approach responses (e.g. towards a light cue predictive of food) also appears to be dependent on the processes mediated by the DA system. This behaviour cannot be learned by rats with lesions of DA terminals or the NAc core region (Parkinson et al., 1999). Similarly, lesions of the CeN also impair the acquisition of this task (Parkinson et al., 2000b). The electrophysiological and lesion data, together, support the hypothesis that modulation of DA transmission in the NAc by the CeN may be a mechanism by which the incentive properties of the light stimulus influence the conditioning of orienting and approach behaviours. This, in turn, is in agreement with Berridge's proposal that the CeN is important in directing the attribution of incentive salience to external stimuli, whose incentive motivational value has been established through associative learning (Berridge, 1996).

Modulation of mesotelencephalic DA transmission by the CeN also may be an important mediator of ingestive behaviour guided by the incentive value of the (intrinsic) sensory properties of food (i.e., in contrast to a light cue that has incentive value extrinsic to the food). In Experiment III, inactivation of the CeN lead to an abnormal pattern of food intake that appeared to reflect an inability to respond to the association between the

olfactory and gustatory properties of food (i.e., the intrinsic CS) with its hedonic properties (the US). This failure to establish appropriate incentives may have disrupted the overall expression of sensory-specific satiety and the associated changes in DA efflux in the NAc and the mPFC (Figures 16 and 17). These findings suggest that without the CeN, CS-US associations can provide, at best, marginal influences on attribution of incentive salience.

The BLA subregion of the amygdala

In contrast to the CeN which interacts with DA neurons, the BLA sends excitatory inputs to two areas of the brains that receive DA terminals, namely the NAc and mPFC (Kelley et al., 1982; McDonald, 1991a). Electrical stimulation of the BLA can evoke significant increases in DA efflux in the NAc and mPFC (Floresco et al., 1998; Jackson and Moghaddam, 2001). Concurrent increases in glutamate efflux from putative BLA afferents (Jackson and Moghaddam, 2001) suggest the possibility that activation of glutamate receptors on DA terminals may presynaptically modulate phasic changes in DA efflux (Gracy and Pickel, 1996; Floresco et al., 1998). Inactivation of the VTA has no effect on evoked increases in DA efflux (Floresco et al., 1998) indicating that this effect is independent of impulses originating from DA neurons in the VTA.

BLA afferents to the NAc core region have been suggested to mediate the “transfer” of CS-US associative information necessary for the acquisition of instrumental behaviours, such as conditioned reinforcement (Everitt et al., 1999). Responding for conditioned reinforcers can be greatly potentiated by infusions of amphetamine, an indirect DA agonist (Cooper et al., 1996) into the NAc (Taylor and Robbins, 1986). Although BLA afferents can be stimulated to evoke increased DA efflux in the NAc

(Floresco et al., 1998; Jackson and Moghaddam, 2001), this pathway does not appear to mediate the potentiative mechanism revealed by the effect of intra-NAc amphetamine infusions on conditioned reinforcement. Rather, a circuit involving the CeN and the DA projections to the NAc appears to mediate the potentiation of conditioned reinforcement by amphetamine because lesions within this circuit greatly attenuate this effect (Everitt et al., 1999). Thus, in addition to its role in Pavlovian conditioning, modulation of DA efflux in the NAc by the CeN may have an additional role in *moderating* the expression of instrumental behaviours.

Despite the growing number of reports describing the modulatory effects of the BLA on DA transmission in the NAc and mPFC (Floresco et al., 1998; Floresco et al., 2001b; Jackson and Moghaddam, 2001), a behavioural context in which these interactions may play a role remains to be elucidated. However, several arguments can be made in favour of a neural circuit involving the BLA and DA transmission in the NAc in the expression of complex behaviours. First, the CeN-VTA-NAc pathway may not be the unique mediator of the potentiative effects of intra-NAc amphetamine in conditioned reinforcement. The pharmacological effects of amphetamine are thought to involve the inhibition of reuptake and reversal of the DA transporter (Cooper et al., 1996; Jones et al., 1998), both of which contribute to increased extracellular levels of DA in the NAc in an impulse-independent manner. The BLA has also been shown to modulate DA efflux presynaptically in the NAc, independent of impulses originating from DA neurons in the VTA (Floresco et al., 1998). The similarity of mechanisms by which amphetamine and the BLA evoke DA efflux in the NAc raises the possibility that the facilitatory effects of amphetamine on instrumental behaviour may be mediated by direct BLA projections to

the NAc. Second, interactions between the BLA and the DA system appear to be more complex than the polysynaptic circuit originating in the CeN. Tentanic stimulation of the BLA has recently been shown to potentiate the postsynaptic excitatory effects of subsequent BLA inputs to the NAc by enhancing DA efflux (Floresco et al., 2001b). This finding indicates that this mechanism may mediate the selective facilitation of BLA inputs while suppressing other limbic glutamatergic inputs to the NAc. It is of interest to note that a similar selective facilitation effect has been observed following tentanic stimulation of the ventral subiculum (Floresco et al., 2001a), the major output pathway of the ventral hippocampus. Further complexity between glutamate and DA interactions in the NAc is suggested by the possibility that, in addition to limbic modulation of DA efflux, DA may influence glutamate release from limbic terminals. Little is known regarding the latter interaction. However, in an earlier study, infusion of DA or DA agonists in the NAc was reported to depolarize hippocampal axon terminals in the NAc (Yang and Mogenson, 1983). Thus, the identification of similar mechanisms involving the BLA would provide the basis to suggest a complex bi-directional interaction between DA terminals and BLA glutamatergic afferents in the NAc.

The BLA is anatomically connected with cortical regions of the brain, such as the orbitofrontal cortex (i.e., the region surrounding the rhinal sulcus in the rat) (Krettek and Price, 1977) and mPFC (McDonald, 1991a). Interactions between the BLA and the orbitofrontal cortex have been implicated in the representation of incentive value (Gallagher et al., 1999; Holland and Gallagher, 1999) and an animal's ability to adjust goal-directed behaviour according to changes in incentive value (Schoenbaum et al., 1998). In contrast to the orbitofrontal cortex, the behavioural relevance of the anatomical

relationship between the BLA and mPFC remains to be investigated. However, some observations regarding the interaction of these two structures suggest potential relevance for behaviour. A recent study showed that stimulation of the BLA results in phasic increases in DA efflux in the mPFC (Jackson and Moghaddam, 2001) while Experiment II revealed that inactivation of the BLA triggers long-lasting oscillatory changes in DA efflux in the mPFC. Thus, the BLA may have phasic and tonic influences on neuronal activity in the mPFC, via modulating levels of DA efflux. In conclusion, in contrast to the CeN, the cellular mechanisms of interaction between the BLA and the mesotelencephalic DA system appear to be more complex and accordingly, suggest the potential to support cognitive processes (e.g., representation of incentive value, prospective planning of action) required for the expression of intentional voluntary behaviour.

Stress-induced behavioural sensitization and the DA system

This thesis has argued that fine-tuning of activity within the mesotelencephalic DA system by inputs from the amygdala is critical to the appropriate expression of simple and complex incentive-motivated behaviours. The behavioural sensitization literature suggests that repeated (abnormal) activation of the DA system can exert disruptive effects on these same behaviours. In particular, repeated administration of psychostimulants (Fiorino and Phillips, 1999a,b; Robinson and Berridge, 1993) or repeated exposure to stress (Doherty and Gratton, 1992; Prasad et al., 1995) results in altered function (i.e., sensitization) of the mesotelencephalic DA system, such that subsequent encounters with a sensitizing stimulus can elicit potentiated behavioural responses which are accompanied by augmented DA transmission (Doherty and Gratton, 1992; Fiorino and Phillips, 1999a).

Unlike behavioural sensitization induced by psychostimulants, which have the ability to increase DA transmission acutely (Pierce and Kalivas, 1997; Robinson and Berridge, 1993), stress-induced sensitization requires the involvement of an intermediary, which interacts with the DA system. Stress activates the HPA-axis (Koob et al., 1993; Chrousos, 1998), which in turn regulates the release of glucocorticoids (Piazza et al., 1996). These final effectors of the HPA-axis have been suggested to function as "endogenous psychostimulants" (Piazza et al., 1996), which implies that glucocorticoids also have the ability to increase DA transmission. In support of this, DA neurons express receptors for corticosterone, the major stress hormone in the rat (Häfstrand et al., 1986). Furthermore, administration of corticosterone at doses similar to stress-induced plasma concentrations, inhibits DA reuptake (Gilad et al., 1987), reduces DA catabolism (Rothschild et al., 1985) and leads to increased basal and food-evoked DA efflux in the NAc (Imperato et al., 1989; Piazza et al., 1996).

An important aspect of behavioural sensitization protocols is the *repeated, intermittent* administration of a sensitizing agent, such as amphetamine or cocaine (Fiorino and Phillips, 1999a; Kalivas and Stewart, 1991; Prasad et al., 1995). Similarly, repeated brief exposures to mild stressors in rats, such as tail pinch or restraint, can also result in several manifestations of stress-induced behavioural sensitization, including hyperphagia (Heinrichs et al., 1992; Badiani et al., 1996). Food restriction is also considered to be a form of mild stress, which stimulates food intake (Del Prete et al., 1994; Specker et al., 1994). The amount of food intake can be significantly potentiated (i.e., lead to hyperphagia) when prolonged exposure to food restriction is interrupted by periodic opportunities to eat unlimited quantities of food (Hagan and Moss, 1997).

Furthermore, Experiment IV demonstrated that rats that experienced similar cycles of food restriction and binge opportunities did not display sensory-specific satiety, a pattern of feeding that depends on the relative devaluation of the incentive value of one food and inflation of the incentive value of other foods.

Given that food restriction is associated with glucocorticoid release (Marinelli et al., 1996), it is conjectured that repeated switches from food restriction to binge feeding and vice versa, which is presumably stressful, is paralleled by repeated glucocorticoid-mediated activation of the DA system. The dysregulatory effects of repeated restriction-binge cycles on food intake, considered in conjunction with the hyperphagic effects of other stressors known to increase HPA-axis activation and glucocorticoid release, suggest that glucocorticoid-mediated activation of the DA system may be a common mechanism by which stressors may induce behavioural sensitization. I hypothesize that stress-sensitized responses to food, like amphetamine-induced behavioural sensitization, may be accompanied by augmented (sensitized) DA transmission. When sensitized rats are tested for sensory-specific satiety, the absence of satiety (i.e., hyperphagia) may be associated with enhanced increases in DA efflux in the mPFC and NAc. These findings would suggest the possibility that the attribution of incentive salience, proposed to be mediated by the DA system (Berridge and Robinson, 1998), may not be regulated by the incentive sensory properties of food or by satiety factors. These hypotheses will be tested in future experiments.

Conclusion and future direction

The present results indicate that the CeN and BLA subregions within the amygdala independently and differentially modulate DA transmission in the NAc and mPFC. Based on these findings and other research which indicate a functional dissociation of the CeN and BLA (Everitt et al., 1999; Holland and Gallagher, 1999), this thesis contends that these nuclei are critical aspects of a neural system that mediates the expression of a wide range of incentive-motivated behaviors. Outside the bounds of controlled experimental conditions, constant changes in the real environment require an animal to make repeated behavioural adjustments, rather than to engage continuously in well-learned habits. This general adaptive process may be subserved by CeN and BLA interactions with the DA system. These mechanisms may facilitate learning of new strategies, as indicated by the studies by Everitt and his colleagues (1999, 2000), as well as “switching” between existing response strategies. Such behavioural adaptations could involve changing from a simple approach towards an incentive cue, to a more sophisticated use of the same information to learn and perform behaviours instrumental in obtaining a primary reward. In this regard, changes in expression of behaviour may reflect the relative influence of impulse-dependent modulation of DA efflux in forebrain regions by the CeN or BLA-mediated presynaptic modulation of DA efflux in specific terminal regions. Specifically, it is conjectured that the CeN-VTA-NAc pathway facilitates species-specific innate responses, whereas the BLA projections to the NAc and mPFC control the expression of voluntary instrumental behaviours.

This view of amygdalar interactions with the DA system may be extended to integrate other important limbic structures involved in the expression of motivated

behaviour, such as the hippocampus. A neural circuit linking the hippocampus with the NAc, as well as with the mPFC, has been shown to underlie different foraging strategies used by rats in spatial learning tasks on a radial-arm maze (Floresco et al., 1997). The performance of these tasks may involve hippocampal modulation of DA transmission in the NAc and mPFC. In support of this, infusions of the DA D₁ receptor antagonist SCH 23390 into the NAc and mPFC have been shown to dose-dependently increase the number of errors in entering the correct arm to retrieve food pellets (Floresco and Phillips, 1999; Floresco et al., 1997). Additionally, we observed increased DA efflux in the same terminal regions during the performance of these tasks (Ahn et al., 2000; Phillips et al., 2001). As already noted, recent findings by Floresco and his colleagues (2001a) have identified DA-dependent cellular mechanisms for the control of interactions amongst converging cortical limbic inputs, particularly those arising from the BLA and hippocampus, which then may influence neural activity in the NAc. Future experiments should investigate the relative contribution of cortical limbic structures and their "use" of the DA system to facilitate behavioural learning and expression. For instance, functional interactions of the hippocampus and amygdala with the DA system may be examined in a behavioural test which involves a spatial learning component (e.g., radial arm maze) and a positive incentive contrast learning component (e.g., sensory-specific satiety or the "Coolidge effect"). Alternatively, a recent study by Gall and her colleagues (Gall et al., 1998) suggests that, rather than mediating information-specific learning, the degree of prior learning in general (i.e., novice versus overtrained) may be a primary determinant of whether amygdalar or hippocampal afferents to the NAc are preferentially activated. This study employed expression of the immediate early gene product c-fos as an index of

neuronal activity in the hippocampus and amygdala during different stages of a two-odour discrimination task. Specific amygdalar nuclei were preferentially activated during early stages of acquisition, whereas regions of the hippocampus were more active during maintenance of the task, which involved exploration and habit-based responding, respectively. These observations suggest the possibility that the DA-dependent cellular mechanisms may also contribute to the development and fine-tuning of behaviour mediated by the different limbic regions during distinct stages of behavioural learning.

Questions related to the neurochemical basis and functional significance of limbic interactions with the mesotelencephalic DA should be guided by general brain mapping studies, similar to those of Gall and her colleagues (1999), and cellular mechanisms of limbic-DA interactions, reported by Floresco and his colleagues (2001a). Such questions may be best examined using the present strategy of complementary neural manipulation techniques, such as transient chemical inactivation and chemical or electrical stimulation techniques, in conjunction with *in vivo* monitoring techniques, such as microdialysis or electrochemistry.

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APPENDIX:

**THE USE OF *IN VIVO* MICRODIALYSIS TO MONITOR
EXTRACELLULAR CONCENTRATIONS OF DOPAMINE IN THE
BRAIN**

In vivo microdialysis is a widely accepted and used technique that allows estimates of both basal and evoked changes in neurotransmitter concentrations in the extracellular fluid. In principle, this technique mimics a capillary blood vessel (Ungerstedt and Hallström, 1987): a physiological Ringer's solution is continuously perfused through silica inlet tubing contiguous with a semi-permeable dialysis membrane tip. Any analyte within the molecular cut-off weight range of the membrane diffuses down its concentration gradient into the probe, and is then carried out by the perfusate through silica outlet tubing (see Microdialysis and high pressure liquid chromatography in the General Methods section for further details). The collected sample is separated into its component species by high-pressure liquid chromatography (HPLC) and subsequently quantified by electrochemical detection (ED).

Regional specificity reflected by microdialysis samples

Microdialysis continues to be particularly successful in monitoring changes in monoamine levels in the brain, such as DA, noradrenaline and serotonin. It allows changes in neurotransmitter levels to be monitored within a relatively localized environment. One factor that significantly determines the area of sampling depends on the physical parameters of the probe itself. In the present studies, the diameter of the probe membrane was fixed at ~250 μm but the length of the dialysis membrane was

adjusted according to the dorsal-ventral extent of the region of interest (see Reverse-dialysis section in the General Methods section).

Studies of the microenvironment surrounding the microdialysis probe suggests that the samples reflect changes in analyte levels relatively close to the probe. For example, in the striatum, evidence indicates that samples reflect fluctuations in DA levels for a distance of up to 1 mm from the surface of the membrane (Blaha, 1991).

Methods of estimating extracellular concentration of analytes

The amount of an analyte recovered through a microdialysis probe depends on the flow rate of the perfusion and the yield (or recovery) of the probe. The methods of estimating the extracellular concentration of analytes in a local environment tend to be variable in accuracy, as *in vivo* recovery rates may differ from those obtained *in vitro*. One of the earliest methods involved the calculation of a relative ratio of analyte concentration recovered by the probe versus that present in a beaker. However, this method has been criticized for its tendency to underestimate the actual concentrations of analytes in the extracellular fluid (Benveniste and Hüttemeier, 1990). The “no-net flux” method, originally described by Lönnroth et al. (1987), provides greater accuracy. This approach is based on the assumption that when the concentration of an analyte within the probe equals that outside the probe at a constant flow, there is no net diffusion across the dialysis membrane (i.e., the point of no-net flux). If the perfusate contains lower concentrations of the analyte than in the extracellular fluid, then the diffusion gradient leads to an influx of analyte into the dialysate relative to the perfusate. If the perfusate contains higher concentrations of the analyte, then the diffusion gradient becomes reversed, and the amount of substance in the dialysate is diminished relative to the

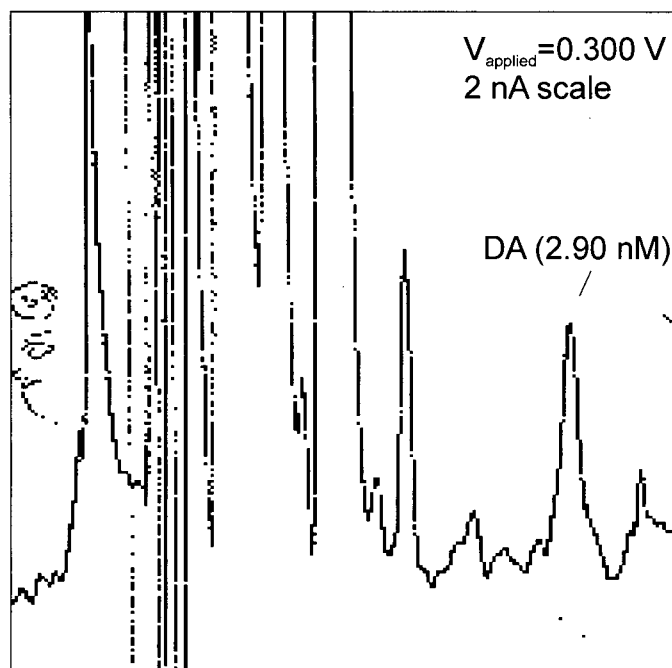
perfusate. By plotting the difference between the “in” and the “out” concentrations against the “in” concentration, the exact extracellular concentration can be estimated at the point of no net flux. This technique has been used to determine basal and evoked changes in extracellular concentrations of DA (Pettit et al., 1990; Parsons et al., 1991; Kalivas and Duffy, 1990).

Basal levels of DA in the extracellular fluid of the mPFC and NAc

In the present experiments, samples from the NAc were analyzed by a Coulochem HPLC-ED system (ESA, Chelmsford, MA). Samples from the mPFC were analyzed by a different system made by Antec-Leyden (Leyden, The Netherlands) as the approximately 20 fold lower concentration of DA in the mPFC sample challenged the detection limits of the Coulochem system (~0.1 nM).

Given the difficulties associated with determining “true” basal concentrations of extracellular analytes in the brain, only basal values uncorrected for recovery were reported in the results of the experiments in this thesis. In the NAc, basal levels of DA ranged from 2.78 to 3.16 nM. In the mPFC, basal levels of DA ranged from 0.13 to 0.18 nM. Figure 24 shows representative chromatograms from analysis of NAc samples by the Coulochem system (2.9 nM) and a mPFC sample (0.13 nM) by the Antec system. Each sample represents a sample taken during baseline conditions.

Nucleus accumbens



Medial prefrontal cortex

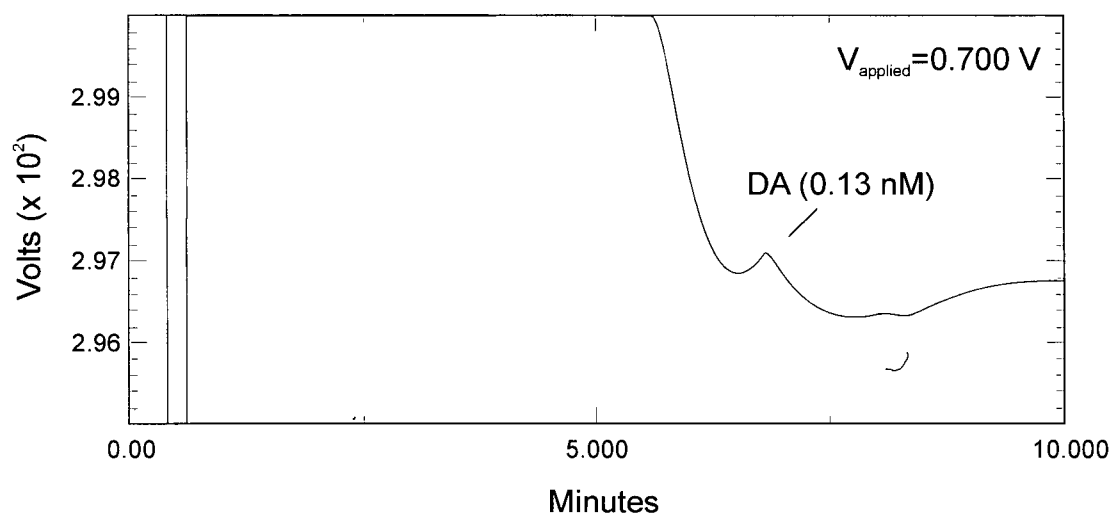


Figure 24. Representative chromatograms of samples from the NAc and mPFC. The peak in each chromatogram represents the DA content in a 10 μL sample from the NAc analyzed with a Coulochem HPLC system and from the mPFC by an Antec-Leyden HPLC system. Equivalent concentration values are shown for each DA peak.