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Dopaminergic Correlates of Motivated Behavior: Importance of Drive

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In vivo **brain microdialysis was used to monitor changes in dopamine (DA) release in the nucleus accumbens (NAc) during anticipatory and consummatory components of feeding behavior. During 10 daily training sessions, rats were first confined to one compartment of a testing chamber for 10 minutes. During this period (anticipatory phase) they were prevented from gaining access to a highly palatable liquid meal by a wire mesh screen. The screen was then removed and the animals were permitted to consume the meal for 20 min (consummatory phase). On removal of the screen, the latency to begin drinking decreased and the amount consumed increased as a function of days of training, both measures reaching asymptotic levels by day 7. Trained animals were implanted with dialysis probes in the NAc on day 10, and on day 12 DA release was monitored during the feeding session. Compared to controls, trained animals failed to show significantly greater increases in accumbal DA release during the anticipatory phase, all groups showing small (~10%) increases on being placed in the test chamber. In contrast, compared to controls, DA release increased significantly in the NAc during consumption of the palatable meal. The magnitude of this increase was significantly enhanced (30% vs 71% peak increase) in animals that were 20 hr food deprived at the time of testing. The latter animals also showed a statistically significant increase (24%) in DA release during the anticipatory phase. A subsequent experiment in which consumption of the palatable liquid was limited to 5 ml in deprived and nondeprived animals indicated that only part of the deprivation-induced potentiation of accumbal DA release could be attributed to the larger volume consumed by the deprived animals. That is, the same volume and rate of consumption of a small amount of the liquid diet produced a significantly greater increase in accumbal DA release in deprived than in nondeprived animals (42% vs 23% peak increase). Feeding-induced increases in accumbal DA release were not due to postingestional factors as direct injections of the liquid diet into the stomach by gavage failed to produce this effect. The results of these experiments indicate (1) that consummatory rather than anticipatory aspects of feeding are robustly associated with increases in DA release in the NAc, and (2) that motivational state can influence the magnitude of the neurochemical events that are associated with goal-directed behaviors.**

[Key words: nucleus accumbens, dopamine, microdialysis, feeding, consummatory behaviors, anticipatory behaviors, motivation]

On the basis of lesion studies with 6-hydroxydopamine (6-OHDA) it has been known for more than 20 years that lesions of the dopaminergic mesotelencephalic system produce a sensorimotor deficit syndrome that includes aphagia and adipsia (Ungerstedt, 1971; Zigmond and Stricker, 1972; Fibiger et al., 1973; Marshall et al., 1974; White, 1986). While extensive lesions of these dopaminergic neurons or high doses of dopamine (DA) receptor antagonists can profoundly influence all aspects of feeding, a large body of evidence indicates that the so-called "anticipatory" (also termed "instrumental" or "preparatory") aspects of feeding are more easily disrupted by these manipulations than are the "consummatory" components (see Salamone, 1992; Fibiger, 1993, for reviews). For example, instrumental responding for food is attenuated by 6-OHDA lesions of dopaminergic neurons in animals that are not aphagic (Fibiger et al., 1974; Heffner and Seiden, 1983) and by low to moderate doses of neuroleptics that do not disrupt feeding per se (Wise et al., 1978; Tombaugh et al., 1979; Wise and Schwartz, 1981).

The introduction of *in vivo* procedures such as brain microdialysis has provided exciting new opportunities to investigate regional changes in DA release in freely moving animals during feeding or the performance of food motivated tasks. In one of the first studies of this kind, Church et al. (1987) reported that rats trained on a feeding schedule showed increases in interstitial concentrations of DA in the striatum during and after the feeding sessions. This preliminary finding has subsequently been confirmed by other laboratories. For example, Radhakishun et al. (1988) examined the effects of scheduled eating on DA release in the nucleus accumbens (NAc) of rats that were maintained on a food deprivation schedule and found increased interstitial concentrations of DA during feeding. Interestingly, in this experiment there was no evidence of enhanced DA release in the period immediately preceding food availability, a period during which there was a conditioned increase in locomotion and rearing. These data indicate that scheduled eating by food-deprived rats increases DA release in the NAc. This effect appears to be somewhat specific to feeding since DA release did not change during the period of increased locomotor activity that preceded

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food presentation. In a study by Hernandez and Hoebel (1988) animals were trained to lever press for food in a situation in which a light in the test chamber indicated the availability of food. In the condition in which the light was turned on and the animals lever pressed for food for 20 min, DA release increased significantly in the NAc. However, in another condition in which the light was turned on but food was not available, lever pressing produced no increases in DA release in this structure. These findings are generally consistent with the data obtained by Radhakishun et al. (1988) in that they indicate that stimuli that predict availability of food, and that produce increases in either locomotor activity or lever pressing, do not by themselves increase DA release in the NAc. These results are not easily accommodated by the hypothesis that anticipatory aspects of feeding behaviors are preferentially associated with changes in DA metabolism and/or release in the NAc (Blackburn et al., 1989).

The present experiments were undertaken to examine further the extent to which anticipatory and consummatory aspects of feeding are associated with changes in DA release in the NAc. This was accomplished by monitoring DA release during the performance of a feeding protocol in which fixed anticipatory and consummatory components were imposed. A second goal of these studies was to examine the effects of different levels of "drive," as defined by the presence or absence of food deprivation, on DA release in the NAc.

Materials and Methods

Subjects. Male Long-Evans rats weighing 200–250 gm at the beginning of experiment were housed individually under a 12:12 light–dark cycle (lights on at 7:00 hr) at 23°C. Food and water were available ad libitum unless otherwise indicated. Animals were assigned to the following training and test procedures (experiments I–III).

Experiment I. During behavioral training, individual rats were placed in a Plexiglas chamber (35 × 30 × 40 cm). The floor of the cage was covered with animal bedding (Andersons). A removable plastic-mesh screen divided the chamber into two compartments. A removable Richter tube that contained a chocolate-flavored liquid diet (Sustacal, Mead Johnson) was attached on the outer side of the wall of the smaller compartment (15 × 30 × 40 cm). Sustacal was made available via a drinking tube protruding into the cage through a hole in the wall that was parallel to the partition. The animals were water-deprived 24 hr before an habituation session. During this session each rat was placed in the chamber for 30 min and allowed to explore both compartments and taste the Sustacal. At the conclusion of this and all other training sessions each rat was returned to the vivarium where it was housed individually and given free access to food and water. The next day, which was the first training session (day 1), each rat was confined to the large compartment of the chamber for 10 min (anticipatory phase). The screen was then removed and drinking was allowed for 20 min (consummatory phase). During the latter phase, the latency to initiate drinking (i.e., the time in sec between the removal of the partition and the beginning of drinking) and the amount (volume in ml) of Sustacal consumed were recorded. Each rat was trained on this schedule for 10 consecutive days. All the training sessions were performed between 10:00 and 14:00. From 2 to 3 hr after the tenth training session (day 10), each rat was implanted with a microdialysis probe. Following surgery, the rats were housed individually in Perspex holding ("home") cages (25 × 35 × 35 cm) with food and water available ad libitum. In contrast to the training chamber, the floors of the home cages were covered with Crown Animal Bedding. Twenty to 24 hr after implantation of the microdialysis probe (i.e., on day 11), an additional training session was performed. Approximately 48 hr postimplantation (i.e., on day 12) the behavioral test with concurrent microdialysis sampling was performed. During this final test session the anticipatory phase was increased from 10 to 20 min so as to permit two microdialysis samples to be obtained during this phase. Immediately after a subsequent 20 min consummatory phase, the rat was returned to the home cage for the remainder of the experiment.

A separate group of animals trained as described above was prevented from consuming Sustacal on the test day by blocking the outlet of the Richter tube (extinction group). In addition, a control group was subjected to the same behavioral training and testing as described above except that the Richter tube contained water instead of Sustacal during all phases of the training and testing procedures (water group). Animals in this group did not consume significant quantities of water (i.e., <1 ml) during either the training or test sessions.

A fourth group of animals (food-deprived group) was subjected to similar behavioral training until day 6. On the subsequent sessions, immediately following the training session these animals were returned to the vivarium but with no food available. Two hours later food pellets were placed inside the vivarium cages for 2 hr after which they were removed. The amount of food consumed (gm) during the 2 hr period was recorded. This schedule was maintained on days 7–9 and day 11. Thus, animals were food deprived for about 20 hr prior to the Sustacal training sessions on these days. On day 10, animals in this group were implanted with a dialysis probe approximately 2 hr after the training session. Following surgery 6 gm of food was placed in the animal's home cage. Throughout the behavioral training of the food-deprived group a wire mesh floor was placed inside the training chamber to prevent any ingestion of the bedding. For the same reason, a plastic mesh floor was placed inside the home cages in which these animals were housed following implantation of the microdialysis probes. On day 12, the animals (20 hr food deprived) were tested with Sustacal as described above. Two hours after the rats were returned to their home cages, they were subjected to a further feeding test in which food pellets were placed inside the home cage for 2 hr. DA and its metabolites were continuously monitored on line throughout the test session.

Experiment II. This experiment sought to determine the effects of food deprivation on feeding-induced increases in DA release in the nucleus accumbens when the volume of food intake was controlled for. Animals were trained as described in experiment I; however, on day 7, and for all subsequent training and test sessions, these animals were only permitted to consume 5 ml of Sustacal. This group was then further divided after the training session on day 6 into food-deprived and non-food-deprived subjects. The food deprivation schedule was identical to that in experiment I.

Experiment III. The purpose of this experiment was to examine possible postingestional effects of the liquid diet on DA release in the nucleus accumbens. Animals were trained as previously described; however, the partition was not present in the training chamber. Subjects received Sustacal (5 ml) by gavage using a feeding tube attached to a syringe. Immediately following a waiting period of 10 min corresponding to the anticipatory phase of experiment I, rats were picked up, the feeding tube was inserted, and Sustacal was delivered over a period of 1 min. Rats were then returned to the test cage for a further 20 min, corresponding to the consummatory periods in other experiments. On the test day (day 12), the duration of the anticipatory (20 min) and consummatory (20 min) phases was the same as in the other experiments except that no partitioning screen was present and Sustacal was administered by gavage.

Surgery and microdialysis. Two to 3 hr after the tenth training session (day 10), each rat from experiments I–III was anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and implanted stereotaxically with a vertical microdialysis probe aimed at the NAC (coordinates of the probe tip relative to bregma were AP +3.6 mm, ML ±1.5 mm, DV −8.2 mm according to the atlas of Pellegrino et al., 1979). The microdialysis probe was a variant of the concentric design (Robinson and Whishaw, 1988; Brown et al., 1991a; Nomikos, 1991). Dialysis occurred through 2.0 mm of a semipermeable hollow fiber (copolymer of acrylonitrile and sodium methyallyl sulfonate AN69, ID = 0.24, 40,000 Da, Hospal). The *in vitro* recoveries of DA and metabolites with these probes are in the order of 5–7% (Nomikos, 1991). The probe was secured with dental cement to three anchoring skull screws and the skin was sutured.

A behavioral test with concurrent microdialysis sampling was conducted on the second day after surgery (day 12). Microdialysis and subsequent chemical analysis were performed using an automated on-line sample injection system (Damsma et al., 1989). The inlet cannula of the dialysis probe was connected to the perfusion pump (Harvard Apparatus), and the outlet cannula was directly connected to the injector (Valco, EC10W) of the analytical equipment. The microdialysis perfusion solution contained (in mM) NaCl 147, KCl 3.0, $CaCl_2$ 1.3, $MgCl_2$ 1.0, and sodium phosphate 1.5 mM, pH 7.3, and was delivered at a rate of 5 μl/min. The dialysis samples were injected automatically every 10 min (regulated by an adjustable timer; Valco), and analyzed for their

contents of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by HPLC-ECD. Separation of DA and its acid metabolites was achieved by reverse phase liquid chromatography (150 × 4.6 mm, Nucleosil 5 μm, C18; Chrompack) with a mobile phase consisting of 0.1 M sodium acetate (pH 4.1) with 0.4–0.5 mM octanesulfonic acid, 0.01 mM Na_2EDTA, and 100 ml/liter methanol. The mobile phase was delivered by an HPLC pump (Bio-Rad, 1350) at 1.5 ml/min. A pulse-dampener (SSI) was placed between the HPLC pump and the injector. Electrochemical detection was accomplished using a coulometric detector (5100A coulochem, ESA Inc.) with a High Sensitivity Analytical Cell (5011). In the latter system detection of the amines was achieved by the sequential oxidation and reduction of samples (first electrode +0.4 V; second electrode −0.2 V). This arrangement allowed DOPAC and HVA to be detected at the coulometric cell, and DA at the subsequent electrode. Chromatograms were recorded on a two-pen chart recorder (Kipp and Zonen, BD41).

After a stable output (<5% variation) of DA and its metabolites had been obtained for at least four consecutive samples in the home cage, each rat was transferred to the training chamber and the test session was performed as described above for experiments I–IV. Throughout the test session DA and its metabolites were continuously determined on line.

Upon completion of the experiments, the animals were sacrificed, and the brains were processed histologically (50 μm slices, Nissl stain) to verify the placement of the microdialysis probes. Animals in which the probe was located outside the nucleus accumbens were excluded.

Statistical analyses. Basal dialysate values for DA and its metabolites were expressed in fmol/min. The data in Figures 2–8 are presented as mean percentage changes from baseline. For these purposes the average absolute value of the first three baseline samples was considered as baseline and defined as 100%. The baseline sample immediately preceding the anticipatory phase was not used in the definition of 100% and was used in the statistical analyses. Thus, the last baseline plus the 12 subsequent samples were used for statistical evaluation. Data were analyzed using analysis of variance (ANOVA) with repeated measures followed by post hoc comparisons (Tukey's test) when significant time effects were revealed. The Huynh-Feldt adjustment to degrees of freedom was used to account for the use of time as a repeated measure. A significance level of 0.05 was used for all tests.

Results

The latency to initiate feeding as well as the volume of the liquid diet consumed during the training and test days (days 1–12) by food-deprived and nondeprived animals in experiment I are presented in Figure 1. The time elapsing between the removal of the screen and the beginning of feeding gradually decreased and reached asymptotic levels of approximately 10 sec over the first 6 d of training. During the same period, the volume of Sustacal consumed gradually increased and plateaued at approximately 7 ml in the nondeprived groups and at 18 ml in the food-deprived group. The weight of food pellets consumed by the food-deprived animals after the training sessions on days 6–12 is also presented in Figure 1. During the training period (days 7–11) the food-deprived animals consumed between 8 and 10 gm in their home cages after the Sustacal training session except on the final test session (day 12) when they consumed approximately 13 g. Probe implantation did not adversely affect either the latencies to begin feeding or the amount of Sustacal or food consumed on days 11 or 12 (Fig. 1).

When the Sustacal trained (food and non-food-deprived) animals were placed into the chamber on the test day, active exploratory activity was observed during the anticipatory phase. This included sniffing, locomotion, and rearing, as well as nose-poking through the mesh of the plastic screen partition. After the screen was removed the animals started to drink the liquid diet within 5 sec and this continued in bouts throughout the 20 min test period. On the test day, the nondeprived animals consumed 8.5 ml of the liquid diet while the food-deprived animals consumed 19.6 ml ($p < 0.01$). There were no statistically significant differences between the groups in the latency to initiate drinking. During the training and the test days, the control animals that were given access to water rather than Sustacal did not consume significant quantities of water (<0.5 ml).

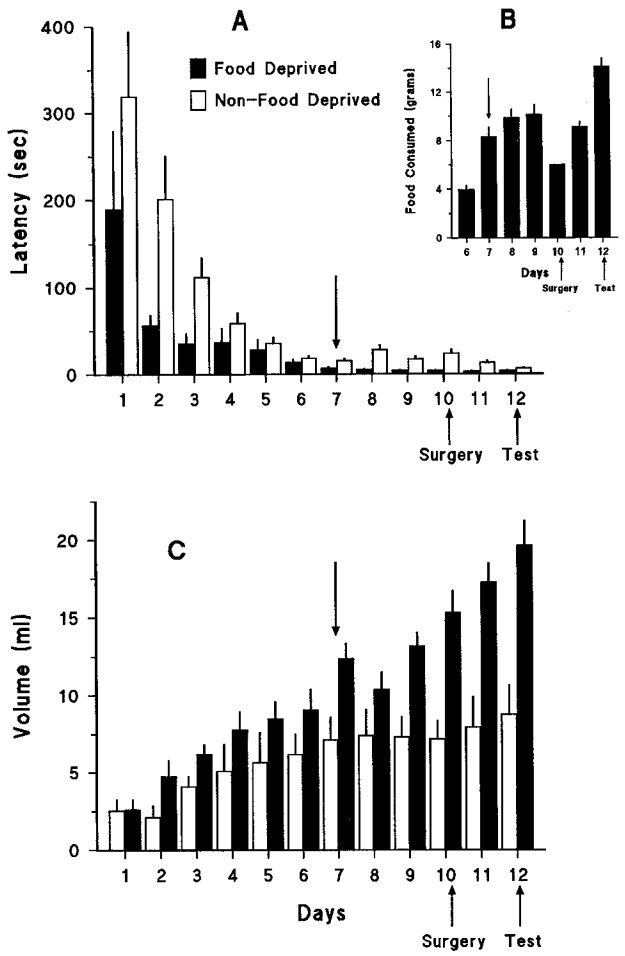

Figure 1. Mean latencies (±SEM) to initiate feeding (*A*) and mean volumes of liquid diet (Sustacal) consumed (*C*) as a function of days of training in food-deprived (*solid bars*, $n = 8$) and non-food-deprived (*open bars*, $n = 24$) rats. *Downward arrows* (day 7) indicate the start of the food deprivation schedule for the food-deprived group. The small panel (*B*) shows the amount of solid chow consumed (grams) by the food-deprived animals over a 2 hr period that began 2 hr after the consumption of the liquid diet.

Figure 2 shows the percentage changes in dialysate concentrations of DA obtained from the NAC of the control, food-deprived, and nondeprived animals during the test session. The results of this experiment generated significant group [$F(3,25) = 11.37$, $p < 0.01$], time [$F(6.78, 169.55) = 17.06$, $p < 0.01$], and group × time interactions [$F(20.35, 169.55) = 3.91$, $p < 0.01$]. In the nondeprived group trained on Sustacal DA concentrations increased to 110% of baseline during both 10 min samples of the anticipatory phase. When compared to baseline values, neither of these increases was statistically significant ($p > 0.05$). A further gradual increase that reached 125% of baseline occurred during the 20 min feeding period. During the second 10 min period of the consummatory phase dialysate concentrations of DA were significantly greater in the nondeprived animals consuming Sustacal than in the water or extinction groups ($p < 0.01$). Interestingly, the maximal DA increase (130%) occurred during the 10 min immediately after the ani-

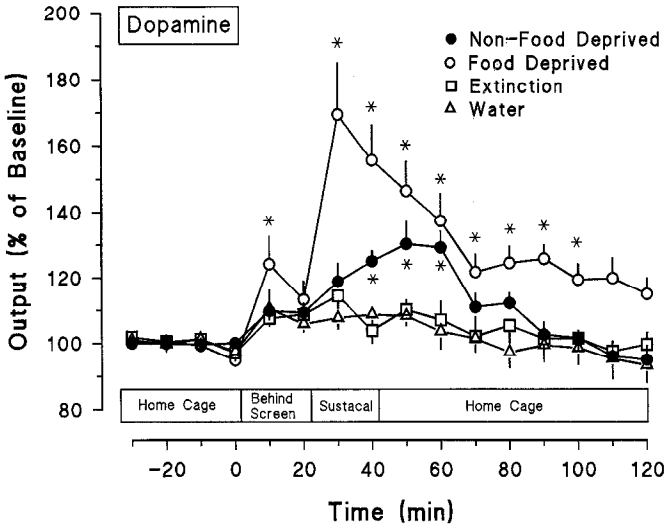

Figure 2. Changes in dialysate concentrations of dopamine obtained from the nucleus accumbens during the final test session in nondeprived rats trained and tested on Sustacal (*solid circles*, non-food-deprived), food-deprived rats trained and tested on Sustacal (*open circles*, food deprived), rats trained but not tested on Sustacal (*open squares*, extinction), and rats trained and tested on water (*open triangles*, water). Data represent the mean (±SEM, n = 7–8 in all groups) percentage changes from home cage baseline values. There were no significant differences between the groups with respect to basal concentrations of DA in the dialysates. The overall mean (±SEM) basal value of DA in the dialysates was 5.67 ± 0.64 fmol/min (n = 29). *, Significantly different from last baseline (home cage) value, $p < 0.05$.

mals were returned to their home cages, after which DA concentrations gradually returned to baseline values over the subsequent 40 min.

The food-deprived animals exhibited a 24% increase in dialysate DA concentrations during the first 10 min of the anticipatory phase. This was a statistically significant increase compared to the baseline values of this group ($p < 0.01$) but was not significantly greater than the modest 10% increases observed in the other groups during the first 10 min of the anticipatory phase. During the first 10 min of the consummatory period DA release increased markedly (74%) in the NAc of the 20 hr food-deprived animals. This was highly statistically significant compared to the dialysate concentrations obtained from the NAc of the nondeprived animals during the same period ($p < 0.001$). Extracellular DA concentrations in the NAc decreased slightly in the second 10 min of the consummatory phase and over the subsequent 80 min in the home cage but failed to return to pretest session baseline values (see also Fig. 4). Thus, compared to their baseline values, extracellular DA concentrations in the NAc were significantly elevated ($p < 0.01$) and during the subsequent 70 min in the home cage ($p < 0.05$). During the second feeding session involving only those animals that were food deprived, DA concentrations increased to about 170% within the first 10 min after the presentation of food pellets (see Fig. 4). DA declined slightly thereafter, but again did not reach baseline levels within 2 hr.

The animals that were trained to drink Sustacal but were not permitted to drink during the test session (extinction group), also showed a nonsignificant increase in DA of approximately 10% during the anticipatory phase. DA concentrations subsequently increased slightly during the first 10 min after removal of the screen, this being followed by a gradual decrease towards baseline (Fig. 2). In the control animals that had access to water instead of Sustacal during the training and test sessions, dialysate DA concentrations also increased by 10% during the first 10 min after being placed into the test chamber and then gradually returned to baseline values over the next hour (Fig. 2).

Interstitial concentrations of DOPAC and HVA increased in response to feeding behavior in all four groups (Fig. 3). Thus, ANOVA indicates that for both metabolites there was a significant effect of time [DOPAC: $F(5,36, 134.05) = 23.98$, $p < 0.01$; HVA: $F(5.04, 126.01) = 20.12$, $p < 0.01$]. However, neither metabolite showed significant group differences [DOPAC: $F(16.09, 134.05) = 1.19$, $p = 0.285$; HVA: $F(15.12, 126.01) = 1.05$, $p = 0.413$].

Experiment II

Figure 5 shows the percentage changes of dialysate DA concentrations obtained from the NAc of food-deprived and nondeprived animals that were only permitted to consume 5 ml of Sustacal during the training and test sessions. ANOVA indicated that while the overall group effect was not statistically significant [$F(1,14) = 2.30$, $p = 0.152$], there was a significant effect of time [$F(7.58, 106.05) = 10.61$, $p < 0.01$] and the group × time interaction was significant [$F(7.58, 106.05) = 2.37$, $p < 0.05$]. Compared to baseline values, both groups showed nonsignificant 10–15% increases in accumbal DA release during the anticipatory phase. Both groups showed further, statistically significant increases from baseline during the consummatory phase ($p < 0.01$), the peak increase being to 124% of baseline values in the nondeprived group and to 139% in the food-deprived group (Fig. 5). Accumbal DA release was significantly higher in the food-deprived than in the nondeprived group during the second 10 min period of the consummatory phase ($p < 0.01$). In addition, the food-deprived animals continued to show significantly higher dialysate concentrations of DA during the first 20 min of the postingestional (home cage) period ($p < 0.05$; Fig. 5).

There were also significant differences between the deprived and nondeprived groups in dialysate concentrations of DOPAC and HVA obtained from the NAc (Fig. 6). Thus, with DOPAC there were significant group [$F(1,14) = 11.12$, $p < 0.01$] and time [$F(5.81, 81.33) = 13.96$, $p < 0.01$] effects, as well as a significant group × time interaction [$F(5.81, 81.33) = 7.32$, $p < 0.01$]. DOPAC concentrations were significantly higher in the food-deprived group beginning in the second 10 min period of the anticipatory phase and for the remainder of the experiment (Fig. 6). The same pattern of results pertained to HVA, there being significant group [$F(1,14) = 4.18$, $p < 0.05$], time [$F(3.93, 55.03) = 8.14$, $p < 0.01$], and group × time interaction [$F(3.93, 55.03) = 5.02$, $p < 0.01$] effects (Fig. 6).

The percentage changes in dialysate concentrations of DA obtained from the NAc of the two food-deprived groups, one having unlimited access to the liquid diet and the other allowed to consume only 5 ml during the test session, are compared in Figure 7. ANOVA confirmed that there were significant differences between these groups, there being significant group [$F(1,15) = 6.20$, $p < 0.025$], time [$F(6.20, 93.02) = 19.18$, $p < 0.01$], and group × time interaction [$F(6.20, 93.02) = 3.17$, $p < 0.01$] effects. Compared to the food-deprived animals that were restricted to 5 ml, the food-deprived animals that consumed approximately 18 ml of Sustacal during the feeding test showed significantly greater increases in accumbal DA release during the consummatory and the entire postingestional phases.

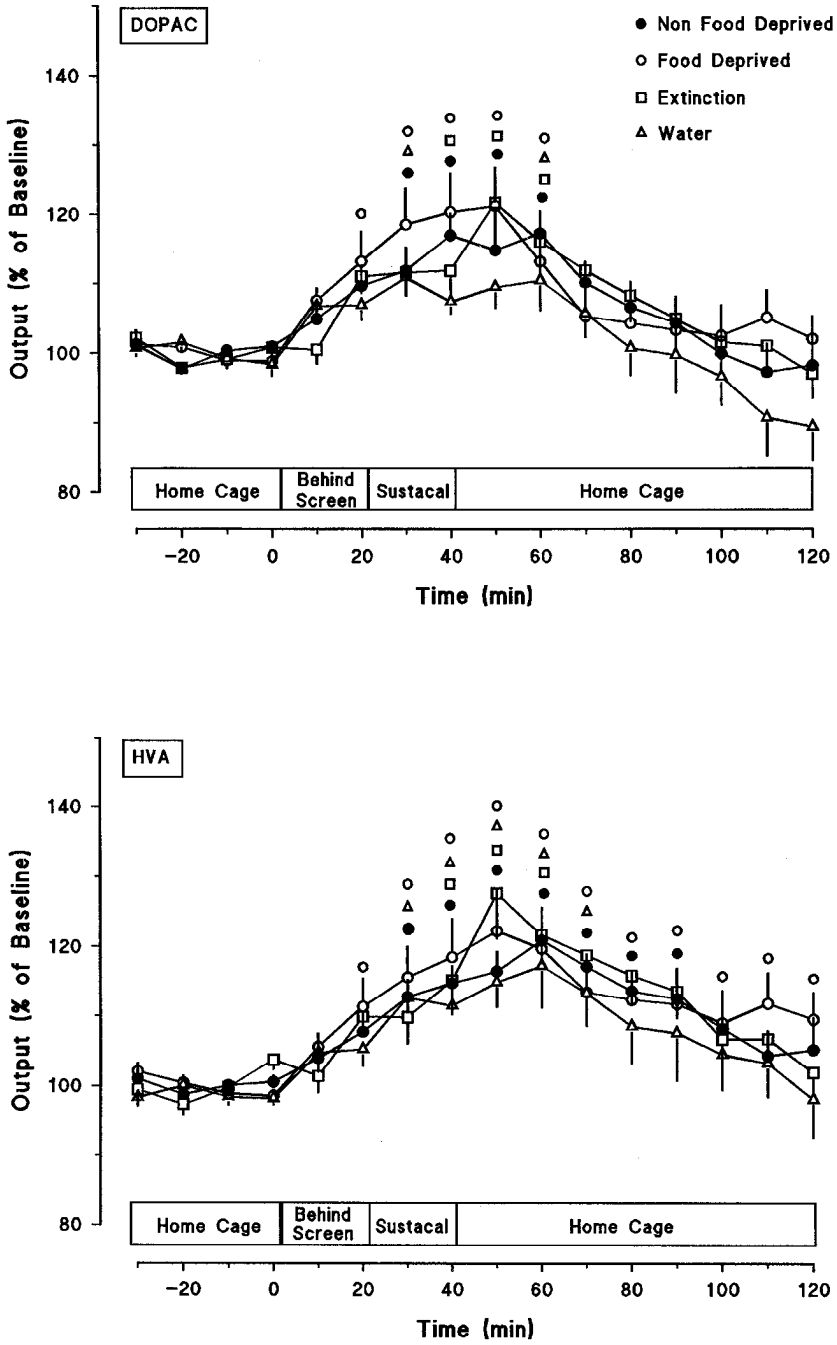

Figure 3. Changes in dialysate concentrations of DOPAC and HVA obtained from the nucleus accumbens of rats during a test feeding session. The experimental groups and the group sizes are indicated in the Figure 2 caption. Data represent the mean (±SEM) percentage changes from baseline. There were no significant group differences in basal DOPAC and HVA values. The overall mean (±SEM) basal values in the dialysates were 794 ± 51 and 361 ± 22 fmol/min for DOPAC and HVA, respectively. Each time point at which there was a statistically significant difference ($p < 0.05$) from the last baseline value is indicated by the homologous group symbol.

Experiment III

Figure 8 shows the percentage changes in dialysate concentrations of DA and its metabolites obtained from the NAc of animals that received 5 ml of Sustacal by gavage. This tube feeding procedure did not significantly affect extracellular concentrations of DA [$F(5.64, 33.82) = 2.16$, $p < 0.076$] or HVA [$F(2.76, 16.57) = 2.73$, $p < 0.080$]. However, ANOVA performed on DOPAC output revealed a significant effect over time [$F(3.88, 23.29) = 5.22$, $p < 0.004$]. Post hoc analysis showed that compared to baseline DOPAC concentrations were significantly elevated between 20 and 70 min after the stomach loading procedure.

Discussion

In agreement with earlier reports, the present data demonstrate that feeding behavior is associated with increases in DA release in the NAc as estimated by *in vivo* brain microdialysis (Church et al., 1987; Hernandez and Hoebel, 1988; Radhakishun et al., 1988; Yoshida et al., 1992). The present findings extend these earlier observations by demonstrating that the magnitude of the enhanced DA release is determined in part (1) by the amount of food consumed and (2) by the motivational state of the animal at the time of feeding. Thus, food-deprived animals that had unlimited access to the meal during the test session and consumed nearly 20 ml of Sustacal showed significantly greater increases in DA release in the NAc during and after feeding than did food-deprived animals whose intake was restricted to 5 ml (Fig. 7). In addition, animals that were food deprived for 20 hr prior to the test session showed significantly greater increases in DA release (Fig. 5) and metabolism (Fig. 6) in the NAc than did nondeprived animals despite the fact that both

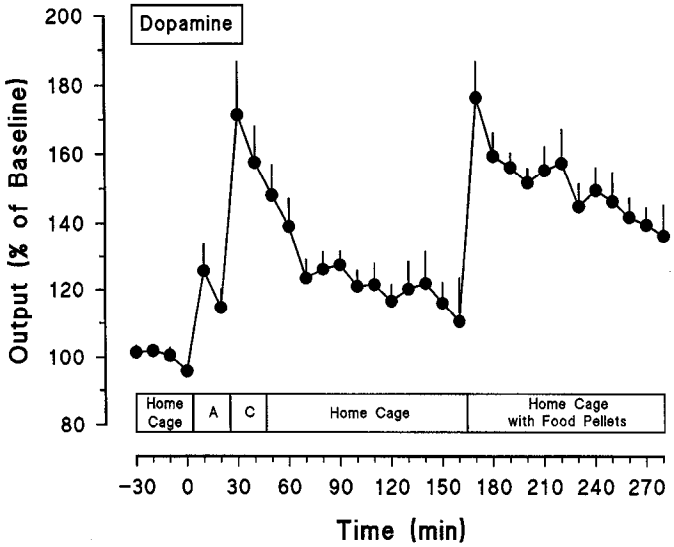

Figure 4. Dialysate concentrations of dopamine obtained from the nucleus accumbens of food-deprived rats during baseline (home cage), anticipatory (*A*), consummatory (*C*), home cage, and second feeding (solid chow) phases of the experiment. Amounts of food consumed are presented in Figure 1. Data represent means (±SEM) of eight animals. Baseline values were 5.08 ± 0.72 fmol/min.

groups consumed the 5 ml of Sustacal over very similar periods of time (nondeprived 154 ± 14 sec vs deprived 141 ± 9 sec). These data indicate that motivational state (i.e., deprivation-induced drive) can influence the magnitude of a neurochemical correlate of a goal-directed behavior. The neural mechanisms underlying "drive" and the manner by which they regulate stimulated DA release and metabolism in the NAc remain to be determined. A potentially interesting lead, however, is that food deprivation has recently been shown to enhance DA mediated

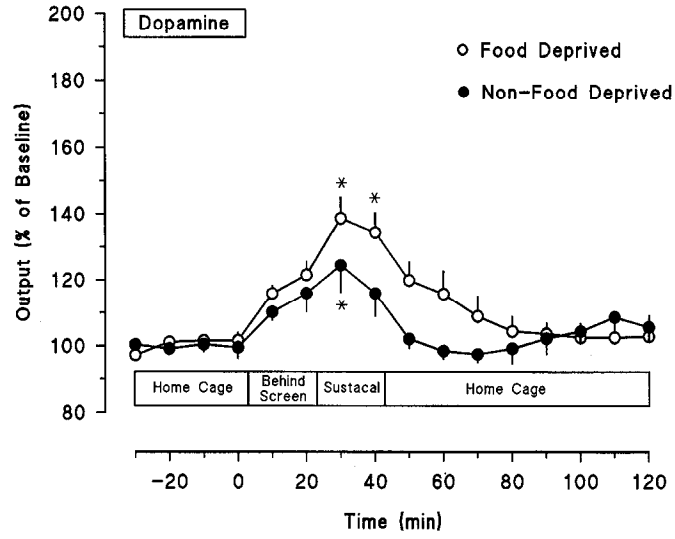

Figure 5. Mean dialysate concentrations of dopamine sampled from the nucleus accumbens in food-deprived (*open circles*) and nondeprived (*solid circles*) rats that were given limited access (5 ml) to a liquid diet (Sustacal). Data represent means (±SEM) of seven or eight animals in each group. There were no significant differences in basal DA values between the two groups. Basal values of the combined groups (n = 15) were 5.51 ± 0.71 fmol/min. *, Significantly different from last baseline (home cage) value, $p < 0.05$.

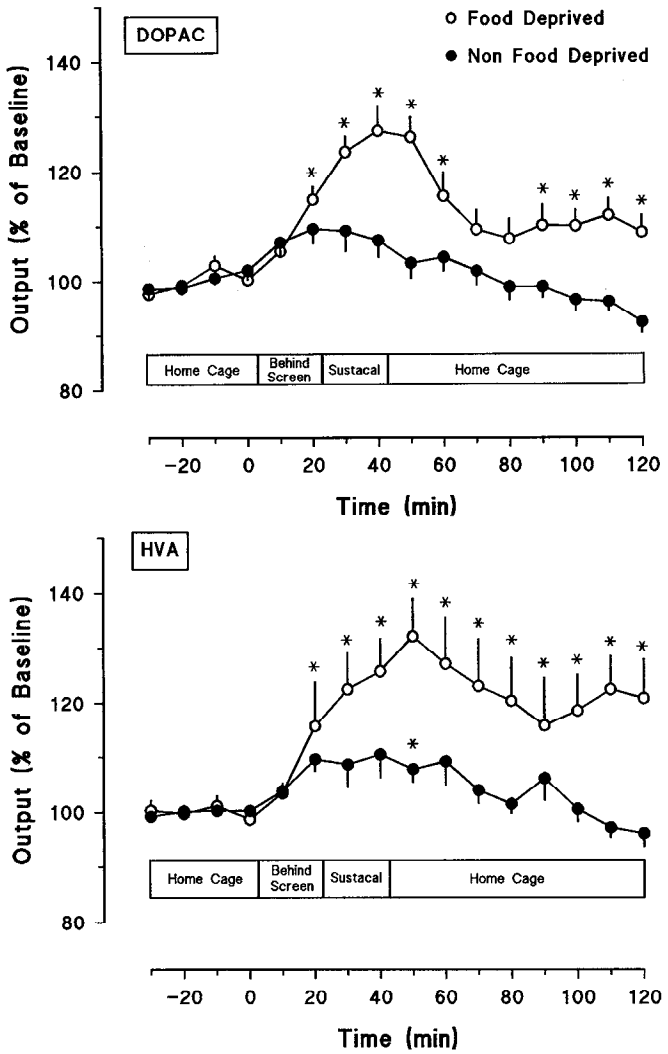

Figure 6. Mean dialysate concentrations of DOPAC and HVA sampled from the nucleus accumbens in food-deprived (*open circles*) and nondeprived (*solid circles*) rats that were given limited access (5 ml) to a liquid diet (Sustacal). Data represent means (±SEM) of seven or eight animals in each group. There were no significant differences in basal DOPAC and HVA values between the two groups. Absolute basal values of the combined groups (n = 15) were 639 ± 77 (*DOPAC*) and 317 ± 55 (*HVA*) fmol/min. *, Significantly different from last baseline (home cage) value, $p < 0.05$.

behaviors via a corticosterone dependent mechanism (Deroche et al., 1993).

The two control groups in experiment I (i.e., the water and extinction groups) provide important information with regard to the interpretation of these results. First, the fact that the water group, which was never reinforced in the training procedure, showed the same modest 10–15% increase in DA release during the anticipatory period as did the non-food-deprived and extinction groups, suggests that this increase was not related to the reinforcement history of the animal and was most likely an arousal response related to being handled and moved from the home to the test chamber. Indeed, with one exception in this series of experiments, there was no evidence of an increase in DA release in the NAc associated specifically with anticipation of reward. The exception occurred in the food-deprived animals that had unlimited access to Sustacal during the test session (Fig. 2). Compared to their home cage baseline values, these animals

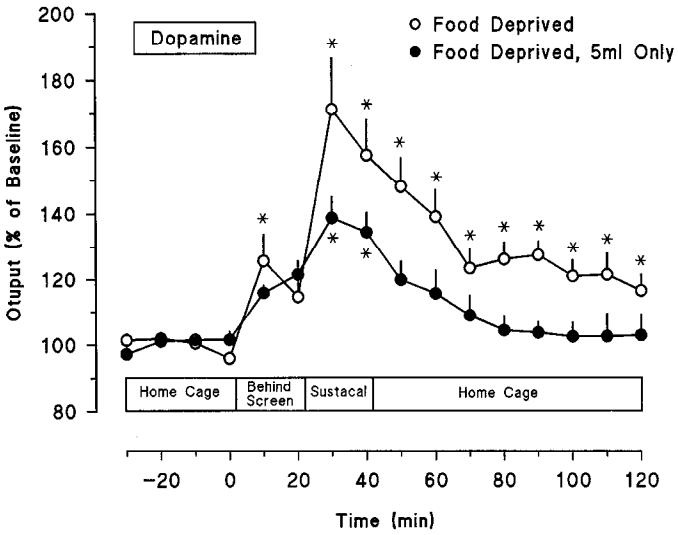

Figure 7. Comparison of dopamine release in the nucleus accumbens of food-deprived rats that were given unlimited access to Sustacal on the test session (mean consumption 20 ml) and food-deprived rats that were given limited access (5 ml) to Sustacal on the test session. *, Significantly different from last baseline (home cage) value, $p < 0.05$.

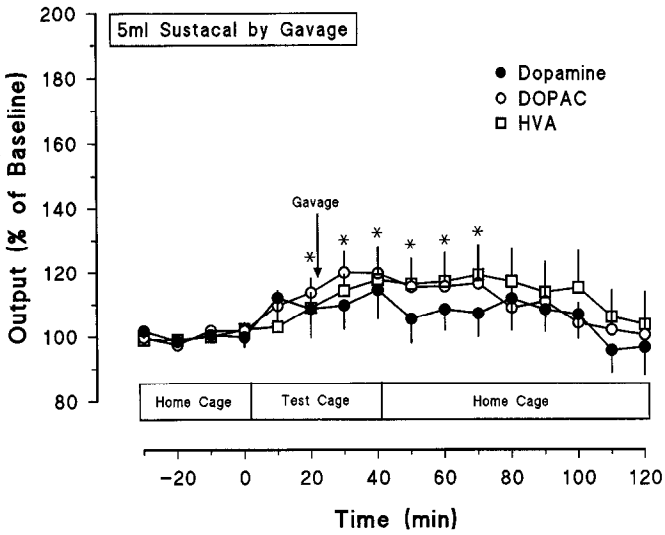

Figure 8. Effect of intragastric loading of Sustacal (5 ml) on extracellular concentrations of dopamine, DOPAC and HVA in the nucleus accumbens. Basal values ($n = 7$) were 6.27 ± 1.32 (DA), 634 ± 102 (*DOPAC*), and 299 ± 58 (*HVA*) fmol/min. *, DOPAC significantly different from last baseline (home cage) value, $p < 0.05$.

showed significant increases in DA release in the NAc during the first 10 min of the anticipatory phase. It appears, therefore, that under conditions of high motivational drive such as that which presumably occurs after 20 hr of food deprivation, the incentive motivational properties of stimuli that are predictive of food availability can produce small but significant increases in DA release in the NAc. It is noteworthy, however, that significant increases in DA release were not observed during the anticipatory period in the food-deprived group that was trained and tested on 5 ml of Sustacal (Fig. 5). This suggests either that increased DA release in the NAc associated with incentive motivation is not a robust phenomenon even under conditions of high drive, or that the size of the anticipated meal (and therefore perhaps the level of incentive motivation) determines the extent to which DA is increased in the NAc during the anticipatory phase. Further studies are required to distinguish between these possibilities.

Data from the extinction group in experiment I indicate that the increase in accumbal DA release observed in the non-food-deprived group was indeed associated with feeding as opposed to being a delayed neurochemical response generated by the anticipatory phase. Thus, despite having identical training histories and experiencing identical conditions during the anticipatory phase of the test session, only the animals that subsequently consumed Sustacal showed significant elevations in interstitial concentrations of DA in the NAc (Fig. 2). It is also noteworthy that increased accumbal DA release did not only occur during consumption of the palatable liquid meal. Thus, when the food-deprived animals that had consumed nearly 20 ml of Sustacal 2 hr earlier were presented with and ate solid laboratory rat chow, DA release in the NAc again showed substantial increases that were of the same peak magnitude as that observed during Sustacal consumption (Fig. 4).

In experiment I increases in DA release and metabolism associated with the consumption of food clearly persisted beyond the feeding period itself (Figs. 2, 3). This raises the possibility that postingestional or nutritive factors may have contributed to these increases. However, the results of experiment III suggest that this was not the case. Thus, animals that received 5 ml of Sustacal intragastrically failed to show significant increases in DA release or metabolism in the NAc (Fig. 8). This is consistent with findings by Heffner et al. (1980) who observed an increase in DA metabolism in the NAc in food-deprived rats following normal feeding but not after intragastric loading. It is noteworthy that the same volume of Sustacal consumed orally produced significant increases in DA release in the NAc (Fig. 5). These results indicate that neither the increase in accumbal DA release occurring during the consummatory period nor that which persists after this period can be attributed to nutritive factors alone. The fact that similar, relatively long-lasting postconsummatory effects are observed after sexual behavior in male rats is consistent with this conclusion (Pfaus et al., 1990; Damsma et al., 1992; Wenkstern et al., 1993). The significance of the slow rate at which consummatory behavior associated increases in accumbal DA release return to baseline values during the postconsummatory period remains to be determined. It does raise the interesting possibility, however, that at least part of the interstitial DA monitored by microdialysis in the NAc is related more to "neurohumoral" than to classical neurotransmitter functions.

The present results, showing that increased DA release in the NAc is associated more with the expression of consummatory than with anticipatory components of goal directed behaviors is consistent with other microdialysis studies. For example, Radhakishun et al. (1988) did not detect changes in DA release in the NAc an hour before food availability, although the animals showed pronounced anticipatory motor activity and arousal. Similarly, in a study by Hernandez and Hoebel (1988) a discriminative stimulus light that signaled food delivery did not itself increase DA release in the NAc. In addition, thirsty rats trained to drink water in a distinctive environment failed to show significant increases in DA release in the NAc during an anticipatory period prior to having access to water (Young et al., 1992). In contrast, and in agreement with the present results, drinking itself produced robust increases in accumbal DA release. The results of recent microdialysis studies on the neurochemical substrates of sexual behavior are also consistent with

the above. Thus, when male rats are placed in an environment where they have learned to expect access to a sexually receptive female, DA release in the NAc undergoes small increases during the anticipatory period and much larger increases during the consummatory (copulation) phase of the test session (Pfaus et al., 1990; Damsma et al., 1992). It is evident, therefore, that with feeding, drinking, and sexual behavior maximal increases in DA release in the NAc occur during consummatory behaviors and much smaller (if any) increases occur during anticipatory behaviors. The results of these microdialysis studies stand in contrast to a study by Blackburn et al. (1989) who found that exposure to a conditioned stimulus associated with food delivery increased DA metabolism (i.e., DOPAC/DA ratios in tissue samples) in the NAc, while no such changes were observed during the consumption of a small unsignaled meal. There were important differences between the experimental protocols employed in the present study and those utilized by Blackburn et al. (1989), including the use of discrete conditioned stimuli in the latter study as opposed to contextual conditioning in the present experiments. Perhaps equally importantly, there is considerable evidence that changes in DA metabolism as determined by tissue concentrations of DA and its metabolites DOPAC and HVA are not robustly tied to changes in DA release as measured by brain microdialysis (Brown et al., 1991b; Cumming et al., 1992).

In an important series of neurophysiological experiments, Schultz and his coworkers have recorded from midbrain DA neurons in monkeys during the performance of a variety of food reinforced behaviors (Schultz, 1986; Romo and Schultz, 1990; Schultz and Romo, 1990; Ljungberg et al., 1992; Schultz et al., 1993). The results of these elegant investigations are clearly relevant to the present findings and can be summarized as follows. Dopaminergic neurons in the mesencephalon can be activated both by primary food and fluid rewards and by conditioned incentive stimuli predicting reward (Schultz, 1986; Romo and Schultz, 1990; Ljungberg et al., 1992). Specifically, while midbrain DA neurons initially respond to primary rewards during learning, these responses gradually transfer to the conditioned stimuli that predict reward during the establishment of task performance. Furthermore, after overtraining even the responses to the conditioned stimuli become strongly reduced (Ljungberg et al., 1992; Schultz et al., 1993). From this series of experiments Schultz et al. (1993) have concluded that midbrain "DA neurons respond phasically to alerting external stimuli with behavioral significance whose detection is crucial for learning . . . and are involved with transient changes of impulse activity in basic attentional and motivational processes underlying learning and cognitive behavior" (p. 900). According to this formulation, the functions of mesotelencephalic DA neurons are not specifically related either to incentive motivation or reward; rather, they are part of an "alerting and attention-grabbing" mechanism that is activated by appetitive stimuli associated with the availability of an object of high interest, that is, "motivational arousal" (Schultz and Romo, 1990; Schultz et al., 1993). In the present experiments, contextual stimuli predictive of the availability of food generally failed to increase DA release in the NAc; in contrast, consumption of the palatable liquid produced significant increases. These results could be viewed as being consistent with Schultz's hypothesis if it is assumed that the rats were at a relatively early stage of training at the time of microdialysis. The hypothesis would also predict that with continued training the increase in accumbal DA release would gradually shift from the consummatory to the anticipatory phase of the test session. Furthermore, after overtraining neither the anticipatory nor the consummatory phase would generate significant increases in DA release. The findings of Schultz and colleagues may also partly account for the apparent discrepancy between the present microdialysis data and the earlier DA metabolism results of Blackburn et al. (1989) inasmuch as in the latter study rats received more extensive training (50–60 trials) prior to the test session than in the present experiments (12 trials).

Ljungberg et al. (1991) investigated the ability of an auditory stimulus that occurred during the delivery of a liquid reward (i.e., the noise generated by a solenoid liquid valve) to increase the activity of DA neurons when liquid flow was blocked. This is analogous to the extinction condition in experiment I where the drinking tube was blocked during the test session. In agreement with the present results showing that DA release in the NAc was not altered in the extinction condition (Fig. 2), Ljungberg et al. (1991) found that DA neurons that responded to the liquid reward failed to be activated by the auditory stimulus.

While the present results indicate that increased DA release in the NAc occurs during the consummatory phase of motivated behavior, it does not necessarily follow that enhanced DA release in this structure is a component of the neurochemistry of hedonia. Indeed, Berridge and colleagues have recently argued that mesotelencephalic DA projections are not involved in hedonic processes, and instead mediate a process termed "incentive salience attribution" which they define as the active assignment of salience and attractiveness to intrinsically neutral stimuli (Berridge and Valenstein, 1991; Robinson and Berridge, 1993). Although by no means identical, this has much in common with the motivational arousal hypothesis of Schultz et al. (1993). According to Berridge's formulation the attachment of incentive value to a neutral stimulus occurs through a three stage process that consists of hedonics, associative learning and salience attribution. In normal circumstances, associative learning and salience attribution are triggered by the hedonic event. On the basis of their studies using the taste reactivity paradigm, Berridge and colleagues have concluded that dopamine systems do not mediate the hedonic sensory experience (i.e., taste pleasure) elicited by food. Similarly, data from other laboratories have shown that DA receptor antagonists do not block associative learning (cf. Beninger, 1983). The present dialysis data are equally compatible with a role for the mesoaccumbens DA projection in either the hedonic or the salience attribution aspects of feeding. However, the electrophysiological data of Schultz and colleagues clearly indicate that the conditions under which DA neurons become activated in behaving animals far exceeds those predicted by simple hedonic hypotheses (Wise, 1982, 1985).

It is important to stress that the conclusion that consummatory rather than anticipatory behaviors are associated with increases in accumbal DA release should not be interpreted to suggest that *basal* levels of dopaminergic transmission are not important for the expression of anticipatory behaviors elicited by conditioned incentive stimuli. Indeed, there is overwhelming evidence that both appetitively and aversively motivated behaviors maintained by conditioned stimuli are much more vulnerable to disruption by DA receptor antagonists or by 6-hydroxydopamine lesions of the mesotelencephalic DA system than are unconditioned responses (see Salamone, 1992; Fibiger, 1993). For example, a defining behavioral property of typical

neuroleptic drugs is that they block conditioned avoidance responding at doses that do not block escape responses. In the context of preparatory and consummatory behaviors, the avoidance response can be considered to be a class of anticipatory behavior whereas escape responding is a consummatory response. It has been proposed that the lower vulnerability of consummatory behaviors to disruption by neuroleptics is a function of the fact that these compounds are competitive DA receptor antagonists and that the enhanced DA release that occurs during consummatory behaviors maintains sufficient activity at postsynaptic DA receptors for the expression of the behavior (Fibiger, 1993). On the other hand, given the lower level of DA release occurring during anticipatory behaviors, these behaviors can be predicted to be more vulnerable to disruption by a dose of a neuroleptic that leaves the expression of consummatory behaviors relatively intact. It is evident, therefore, that while anticipatory behaviors are not generally associated with significant increases in DA release in the NAc as estimated by microdialysis, basal levels of DA release in this structure are critically important for their expression. This provides a neurochemical framework for Salamone's (1992, p. 165) important insight that "the behaviors most easily disrupted by DA antagonists are highly activated and highly complex learned instrumental responses that are elicited or supported by mild conditioned stimuli, and maintained for considerable periods of time" (i.e., anticipatory behaviors associated with basal levels of accumbal DA release), while "the behaviors that are most resistant to disruption by DA antagonists are relatively simple and often unlearned responses to intense unconditioned stimuli" (i.e., consummatory behaviors associated with enhanced levels of DA release in the NAc).

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