AN IN VIVO ELECTROCHEMICAL ANALYSIS OF
THE ROLE OF DOPAMINE IN FEEDING BEHAVIORS

By

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We accept this thesis as conforming
to the required standard

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Abstract

The involvement of dopamine in anticipatory and consummatory aspects of feeding behaviors was investigated in the present thesis. All measurements of dopaminergic activity were taken by in vivo electrochemical techniques. In Experiment 1, dopamine efflux in the nucleus accumbens and caudate of male rats was monitored during sessions in which a small, unsignalled liquid meal was consumed. Increases in the electrochemical measure of dopamine activity, which were of similar temporal pattern and magnitude, were observed in both the nucleus accumbens and striatum following meal consumption. These data suggest a possible postingestional role of dopamine in these two brain structures. In Experiment 2, a conditioned feeding paradigm was utilized to study the role of dopamine during a discrete anticipatory phase of feeding. Rats were conditioned to discriminate between a positive conditioned stimulus (CS+) predictive of meal delivery, and a negative conditioned stimulus (CS-) that was not associated with food. Increases in dopamine activity, as determined by changes in electrochemical oxidation currents, were found to be greater during the CS+ than during the CS- in both the nucleus accumbens and caudate. In addition, the magnitude of increase was greater in the nucleus accumbens than the caudate, suggesting that the accumbens may be preferentially
involved in the processing of external incentive stimuli. The results support a role for dopamine in both the nucleus accumbens and caudate during appetitive or anticipatory responding for food in the male rat.
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I would like to thank Chuck Blaha, Mike Jung, Fred LePiane, Jim Pfaus, and Tony Phillips for the generous assistance they provided during the preparation of this thesis.
INTRODUCTION

Numerous studies have implicated dopaminergic systems in feeding behaviour, but currently, there is no clear consensus as to the exact nature of this involvement. The first section of this introduction reviews experimental data that have associated dopaminergic activity with feeding. Theoretical models that have been proposed to explain the role of dopamine in feeding behaviors are discussed next. The introduction concludes with a brief description of, and rationale for the use of, in vivo electrochemical measurements of extracellular dopamine levels during different phases of feeding in the rat.

Experimental Background

Selective destruction of nigrostriatal dopamine (DA) neurons has been shown to produce a syndrome of aphagia and adipsia (Fibiger, Zis, & McGeer, 1973; Ungerstedt, 1971). Similarly, pharmacological blockade of dopamine receptors has resulted in a disruption of feeding behaviours. Xenakis and Sclafani (1981) found that the DA antagonist pimozide suppressed consumption of a palatable saccharin-glucose solution in a dose-related manner after a 4-hr food deprivation period. The DA antagonist spiroperidol also decreased food intake in rats restricted to a 4-hr feeding
period (Heffner, Zigmond, and Stricker, 1977).

Because reduced dopaminergic activity has been linked with the attenuation of feeding, increased DA activity might be expected to facilitate feeding. Evans and Eikelboom (1987) observed significant increases in food intake and meal duration when they administered the DA agonists bromocriptine and d-amphetamine intracerebroventricularly. They also noted that the activation was selective to food intake, as other behaviours (grooming, drinking, activity) were not affected significantly by the drugs. However, Heffner et al. (1977) have shown that the DA agonist amphetamine and apomorphine reduce food intake in food-deprived rats. Electrical stimulation of the ventral tegmental area (VTA) and the substantia nigra (SN) also increases synaptic activity of DA neurons (Fibiger, LePiane, Jakubovic, & Phillips, 1987; Phillips & Fibiger, 1979). In addition, feeding has been elicited by electrical stimulation of the VTA and SN, both origins of ascending dopaminergic pathways (Phillips & Fibiger, 1973).

The evidence presented so far deals solely with the role of DA systems in feeding behaviours of the rat under unnatural conditions. That is, all subjects were tested either in a state of reduced or elevated DA activity produced by the experimenter. More biologically relevant data have emerged from studies that have attempted to correlate dopaminergic activity and feeding under more natural circumstances, without producing pharmacological or
physical alterations of brain activity. One such study was conducted by Heffner, Hartman, and Seiden (1980). Rats maintained on a feeding schedule consisting of 20 hours of food deprivation followed by 4 hours of access to food pellets were decapitated either before food delivery or after one hour of access to food. Dihydroxyphenylacetic acid (DOPAC)/DA ratios were determined by radioenzymatic assay to provide indices of dopaminergic activity. These researchers found elevated ratios in the nucleus accumbens, hypothalamus, and amygdala of deprived rats given access to food compared to animals that were not fed or to non-deprived controls. Ratios remained unchanged in the striatum, olfactory tubercle, frontal cortex, and septum. Chance, Foley-Nelson, Nelson, and Fischer (1987) reported elevated levels of the DA metabolites DOPAC and HVA in the nucleus accumbens and striatum of chronically deprived rats sacrificed following one hour of access to food, as compared to values from control rats sacrificed in a deprived state. In this later study, neurotransmitter and metabolite measurements were conducted on regional brain homogenates using high performance liquid chromatography with electrochemical detection (HPLC/ED). Simansky, Bourbonais, and Smith (1985) also employed HPLC/ED to determine DOPAC/DA ratios in rats under various feeding conditions. They observed that DOPAC/DA ratios were increased in the hypothalamus of rats maintained on a daily 4-hr food-access schedule when they were measured after one hour of access to
food or simply to food-related stimuli alone, as compared to nondeprived controls or deprived rats not exposed to food or food-related stimuli. They did not observe any similar ratio changes in the nucleus accumbens, striatum, olfactory tubercle, or amygdala.

A significant advance in the analysis of dopaminergic activity and feeding behaviours occurred with the application of chronic in vivo techniques to measure extracellular levels of neurotransmitters in freely moving animals. One of these techniques, microdialysis with HPLC/ED, was employed by Church, Justice, and Neill (1987). These researchers found that rats trained on a 1-min fixed interval feeding schedule showed increased extracellular striatal DA levels during and extending 15 to 20 min after the 15-min feeding periods. Using the same technique, Hernandez and Hoebel (1988) observed increased extracellular DA levels in the nucleus accumbens of food-deprived rats during feeding sessions. Extracellular levels of DA in the nucleus accumbens also were elevated, but no changes were seen in the striatum of deprived rats bar-pressing for food. In another study employing microdialysis and HPLC/ED, Radhakishun, van Ree, and Westerink (1988) observed an increase in DA efflux in the nucleus accumbens that was correlated with the onset of a scheduled feeding session. Levels of DA remained elevated during the entire food intake period.

Another in vivo technique, voltammetry, has been used
to study the role of DA in feeding behaviours. Keller, Stricker, and Zigmond (1983) found a variety of exteroreceptive stimuli including tail shock, ice-water bath, and food after a 24 hour deprivation period, to increase voltammetric signals in the rat striatum. Although the electrodes used in this study were not selective for DA (i.e., DOPAC, AA, and DA may have all contributed to the signal obtained), the results of pharmacological manipulations suggested that the changes in the electrochemical signal were due mainly to increased DA efflux. In vivo voltammetric analyses of extracellular levels of the DA metabolite HVA (used as an index of DA activity) were observed recently in the rat caudate nucleus during lever pressing for food reward (Joseph, Hodges, and Gray, 1989). D'Angio and Scatton (1989) found feeding or exposure to food odors alone, both resulted in increased extracellular DOPAC levels in the anteromedial prefrontal cortex as determined by in vivo voltammetric measurements. In vivo voltammetric data obtained by Louilot, Le Moal, and Simon (1986), argued against the hypothesis that the observed increases in dopaminergic activity are due simply to the induction of motor activity. Exposure of a test subject to an aggressive male caused an increase in the DA activity of the test subject, even though it typically assumed a frozen stance.

As indicated by the evidence reviewed so far, it appears that dopaminergic systems are involved in feeding
behaviors. However, there is still uncertainty as to which aspects of feeding are associated with dopaminergic activity and which brain structures are involved. Blackburn, Phillips, and Fibiger (1987) stressed the utility of factoring motivated behaviors into their component parts and analyzing the role of DA within each component. These authors emphasize the major distinction between consummatory and preparatory behaviors. With respect to feeding, they define consummatory behaviors as "those that occur after the animal has made contact with food and that result in its ingestion" and give "biting, chewing, and swallowing" as examples of this category. Preparatory behaviors are defined as "appetitive acts that typically lead to, and make possible, consummatory behaviors". Studies by these and other researchers suggest that dopaminergic activity may play a greater role in preparatory than consummatory feeding behaviors.

Unlike the results of earlier studies with DA antagonists, Blackburn et al. (1987) found that pimozide did not significantly attenuate food intake, but did affect preparatory responses. The procedure followed in this study was based on a conditioned feeding design by Weingarten (1984). Using this experimental procedure, the researchers found that appetitive responses to a conditioned stimulus (CS+) signalling the subsequent onset of food delivery were attenuated by the administration of pimozide, whereas consummatory behaviors appeared normal when the food was
delivered. In a related study, the DA antagonists metoclopramide and thioridazine had differential effects on preparatory behaviour (Blackburn, Phillips, & Fibiger, 1989a). Like pimozide, metoclopramide attenuated preparatory responses to the conditioned stimulus, whereas thioridazine did not. It was suggested that the effects of metoclopramide reflect a preferential action of this drug in the caudate nucleus. In another study employing the same conditioned feeding paradigm, increased DOPAC/DA and HVA/DA ratios were observed in the nucleus accumbens and striatum (though striatal values did not reach statistical significance) after exposure to the CS+ alone (Blackburn, Phillips, Jakubovic, & Fibiger, 1989b). No increases in either ratio were found in rats allowed to ingest an unsignalled meal. All measurements were made on postmortem tissue by HPLC/EC analysis.

Further support for the hypothetical role of DA in preparatory behaviours comes from the attenuation of hoarding, a form of preparatory feeding behaviour, following the disruption of mesolimbic DA neurons by 6-OHDA lesions (Kelley & Stinus, 1985). Others have found that food-related stimuli (odors, sights and sounds associated with scheduled feeding) in the absence of access to food, can elicit increased DA turnover (D'Angio & Scatton, 1989; Simansky et al., 1985). Schultz (1986) used electrophysiological techniques to monitor activity in dopaminergic midbrain neurons of monkeys during an
appetitive task. The majority of these neurons increased their firing rate when the monkeys were presented with visual and auditory cues that signalled the availability of food and also when the monkeys reached out toward the food. However, the firing rates of very few neurons increased above baseline levels when food was actually placed into the mouth.

**Theoretical Background**

Several hypotheses have been proposed to account for the experimental data relating changes in dopaminergic activity to feeding behaviors. The "sensorimotor hypothesis", was developed in response to observations following dopamine depletion caused by 6-OHDA lesions of the nigrostriatal pathway. The hypothesis relates ascending DA system involvement in sensorimotor facilitation (Marshall, Richardson, & Teitelbaum, 1974; White, 1986). That is, DA activity can modulate the ability of sensory stimuli to initiate motor responses. Numerous studies have shown that destruction of this pathway results in locomotor and postural deficits, an inability to orient to external stimuli and a disruption of spontaneously initiated natural behaviors (c.f. Berridge, Venier, & Robinson, 1989). With respect to feeding behaviors, the sensorimotor hypothesis predicts that DA activity would be required for the
production of appetitive and consummatory responses to external food-related cues such as food odors.

An alternative hypothesis, the "response initiation hypothesis", proposes that DA is essential for response production (Fibiger, Zis, & Phillips, 1975; Posluns, 1962). This hypothesis proposes that an animal treated with a neuroleptic is capable of understanding the significance of a biologically relevant environmental cue, but is unable to initiate a motor response to this cue. This hypothesis was developed to account for the observation that neuroleptic-treated animals could not perform avoidance responses to a stimulus that predicted footshock, but could perform short latency escape responses once the shock began (Hunt, 1956). During presentation of the predictive stimulus, the animals squealed and defecated, indicating that they realized the relevance of the cue, yet were unable to perform a response to avoid the impending shock. The ability to escape once the shock began demonstrates that the animal had the physical capacity to perform the response. Fibiger et al. (1975) explain this preserved ability by suggesting that even though an animal is unable to perform an avoidance response, involuntary motor activity caused by the shock itself may be sufficient to initiate an escape response.

Blackburn et al. (1987) suggested that both avoidance and appetitive behaviors may fall under the general category of preparatory responses, whereas escape and ingestion may be classified as consummatory responses. Given this
distinction, the response initiation deficit hypothesis derived from shock experiments could be applied to feeding behaviors. DA activity may be required for the initiation of any preparatory response, whether this response is made to avoid shock or to approach food.

In 1978, Wise, Spindler, DeWit, and Gerber proposed the "anhedonia hypothesis" to explain the action of neuroleptic drugs and possibly the role of ascending dopamine systems. This hypothesis suggested that dopaminergic neurons mediate the rewarding or "hedonic" impact of positive reinforcers, such as food (Wise, 1982, 1985; Wise et al., 1978a). Accordingly, disruption of ascending dopaminergic pathways attenuates the hedonic properties of various positive reinforcers. The anhedonia hypothesis is consistent with the reduction in food-rewarded responses observed in animals treated with the DA antagonist pimozide (Geary & Smith, 1984; Wise, Spindler, & Legault, 1978b; Xenakis & Sclafani, 1981). Therefore, if the reinforcer loses its hedonic impact, the response associated with it will be extinguished. However, this apparent "extinction" effect resulting from DA receptor blockade is not equivalent to the typical extinction seen following a period of nonreinforcement. Specifically, more rapid extinction is observed as the effects of neuroleptics sum with those of extinction by nonreinforcement (Gray & Wise, 1980; Phillips & Fibiger, 1979; Tombaugh et al., 1980).

Results obtained by Blackburn, Phillips, Jakubovic, and
Fibiger (1986) also do not support the anhedonia hypothesis. In this study, increased DA activity was observed following ingestion of a nutritive liquid diet or lab chow, but not after ingestion of a palatable saccharin solution. These results suggest that since saccharin solution is avidly consumed by rats and has been shown to maintain high rates of operant responding, the apparent "reward" associated with saccharin ingestion is not sufficient to increase DA activity.

As mentioned above, Blackburn et al. (1987) noted that pimozide disrupted responses to a conditioned stimulus signalling food, but not responses to the food itself. They argued that the anhedonia hypothesis does not adequately explain why the response to a secondary reinforcer (the conditioned stimulus) should be more susceptible to disruption by pimozide than a primary reinforcer (food). In addition, the hypothesis cannot account for the results obtained from a second experiment in which pimozide-treated animals were given unlimited quantities of food in their home cages. Under this condition, the anhedonia hypothesis would predict that following pimozide treatment, the food would be less rewarding, resulting in decreased consumption. However, this prediction was not confirmed.

In a recent study, Berridge et al. (1989) provided evidence that argues against both the anhedonia and the sensorimotor hypotheses. These researchers employed a taste reactivity paradigm to examine the effects of mesostriatal
6-OHDA lesions. Rats emit positive and aversive responses naturally, depending on the palatability of an ingested substance. By studying the reaction of both lesioned and control rats to solutions over a range of taste palatability and intensity, the researchers sought to discriminate between the sensorimotor and anhedonia hypotheses. They proposed that a decrease in sensorimotor arousal would lead to a general reduction in taste reactivity whereas anhedonia would selectively reduce positive reactions. However, taste reactivity remained unchanged after the lesions, a result that supported neither hypothesis. Berridge et al. (1989) argued that their study "provides evidence that the capacity for hedonics can be neurologically dissociated from motivated appetitive behavior". They proposed an alternative theory based on incentive attribution.

The concept of "incentive motivation" emphasizes the ability of environmental stimuli associated with biologically significant events to elicit behavioral responses (Bindra, 1978; Bolles, 1972; Toates, 1981). Preparatory behavior, including both appetitive and aversive responses, may be initiated by formerly neutral stimuli that have become incentives through experience. The strength of an incentive stimulus is determined by the degree of association between this stimulus and a biologically relevant stimulus (e.g., food, shock), and also by the salience of the biological stimulus. That is, although an internal condition such as "hunger" cannot initiate activity
itself, it can potentiate the effectiveness of a given food-related environmental stimulus in initiating future responses (Wise, 1987). Bindra (1978) argued that physiological conditions are important because they "serve as 'gates' or limits within which certain particular incentive stimuli become effective", but without an incentive or a stimulus predictive of an incentive, there is no motivation or response initiation.

It has been suggested that incentive stimuli not only predict hedonic events, but also acquire some of the hedonic properties of the biologically significant events they are associated with (Berridge & Schulkin, 1989; Berridge et al., 1989; Stewart, de Wit, & Eikelboom, 1984). In a study cited by Stewart et al. (1984), opiate-like effects were reported by former addicts self-injecting saline under high-drug-expectancy conditions. Berridge and Schulkin (1989) found that positive palatability-dependent reactions to a taste previously paired with salt, were enhanced only when a rat was deprived of sodium. This enhancement of palatability occurred even though the conditioned taste became associated with salt during "sodium-balanced" trials, a condition under which rats show aversive reactions to hypertonic salt. The shift in palatability of the conditioned taste resembled the shift that would have occurred with salt itself during sodium depletion, indicating that the conditioned taste had acquired some of the hedonic qualities of the salt.

It has been proposed that the neural substrate of
incentive motivation involves ascending DA systems (Blackburn et al., 1987; Fibiger & Phillips, 1986; Mogenson and Phillips, 1976). According to this hypothesis, dopaminergic activity is required when motivational incentive significance is assigned to neutral stimuli through association with biologically relevant events. Disruption of the neural system involved in the attribution of motivational salience would disrupt motivated behavior even if motor, sensory, and affective systems were intact. The incentive motivation model of DA function asserts that exposure to an incentive stimulus can lead to an increase in the release of DA in the forebrain, which in turn results in a preparatory response (Blackburn et al., 1987). This hypothesis is supported by the observation of increased dopaminergic activity during appetitive behaviors (Schultz, 1986; Blackburn et al., 1989b). It is also consistent with the selective disruption of preparatory, but not consummatory feeding behaviors observed following pimozide treatment (Blackburn et al., 1987).

Rationale for the application of electrochemistry to the study of feeding in the rat

The purpose of the present thesis was to study the role of DA in feeding behaviors in vivo by employing the technique of electrochemistry with stearate-modified carbon
paste electrodes. This technique offers several advantages. Electrochemistry does not depend on postmortem tissue analysis, but can be performed on unanesthetized, freely-moving subjects. Electrochemical signals can be obtained from one animal over an extended period of time (i.e., during both baseline and experimental phases), allowing it to serve as its own control. This also enables the researcher to observe the time course of an observed neurochemical response. In addition, the technique can be used to study neurochemical and behavioral correlations without the need for experimentally manipulated DA levels obtained by electrical stimulation, lesions, or pharmacology.

Although all of the points mentioned so far could apply equally to both electrochemistry and microdialysis, each technique has its own unique advantages. The temporal resolution of chronoamperometry is superior to microdialysis because samples can be taken every few seconds (usually 15 to 20 s) as opposed to the 5 to 20 min sampling time in microdialysis. Also, chronically implanted electrochemical electrodes tend to remain viable for much longer periods than microdialysis probes (often, weeks as opposed to days), thus facilitating replication. Finally, electrochemical electrodes are generally smaller than dialysis probes and as a result, cause less damage during implantation. However, the exact nature of the substance contributing to signals obtained by electrochemical methods is not firmly
established, whereas the results obtained by microdialysis with HPLC/ED analysis are unequivocal. Stearate-modified carbon-paste electrodes have been developed for the selective monitoring of extracellular DA levels in "DA-rich" brain regions (Blaha & Lane, 1983). This gives them an advantage over other electrochemical electrodes that are either nonselective, or that monitor DA metabolites as indices of DA turnover.
GENERAL METHODS

Electrode preparation

A three-electrode electrochemical detection system was used in all experiments. Recording electrodes were made from Teflon-coated stainless steel wire (0.008" bare, 0.011" coated). The stainless steel was withdrawn approximately 0.5mm through the Teflon coating leaving a well at one end. This well was packed with a graphite paste mixture consisting of graphite powder, stearic acid, and liquid paraffin (Nujol). Reference electrodes were made from Teflon-coated silver wires. These electrodes had the Teflon coating stripped for 1-2mm at one end, and the exposed silver wire was silver-chlorided. The third type of electrode used, was the auxiliary electrode, consisting of a bare stainless steel wire anchored to a skull screw. The free ends of each type of electrode were soldered to gold connector pins.

Surgery

All rats used in the following experiments had two cranial electrodes implanted under sodium pentobarbital anesthesia (Somnitol, 60mg/kg i.p.) One recording electrode was implanted stereotaxically in the nucleus accumbens,
1.2mm anterior to bregma, 1.2mm lateral (left hemisphere), and 6.5mm ventral to the surface of the cortex with the incisor bar set at -3.3. The second recording electrode was implanted into the striatum, 1.0mm anterior to bregma, 1.7mm lateral (right hemisphere), and 4.0mm ventral to the cortical surface.

For each rat, a single reference electrode was implanted approximately 2-3mm posterior and lateral to bregma into the right hemisphere. The reference electrode was lowered until the chlorided tip disappeared into the cortex. A separate auxiliary electrode was wrapped several times around one skull screw. Three to four additional screws were anchored to the skull. The gold pin from each electrode was snapped into a plastic holder. This whole assembly was cemented together with dental acrylic. The upper edge of the plastic holder containing the gold pins was threaded so that a lead used for electrochemical recording could be screwed on. The rats were allowed to recover from surgery for at least 2 days before being used in an experiment.

Recording procedure

Chronoamperometric recordings were taken by applying square-wave potential pulses to each recording electrode at 20-s (Expt. 1) or 30-s (Expt. 2) intervals and measuring the
current after 1 s. The voltage parameters for the pulses were determined by first obtaining voltammetric sweep records. In a sweep, the applied voltage was ramped at +10mV/s, and the resulting current was recorded. When the record was semidifferentiated, any current due to the oxidation of a given substance at the electrode tip showed up as an exaggerated peak superimposed upon a background curve (Figure 1). The pulse parameters for chronoamperometry were set by determining the window within which the peak of interest lay (e.g., -100mV to +175mV for the proposed DA peak in Figure 1).

When voltammetric sweep records themselves were used to record changes in DA levels during a test session (in Expt. 2 only), a sweep interval of 10 min was chosen to allow sufficient time for equilibration of DA at the electrode tip. Peak height was used as an indication of extracellular DA levels.

Histology

At the conclusion of an experiment, each rat was sacrificed in a CO2 chamber and its brain was removed and stored in a 10% formalin solution for several days. Later, each brain was frozen, sectioned, and mounted in order to allow histological confirmation of electrode placements in accordance with the Paxinos and Watson atlas (1982).
Figure 1 Example of a typical voltammetric sweep, ramped at 10mV/s (-150 to +450mV). The peak occurring at approximately 100mV represents the putative DA peak.
EXPERIMENT 1

Effects of an unsignalled liquid meal on electrochemical measures of DA activity

Experiment 1 was designed to determine whether any changes in the dopaminergic electrochemical signal could be detected in the rat caudate and nucleus accumbens before, during, and after the delivery and consumption of a small unsignalled meal of a palatable liquid diet. The simple procedure followed in this experiment allowed observation of the time course of any effects of feeding on extracellular DA levels. One advantage of this experiment over past studies correlating feeding and DA release was that food deprivation was not a factor here. Each rat was allowed free access to food except during the actual test sessions, resulting in a more normal physiological, and possibly neurophysiological, state during testing. Based on the results of other feeding studies mentioned earlier, increases in DA activity would be predicted to occur in response to the meal presented in the current study.
Methods

Subjects

Four male hooded Long-Evans rats from Charles River Laboratories were used in this study. At the beginning of the experiment, the rats weighed between 350g and 450g.

Apparatus

During the daily recording sessions, individual rats were placed inside a Plexiglas testing chamber located within a larger Faraday cage to reduce electrical noise. The bottom of the chamber consisted of a wire grid floor with a bed of San-i-cel beneath it. At one side of the compartment, a hole was drilled and a brace attached to accommodate a removable Richter tube containing the liquid diet, Sustacal (Mead Johnson), used in the study. A commutator for electrochemical recording extended down from the roof of the Faraday cage to just above the roof of the Plexiglas compartment. A recording lead was attached to this commutator at one end and to the rat's electrode assembly at the other. Outside of the Faraday cage, leads originating from the commutator were connected to an electrochemical recording box. Recorded electrochemical
Signals were translated by, and viewed on, a micro-computer.

On each trial, the rats' behavior was observed via a video camera in front of the test chamber. The room was lit by a bulb turned away from the rats and dimmed as much as possible while still allowing an adequate video recording of the rats' behavior.

Procedure

In order to prevent neophobia during test sessions, all rats were preexposed in their home cages to 10-20ml of the chocolate-flavored liquid diet in addition to their usual diet of lab chow pellets and water. Rats that consumed the liquid diet overnight or upon a second exposure, were used in the experiment.

During a given experimental session, an individual rat was connected to a recording lead and placed inside the test chamber. At the beginning of a test session, the rat had no access to food nor water. Each day, voltammetric sweep records were first obtained and chronoamperometric parameters were then determined from these sweep data. After a stable baseline (less than 1 nA peak to peak fluctuation) of at least 10 min was recorded at a 20 s pulse interval rate, the test box was opened and a 10ml portion of the liquid diet was placed inside. The electrochemical signal from the rat was recorded for another 18-30 min.
During all phases of the experiment, the rat remained undisturbed in a closed room except during the brief food delivery period. Each session was videotaped and the tapes were used to analyze the time spent on feeding behavior. At the conclusion of a test session, the rat was returned to its home cage and given free access to lab chow and water until the following session. Each rat was tested in this manner, once per day, for seven consecutive days.

Results

Each of the 4 rats used in this experiment showed an increase in chronoamperometric oxidation current of approximately 0.5 to 2.0 nA over baseline levels from both the n. accumbens and caudate in the period following the introduction of Sustacal inside the test chamber (Figures 2, 3, 4, and 5). Each figure represents data obtained from 1 of the 4 single rats averaged over test days 2 through 7. Data from the first test day for each rat were not included in this average because the feeding response was typically not immediate nor as vigorous on this day as on the following sessions, when the rat was more familiar with the test chamber and procedure. In fact, none of the four subjects showed any recorded increases in either the n. accumbens or caudate on the first day of testing (Figure 6). Aside from the differences observed between day 1 and days
Figure 2  Chronoamperometric signals recorded from the caudate (squares) and nucleus accumbens (crosses) before and after the delivery of a 10cc Sustacal meal. The figure represents averaged data obtained from rat #1 over days 2-7. The point at time zero was adjusted to 0nA to facilitate comparison of oxidation current changes between the two recording channels. The average meal duration from days 2-7 is bounded by time zero (the point of food delivery) and the arrow indicating the end of the feeding bout.
Figure 3  Chronoamperometric signal recorded from the caudate (squares) and nucleus accumbens (crosses) before and after the delivery of a 10cc Sustacal meal. The figure represents averaged data obtained from rat #2 over days 2-7. The point at time zero was adjusted to 0nA to facilitate comparison of oxidation current changes between the two recording channels. The average meal duration from days 2-7 is bounded by time zero (the point of food delivery) and the arrow indicating the end of the feeding bout.
Figure 4  Chronoamperometric signal recorded from the caudate (squares) and nucleus accumbens (crosses) before and after the delivery of a 10cc Sustacal meal. The figure represents averaged data obtained from rat #3 over days 2-7. The point at time zero was adjusted to 0nA to facilitate comparison of oxidation current changes between the two recording channels. The average meal duration from days 2-7 is bounded by time zero (the point of food delivery) and the arrow indicating the end of the feeding bout.
Figure 5  Chronoamperometric signal recorded from the caudate (squares) and nucleus accumbens (crosses) before and after the delivery of a 10cc Sustacal meal. The figure represents averaged data obtained from rat #4 over days 2-7. The point at time zero was adjusted to 0nA to facilitate comparison of oxidation current changes between the two recording channels. The average meal duration from days 2-7 is bounded by time zero (the point of food delivery) and the arrow indicating the end of the feeding bout.
Figure 6  Representative chronoamperometric record from the caudate (squares) and nucleus accumbens (crosses) of rat #2 on the first day of testing. Time zero indicates the point of delivery of a 10cc Sustacal meal and the arrow indicates the end of the first feeding bout.
Figure 7 Chronoamperometric signal recorded before and after the delivery of a 10cc Sustacal meal. The figure represents averaged data obtained from rats 1, 2, 3, and 4 over days 2-7. The point at time zero was adjusted to 0nA to facilitate comparisons of oxidation current changes between the 2 recording channels. The average meal duration is bounded by time zero (the point of food delivery) and the arrow indicating the end of the feeding bout. The symbols (crosses = n. accumbens, squares = caudate) represent averaged data from the 4 rats, and the solid lines represent standard error of the mean. a) n. accumbens b) caudate
Figure 8  Electrode placements directed at the left nucleus accumbens (n=4) and right caudate (n=4) in Experiment 1.
2-7, no further developments across sessions were observed.

For a given rat, the time course and the magnitude of the recorded increases in chronoamperometric current were very similar in both the n. accumbens and caudate. In addition, the pattern of change seen in each of the four rats was comparable. The average change in recorded oxidation current across all 4 subjects is presented in Figure 7.

The time for a rat to complete its first intense feeding bout was defined as ending when the rat had spent at least 30s away from the feeder (generally, the rat had consumed most or all of the meal at this point). In three of the four figures (Figures 2-4), the recorded current appears to be returning to baseline levels approximately 25-30 minutes after food delivery. Although Figure 5 does not indicate a complete return to baseline, this figure represents data collected for less than 20 minutes, which may not have been sufficient to observe a return to baseline in this subject.

Analysis of the histological data revealed that all of the electrode tips were located either in the right dorsomedial striatum or the left nucleus accumbens (Figure 8).
Discussion

The results of Experiment 1 indicate that feeding has an effect on dopaminergic activity in both the nucleus accumbens and striatum of the rat. In both cases, estimates of extracellular DA levels derived from chronoamperometric records increased after consumption of a liquid meal. Both the magnitude and time course of the recorded increases were similar in the n. accumbens and striatum, suggesting that the two recording sites may share a similar role in feeding behaviors.

Two factors in this study indicate that the DA response may be associated with a postingestive stage of feeding. First, there appeared to be a latency between the initiation of food consumption and the observation of a notable increase in the chronoamperometric signal. Second, the DA levels remained elevated between 20 and 30 min after the meal had been completed.

One potentially confounding factor in the present study arises from the possibility that the "baseline" values recorded at the beginning of each test session represent an extracellular DA concentration that has already been elevated simply by placing the rat into the test chamber. The combination of handling, exposure to a novel environment complete with odors from previous feeding experiments in the same test chamber, and possibly, anticipation of a palatable meal, could have resulted in an increase in DA activity.
This elevation could have influenced the observation of any further increase in DA activity due to the presentation and consumption of food. That is, the changes from true "baseline" levels may have been even larger than those that were actually observed.

One aspect of feeding that this study does not explore is the role of DA in anticipatory stages of feeding. In Experiment 1, the rats began to consume the unsignalled meal within seconds of delivery. As noted in the Introduction, earlier studies have indicated that DA systems may be selectively involved in preparatory aspects of feeding behaviors (Blackburn et al., 1987, 1989a). This distinction between preparatory and consummatory feeding stages was investigated in Experiment 2.
EXPERIMENT 2

Electrochemical measures of dopamine activity during anticipatory and consummatory phases of feeding behaviors in the rat

This experiment was designed to study changes in electrochemical measurements of extracellular DA during different phases of a conditioned feeding paradigm developed by Weingarten (1983, 1984). As reviewed in the Introduction, DA has been associated with appetitive responding for food (Blackburn et al., 1987, 1989b; Schultz, 1986). In order to study this proposed correlation, it was necessary to observe the appetitive phase independently of the consummatory response. Weingarten's paradigm accomplished this separation by conditioning the subjects to an external stimulus cue (CS+) that predicted the delivery of a meal. This cue, consisted of a buzzer and a bright light, preceded, and then continued throughout the food delivery period. In the present experiment, during the CS+ period preceding food delivery, the rat's behavior (nosepokes into the feeding niche) was monitored as an indication of appetitive responding. Simultaneously, chronoamperometric records were obtained. As in Experiment 1, the rat's electrochemical signal from both the caudate and nucleus accumbens was recorded during all stages of the
feeding bout, including a distinct "appetitive" phase. One interpretation of the incentive motivation hypotheses of DA function predicts that the CS+ alone should act as an incentive stimulus resulting in increased DA release and subsequently, an approach to the feeding niche (Blackburn et al., 1989a).

Methods

Subjects

All 40 subjects used in this study were male hooded Long-Evans rats weighing 350 to 450g at the beginning of the experiment.

Apparatus

Rats were housed individually within four separate conditioning chambers. Each chamber consisted of a sound attenuating modified Coleman cooler (Sno-Lite Low Boy model) containing a smaller Plexiglas compartment in which the rat was housed. The floor of the inner compartment was made of a wire grid with a bed of absorbent San-i-cel below it. Each compartment had two holes and two Richter tube braces situated at one end wall. Water was freely available to the
rat at all times through one of these two holes. The opposite wall contained a recessed feeding niche with a 1 cm lip enabling it to hold the liquid diet. A small hole was drilled at the back of the feeding niche to accommodate the end of a length of Silastic tubing. Sustacal was delivered through this tubing by a Cole-Parmer peristaltic pump. The sides of the feeding niche were fitted with a photocell. Interruption of the photocell beam indicated a rat's entry into the niche.

Outside of the Plexiglas compartment, each cooler was fitted with a dim houselight (on at 11:00 am, off at 11:00 pm) and a fan (always on). For conditioning purposes, a buzzer, bright cue light and tone generator were also mounted near the ceiling of the cooler. The cues, pump, and photocells were controlled initially by a Nova and subsequently by an INI computer using Manx software.

Two of the four chambers were set up for electrochemical recording. In these chambers, a Faraday cage was placed between the cooler and the smaller Plexiglas compartment. Each cage was fitted with a commutator extending down to the roof of the Plexiglas chamber. A recording lead was attached between this commutator and the rat's head apparatus. Cables were extended from the opposite end of the commutator to an electrochemical recording box outside of the cooler. Recorded signals were translated by and viewed on an IBM compatible personal computer.
Procedure

The procedure followed in Experiment 2 was based on a conditioned feeding paradigm developed by Weingarten (1983, 1984). The present procedure involved exposing individually housed rats to an average of eight signalled 9ml liquid meals per day, a volume sufficient to keep the animals between 90% and 100% free-feeding body weight. Presentation of a meal coincided with the final minute of a 330 second buzzer-light conditioned stimulus (CS+). All meals were delivered into the feeding niche at one end of the cage. When the rat entered the niche, a photocell beam was interrupted and this event was recorded by computer. A second stimulus (CS−), presented as a pure tone, was not paired with food delivery. The intertrial interval ranged from 43 to 105 minutes with a mean of 90 minutes. After several days of training on an alternating CS+/CS− schedule, the rats reliably displayed the ability to discriminate between the two cues by spending much of the CS+ period in the niche, and little or no time in the niche during the CS− period. The amount of time and number of "nosepokes" into the niche during presentation of the CS+ stimulus were considered to be indications of preparatory responding in anticipation of a meal.

Subjects were conditioned to discriminate between the two stimulus cues either before or after electrode
implantation. In both cases, conditioned rats were monitored chronoamperometrically and behaviorally (via nosepoke data) continuously except during a brief daily, and more extensive weekly, maintenance and data collection period. Once a week, the rats were removed temporarily from the conditioned feeding boxes to be weighed and to have their cages cleaned. After initial hook-up to the recording apparatus, a 2-day period was allowed for adaptation to the lead, the novel test box, and for those rats removed for implantation after training, to become reacquainted with the conditioned feeding schedule. If the subject was returned to the colony for any reason, an additional day of adaptation was allowed following reconnection.

Event-marked chronomperometric records were scored blindly for any changes in signal, and later, they were matched with computer data indicating whether event markers represented CS+, CS-, houselights on (11:00 p.m.), or houselights off (11:00 a.m.). For each trial, a chronoamperometric current value (in nanoamps) was obtained by determining the change in oxidation current relative to the baseline level preceding the event.

For one subject, which was showing good behavioral (nosepoke data) and chronoamperometric CS discrimination, additional voltammetric sweep records were obtained during a series of conditioned feeding trials. In the 10 CS+ and 10 CS- trials conducted, the sweep interval was set at 10 min with a voltage ramp of +10mV/s. Each trial was timed such
that the end of one sweep occurred approximately 270 s after
the onset of a CS. The actual sweeps lasted between 40 and
60 s depending on the selected voltage parameters. This
timing was chosen so that one sweep could be obtained as
late as possible in the "CS alone" period which ended with
the onset of food delivery, 270 s after the CS+ began. In
addition to the sweep recorded during the CS period, at
least three sweeps were obtained both before and after this
period.

Three subjects from which several days of good
behavioral and chronoamperometric data had been collected,
were placed on an extinction schedule. During this
condition, two changes were made. First, the food delivery
tubing was disconnected from the back of the feeding niche,
and second, a Richter tube with a daily supply of Sustacal
was fitted into the extra brace in the wall opposite the
niche. Behavioral and electrochemical measurements were
continued throughout the extinction period. In one of the
three subjects, these measurements were continued into an
additional reinitiation period in which the ad lib food
supply was removed and the food delivery tubing reconnected.

Of the total of 40 rats that began this experiment, the
records from 10 rats (3 with 2 good channels) were used in
the statistical analysis. Electrochemical recording
channels were eliminated from the analysis if any one of the
three following conditions occurred before sufficient data
were collected: noisy chronoamperometric records, small
(<1/2 nA) or absent voltammetric peaks, or a pulled electrode cap. Of the rats that developed noisy signals or pulled caps after only a few recording sessions, several showed promising results up until the signal was lost. The three criteria discussed above accounted for the records of all but 2 of the discarded subjects. Although these 2 rats had reasonable voltammetric peak amplitudes and chronoamperometric noise levels, showed behavioral CS+/CS- discrimination, and had histologically confirmed electrode placements, for unknown reasons, they did not appear to exhibit chronoamperometric responses to the CS stimuli. Because no chronoamperometric responses were ever obtained from these 2 rats, they were not included in the following statistical analyses.

Statistical Analyses

For each viable electrochemical recording site (accumbens, n=8; caudate, n=5), a series of 20 consecutive "scorable" CS+ and 20 CS- trials were analyzed. A trial was not scored if in the 10 minutes preceding the event, there was a transient increase in the noise of the signal, a rising baseline, or a spike in the record. Excluding the adaptation days, the first 20 scorable trials were used in the statistical analysis. The mean change from baseline was calculated for each set of 20 CS trials.
The chronoamperometric measures were analyzed using a two-way within subjects ANOVA for repeated measures, with implant location (accumbens vs. caudate) and event (CS+ vs. CS-) as the two factors.

In order to estimate baseline variability, a baseline difference score was calculated for each recording site. Difference scores were determined by using baseline data preceding the first CS+ and CS- trials in a series. The mean baseline value over five points beginning 540 s (30 s intervals) prior to the CS onset was subtracted from the mean value beginning 270 s before the CS. These baseline difference scores were then averaged for each brain site/event combination.

Behavioral discrimination was analyzed with respect to two measures: total time spent in niche during the first 270 s of a CS and latency to enter niche following CS onset. Values for these measures were obtained from the same trials that were used in the chronoamperometric analysis. Average values within each set of 20 trials were calculated and then analyzed in two separate two-way ANOVAs; one for the amount of time spent in niche, and the other for entrance latency. For both measures, the two factors analyzed were implant location (accumbens vs. caudate) and event (CS+ vs. CS-).

Voltammetric sweep records from a single subject were analyzed with respect to peak height ratios. The height of a peak was determined by first drawing a line between the two minimum values on either side of the proposed DA peak.
The peak height value was subsequently obtained by measuring the vertical distance from the peak tip to this line. Ratios were determined between peak height measured during the CS period and peak height in the previous 10 min period. The peak height ratios for CS+ and CS− (10 trials each) were analyzed using an independent variables t-test.

Results

Behavioral discrimination between the two CS conditions is indicated by the results from the two behavioral measures shown in Figures 9 and 10. Whereas during the CS+, the average latency to enter the feeding niche was relatively short, with a substantial amount of time spent in the niche, the reverse was true during the CS−. These results were supported statistically. The ANOVA for the amount of time spent in the feeding niche showed no significant effect of implant location (accumbens vs. caudate) and no significant location by event interaction. However, there was a significant within subjects effect ($F(1,11) = 56.55$, $p<.00001$). The amount of time spent in the niche was significantly greater during the CS+ than the CS− for both groups. The ANOVA for latency to enter the niche also showed no significant effect of implant location or location by event interaction. Again, there was a significant within subjects effect ($F(1,11) = 152.19$, $p<.00001$). Here, the
Figure 9  Mean amount of time spent in the feeding niche during the CS+ and CS- periods prior to the onset of food delivery. Data are the average of 20 consecutive trials on which a good chronoamperometric record was obtained. Error bars represent standard error of the mean.

*p<.0001
The diagram illustrates the time (in seconds) spent in a niche by two different brain regions: caudate and n. accumbens. The x-axis represents two conditions: CS- and CS+. The y-axis represents time in niche. The caudate region shows a lower time spent in the niche compared to the n. accumbens region under both conditions. The n. accumbens region shows a significant increase in time spent in the niche under the CS+ condition (indicated by an asterisk).
Figure 10 Mean latency to enter niche following CS+ or CS- onset on the same trials as shown in Figure 8. Error bars represent standard error of the mean. *p<.0001
Figure 11  Mean changes in DA oxidation current in the nucleus accumbens (n=8) and caudate (n=5) during CS+, CS− and pre-CS baseline periods (bCS+, bCS−). Significant differences were found between values obtained from the nucleus accumbens and caudate (p<.03) and between the CS+ and CS− (p<.0004). The vertical line dividing baseline and event results indicates that no statistical comparison was made between the two conditions, due to large sample size discrepancies. Error bars represent standard error of the mean.
Figure 12 Representative chronoamperometric records obtained from four different subjects during consecutive CS+ and CS- trials. Arrows indicate CS+, CS-, or meal onset. a),b) n. accumbens  c),d) caudate
Figure 13 Behavioral and electrochemical results from extinction subject #1. E = first day of extinction  
a) mean amount of time spent in feeding niche during CS+ period  
b) mean change in oxidation current recorded from the nucleus accumbens during CS+
Figure 14 Behavioral and electrochemical results from extinction subject #2. E = first day of extinction
a) mean amount of time spent in feeding niche during CS+ period b) mean change in oxidation current recorded from the nucleus accumbens and caudate during CS+. 
time in niche (s)

days

0 10 20 30 40 50
0 1 2 3 4 5 6 7
Figure 15 Behavioral and electrochemical results from extinction subject #3. E = first day of extinction, R = first day of reinitiation to conditioned feeding  
a) mean amount of time spent in feeding niche during CS+ period  
b) mean change in oxidation current recorded from the nucleus accumbens and caudate during CS+. Electrochemical signal from the caudate was lost on day 8.
Change in oxidation current (mA)

- n. accumbens
- caudate

Days
Figure 16 Representative CS+ sweep trial from one subject.

The figure presents nine consecutive sweeps ramped at 10mV/s with a 10-min inter-sweep interval. The arrow indicates the DA peak that was obtained during the CS+ period prior to food delivery.
oxidation current (nA)

E (VOLTS VS. Ag/AgCl)
Figure 17 Mean peak height ratios from 10 CS+ and 10 CS- voltammetric sweep trials recorded from the nucleus accumbens of a single rat. Error bars represent standard error of the mean.

*p= .001
Figure 18 Electrode placements directed at the left nucleus accumbens (n=8) and right caudate (n=5) in Experiment 2.
latency to enter the niche following CS onset was significantly greater during the CS- than the CS+ for both groups.

Figure 11 shows the results from the corresponding electrochemical measurements. It is evident from the figure that the CS+ has a greater effect on DA oxidation currents than the CS-. In addition, the chronoamperometric response in the nucleus accumbens appears to be greater than in the caudate. Consistent with this, the ANOVA detected a significant effect of implant location ($F(1,11) = 6.82$, $p<.03$; accumbens > caudate), and of event ($F(1,11) = 30.78$, $p<.0004$; CS+ > CS-), but no significant location by event interaction. Figure 11 also presents average pre-CS baseline difference scores. Although these baseline values were not compared statistically to event scores because of the discrepancy in sample size, they are included to give an indication of baseline fluctuation. The average magnitude of change in oxidation current observed for these baseline difference scores was similar to the average scores in the CS- condition, both of which were substantially smaller than the values obtained for the CS+.

Figure 12 depicts representative chronoamperometric records during CS+ and CS- trials from four different subjects (2 n. accumbens, 2 caudate). These records indicate an immediate increase in DA oxidation current at CS+ onset, with no apparent change in response to the CS-.

Individual data from successive daily tests are
presented for each of the three "extinction" subjects in Figures 13, 14, and 15. In Figures 13a, 14a, and 15a, dramatic decreases in the average amount of time spent in the niche during the CS+ alone period, can be seen on the first day of, and continuing throughout, the extinction period. Although less pronounced, corresponding decreases are found in the average oxidation current measures (Figures 13b, 14b, and 15b). Figure 15 illustrates that when the CS+/food condition was re-established, the amount of time spent in the niche immediately increased to levels that were even higher than those observed during the 2 days prior to extinction. However, in the one remaining viable channel, there is an apparent 2-day lag before any notable increase is detected for the average change in oxidation current.

A representative trial in which linear sweep voltammetric records were obtained, is presented in Figure 16. An increase in peak height can be seen following CS+ onset and continuing for two sweeps. Average peak height ratios were calculated from a series of 10 CS+ and 10 CS- records of this type (CS+ ratio = 1.09, CS- ratio = 0.95) and are presented in Figure 17. The results are presented in Figure 17. The t-test performed on this data detected a significant difference between the CS+ and CS- conditions, with the CS+ peak height ratios being significantly greater than the CS- ratios (t(18)=3.95, p=.001).

Histological confirmation of the caudate and nucleus accumbens electrode placements is presented in Figure 18.
Discussion

The behavioral results from this experiment provide a clear demonstration of the subjects' ability to discriminate between CS+ and CS- conditions. The average amount of time spent in the niche during the CS alone period was substantially greater during the CS+ trials and latency to enter the niche upon CS onset was much greater during the CS- trials. In fact, most rats rarely entered the niche at all during the CS- period. The results of both measures suggest that the rats were responding in anticipation of a meal predicted by the CS+, but not the CS-.

The corresponding electrochemical results indicated that changes in the neurochemical signal also reflected the discrimination between CS+ and CS-. Increased DA release following CS+ onset was indicated by changes in the chronoamperometric records from both the nucleus accumbens and caudate. Generally, the increased oxidation currents in the caudate paralleled those in the n. accumbens, although the magnitude of these increases were lower in the caudate. The results provide evidence that extracellular levels of DA may be affected selectively by salient external environmental cues.

Although the magnitude of change for a given trial varied both within and between subjects, a characteristic pattern could be clearly seen in the chronoamperometric
record. In trials during which an increase could be observed unambiguously, the record typically showed an immediate and rapid ascent to peak levels following CS+ onset. The latency from CS+ onset to peak current approximated 5 min. A more gradual decrease to baseline was observed, usually within 20 to 30 min after stimulus onset.

The extinction experiment consisted of providing ad libitum access to the liquid diet in a Richter tube, coincident with stopping the delivery of food on all CS+ trials. Three rats were tested in extinction and on each extinction day, both the time spent in the niche and the average change in the oxidation current decreased to levels below those obtained for the 2 preceding baseline days. As expected, on the first day of extinction, the amount of time spent in the niche during the CS+ decreased over successive trials. However, even during the first few trials on this day, behavioral responses were substantially reduced relative to the pre-extinction period. One factor that could account for this observation is satiety. Since the rats were given free access to the liquid diet, they may have fed to satiation and as a result, may have been less responsive to external cues signalling food. Although changes were observed in both the behavioral and electrochemical measures, the behavioral changes were much more pronounced. If DA plays a role in the processing of salient external cues and the initiation of goal-directed behaviors, it is possible that some degree of processing was
still occurring in response to the CS+ during the extinction phase. The rats that were placed under extinction had been exposed to the conditioned feeding program for several weeks, allowing for the development of a strong association between the CS+ and food. During extinction, the neural response to the CS+ may have persisted to some extent, although it may not have been sufficient to generate a behavioral approach to the niche.

The individual sweep trials showed relatively small changes in peak height following CS+ onset. However, these changes were consistent with the chronoamperometric records in that the trends were in the same direction. That is, increases were observed during CS+, but not CS- trials. One advantage of a sweep trial is that it allows the observation of potential changes in the size, shape, and position, of the proposed DA peak. However, there are also definite disadvantages to the use of this technique. First, the temporal resolution is limited. It is possible that any change in DA release correlated with the CS+ may be masked by subsequent re-uptake and degradation occurring in the 10 min interval between sweeps. Fortunately, the neural changes examined in this experiment tended to last between 20 and 30 min, as mentioned above, allowing the collection of 2 or 3 sweeps during this period. In the sample sweep trial shown in Figure 15, the two consecutive sweeps following CS+ onset showed increased peak heights, whereas the third peak was considerably diminished. This temporal
pattern of change in peak current is consistent with the changes observed in the chronoamperometric records.

A second disadvantage of linear sweep voltammetry at the sweep rate of 10 mV/s employed here, involves the influence of ascorbic acid (AA) on DA oxidation. Although the oxidation of AA itself does not contribute to the DA peak, AA amplifies the DA peak by catalyzing a reaction that allows repeated regeneration of DA from the orthoquinone, and subsequent reoxidation at the electrode surface (Stamford, 1986). This effect is pronounced at the slow sweep rate, leading to an amplification of the peak. Therefore, the contribution of AA during slow voltammetric sweeps may mask detection of changes in extracellular DA levels. Echizen and Freed (1986) provide evidence that the relative concentration of catecholamine (in this case, DA) to AA determines the amount of amplification taking place. They found that the amplification factor decreased as the catechol concentration increased relative to the AA concentration. As a result, they proposed that recorded changes in catecholamine concentrations were actually much lower than the real changes that were occurring. During square-wave pulse chronoamperometry, catalytic amplification is minimized because of the short duration of electrochemical measurement (Blaha & Jung, in preparation; Stamford, 1986).
GENERAL DISCUSSION

The experiments presented in this thesis provide further evidence for the involvement of dopamine in feeding. The combined behavioral and electrochemical analyses revealed differential changes in DA activity in the striatum and nucleus accumbens during appetitive and consummatory phases of feeding.

This work represents an extension of the conditioned feeding studies conducted previously by Blackburn et al. (1987, 1989a, 1989b). While the behavioral paradigm followed in both this thesis and the Blackburn studies is similar, the approach used to analyze dopaminergic activity is very different. Blackburn's experiments involved pharmacological manipulations and ex vivo tissue analyses, whereas the present investigation employed the technique of in vivo electrochemistry with chronically implanted stearate-modified carbon paste electrodes. This technique permitted the recording of ongoing changes in extracellular DA levels of freely moving subjects.

To begin this discussion, a summary of the major results obtained in the current study is presented. In Experiment I, increases in DA activity were observed in both the nucleus accumbens and the caudate following the presentation of a liquid meal. Changes observed for each of the four subjects were very similar in magnitude and temporal pattern for both brain structures. The similarity
of responses at both recording sites might indicate a common role for the caudate and accumbens in feeding. The fact that the increases in the chronoamperometric record lagged behind the initiation of meal consumption suggests that the observed effects may be due to postingestive factors.

Experiment II allowed a more detailed analysis of dopaminergic activity during feeding by factoring the behaviors into separate anticipatory and consummatory phases. With this approach, it was discovered that changes in electrochemical estimates of extracellular DA levels corresponded to the presentation of an external cue (CS+) which, through previous experience, had become a reliable predictor of meal delivery. Significant increases in DA oxidation currents were observed in both the nucleus accumbens and the caudate, but the magnitude of change observed in the nucleus accumbens during stimuli presentations was significantly greater than in the caudate.

When the behavioral paradigm was altered such that the CS+ was no longer predictive of a meal (the extinction phase), the DA response to the external stimulus diminished accordingly.

The results from Experiment II were consistent with hypotheses associating DA activity and "incentive motivation" as discussed in the Introduction. As predicted by incentive motivational theories of DA function, increases in DA activity were observed following onset of an external stimulus predictive of a meal. In addition, increased
entries into the feeding niche, assumed to be a measure of appetitive responding, were observed during this "incentive stimulus" (CS+) period. Also consistent with incentive theories was the finding that there were significant differences between the DA responses observed following CS+ and CS- onset. Although both the CS+ and the CS- presumably began as neutral stimuli, after repeated presentation of the CS+ with food, the CS+ alone became associated with increases in oxidation currents from the accumbens and caudate that were significantly greater than those following CS- onset.

When the results from Experiments I and II are compared, it can been seen that the average magnitude of change in DA levels, as estimated from absolute changes in the chronoamperometric signal, was greater with the conditioned feeding procedure. There are a variety of factors which could have contributed to this difference. First, subjects in Experiment I may have had elevated extracellular levels of DA before meal delivery due to daily handling, placement into a test box that had previously been used for feeding experiments, or to the anticipation of a highly palatable meal. Therefore, any observed increase following meal delivery may represent a blunted response because of elevated baseline levels. This was not a factor in Experiment II as the subjects lived in the recording chambers. A second explanation for the difference in magnitude may be that the two experiments were investigating
the role of DA in relation to two different aspects of feeding. In Experiment I, subjects were provided with a small liquid meal that was sufficiently palatable to initiate immediate ingestion even in non-deprived animals. Small increases in DA activity, which tended to occur following meal consumption, may have been due to post-ingestional factors. In Experiment II, a distinct anticipatory phase was added to the feeding sessions. The relatively large increases observed during this phase suggest that DA may play a different, and possibly more important role during appetitive responding than during subsequent consummatory and post-ingestive stages of feeding.

The preceding discussion of magnitudes of change in DA oxidation currents must be approached with caution. What exactly does the magnitude represent? Although it may reflect actual quantitative differences in extracellular DA levels, it may also be affected by factors such as the quality of the recording electrode surface, and the amount of scar tissue surrounding an implant. Also, the placement of the electrode would have an influence. It is well established that the striatum, for example, is not a homogeneous system operating in synchrony. The proximity of an electrode to an active site of transmitter release would be expected to affect the magnitude of any recorded changes. In addition, a small change in one structure may have greater functional significance than a large change at a
different neural site. These factors should be kept in mind when generalizations are made concerning relative magnitudes of change, especially when they are based on small sample sizes.

Comparison of the caudate and nucleus accumbens records across both experiments reveals further interesting results. Whereas the pattern and magnitude of increase in DA release following meal delivery are similar for both structures in Experiment I, there is a significant difference between the two brain sites in Experiment II. This result again suggests that we may be investigating two different aspects of feeding behaviors. Despite the aforementioned problems with comparisons of magnitude, it is tempting to present a few speculations. Suppose we assume that the increases obtained in Experiment I are post-ingestional and Experiment II are, at least initially, appetitive. It may be that the caudate and n. accumbens share a similar role during post-ingestive stages of feeding, but that the nucleus accumbens is preferentially involved in appetitive responding.

DA terminals in the n. accumbens may be involved in the processing of, and the initiation of motor responses to, external cues. Could the magnitude of increase in DA levels reflect the salience of the cue? This might explain why small increases (1.1nA) were observed in the nucleus accumbens in response to the CS-. Whereas some degree of anticipatory arousal involving DA could have occurred in response to this cue, it may not have been sufficient to
merit an approach toward the niche. A similar explanation would account for the persistent, though diminished, dopaminergic responses to the CS+ under extinction conditions.

The pattern of change in extracellular levels of DA obtained in these experiments deserves further mention. Whereas Experiment II suggests that DA terminals may be active during appetitive responding, the results also indicate that DA levels remain elevated throughout and even past the completion of a feeding session. Another interpretation of the present results is that increased DA activity during anticipatory stages is associated with an "aroused" motivational state induced by incentive stimuli. This state of arousal could result in an enhanced sensitivity, and increased likelihood of response, to biologically relevant cues.

Would the enhanced motivational state make an animal more responsive to any biological cues, or would it be specific to the event associated with the incentive stimuli? Results from other studies have demonstrated the involvement of DA in different types of anticipatory responding. In particular, Pfaus (1990) has shown that DA activity is increased during anticipatory stages of sexual behavior in male rats. In addition, an investigation by Blackburn (1989) showed DA activity to be associated with preparatory responses to a cue signalling impending shock. Possibly, increased DA activity results in a general state of arousal.
that is common to subjects exposed to incentive cues predicting different consummatory goals.

Summary

This thesis examined the role of DA in feeding behaviors in the male rat. The approach used has allowed the investigation of DA involvement in different aspects of feeding. This approach combined the division of feeding behaviors into appetitive, consummatory, and post-ingestional phases, with an electrochemical recording technique. The temporal resolution of the electrochemical records allowed correlation of ongoing behavioral and neural responses. The results of this thesis provide support for theories based on incentive motivation.
References


