HYPOTHALAMIC DOPAMINE AND SUCROSE SHAM FEEDING

THE EFFECTS OF HALOPERIDOL INFUSIONS INTO THE HYPOTHALAMUS AND NUCLEUS ACCUMBENS ON SUCROSE SHAM FEEDING

By

SYLVIE D. TURENNE, B.A.

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AUTHOR: Sylvie D. Turenne, B.A. (Honours, Psychology)

(Wilfrid Laurier University)

SUPERVISOR: Dr. H.P. Weingarten

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Abstract

The present thesis examined the relative contribution of hypothalamic and nucleus accumbens dopaminergic activity to the control of sucrose sham feeding. Bilateral intracranial infusions of the dopamine antagonist, haloperidol at doses of 1.25, 2.5, 5 and 7.5 ug, into the hypothalamus and nucleus accumbens produced dose-dependent suppressions of sham feeding of a 15% sucrose solution. No differences in temporal pattern of intake were observed between hypothalamic and nucleus accumbens infusions under drug or vehicle conditions. Also, motor deficits were observed with the highest dose of haloperidol (7.5 ug) while the three lower doses inhibited sham feeding without obvious signs of motor impairment. These results suggest that mesolimbic as well as hypothalamic dopaminergic activity mediate the hedonic aspects of feeding.

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INTRODUCTION

Considerable research over the last twenty years has focused on understanding the role of CNS dopamine in reward, palatability and incentive motivation (Smith, 1995). Numerous studies have implicated dopaminergic activity in the rewarding and hedonic aspects of ingestion (Bailey, Hsiao & King, 1986; Blackburn, Pfaus & Phillips, 1992; Duong & Weingarten, 1993; Smith et al, 1987; Towell, Muscat & Willner, 1987; Weingarten, 1993; Wise, 1982).

Using an operant response paradigm, Wise and associates (Wise, Spindler, deWit & Gerber, 1978a; Wise, Spindler & Legault, 1978b) found that treatment with the dopamine antagonist pimozide resulted in a gradual reduction in lever pressing for food reward over successive drug trials. This gradual decrease was similar to the behaviour exhibited by vehicle-treated animals when food reward was withheld (extinction). Wise concluded that neuroleptic treatment decreased operant reponsding by attenuating the rewarding quality of the food (Wise, et al, 1978a; Wise, 1982). An alternate interpretation of these effects is that neuroleptic drugs such as pimozide can produce substantial motor deficits which could result in decreased behavioural responding. Wise argues that motor impairments were not observed with the low doses of pimozide used (0.5 and 1.0 mg/kg) and that a gradual decrease in responding across trials is inconsistent with a motor deficit hypothesis. If sedation impairs lever-pressing then one would expect to see decreased performance equally across testing sessions (Wise, et al, 1978a; 1978b). Based on these results and numerous studies showing that dopamine antagonism interferes with the rewarding effects of psychoactive drugs and brain stimulation reward, Wise (1982, 1985) proposed the anhedonia hypothesis. He suggests that central dopaminergic activity mediates artificial rewards such drug self-administration and intracranial self-stimulation as

well as natural rewards such as feeding.

Since its proposal, the anhedonia hypothesis has stimulated considerable interest in the role of dopamine in food reward and palatability, particularly in mediating the consumption of palatable foods such as sucrose solutions (Xenakis & Sclafani, 1981; Horvitz & Ettenberg, 1989; Wise & Colle, 1984). Xenakis and Sclafani (1981; Sclafani, Avarich & Xenakis, 1982) found that pimozide produced a dose-dependent decrease in consumption of a saccharin-glucose solution as well as a decrease in lick efficiency (fluid intake/lick) similar to that seen with quinine adulteration, suggesting that pimozide reduced the hedonic quality of the sweet solution.

Moreover, similar results have been demonstrated using paradigms which are more sensitive measures of palatability (the organism's evaluation of a food's taste), such as preference tests and sham feeding (Geary & Smith, 1985; Towell, Muscat & Willner, 1987). Using a two-bottle taste preference paradigm (water vs sucrose), Towell, Muscat & Willner (1987) showed that pimozide (0.25 mg/kg) reduced the animals' intake of a preferred sucrose solution while increasing water intake (non-preferred). Overall consumption of the two solutions combined remained constant indicating that motor impairment was not responsible for the pimozide effect on sucrose intake. They also used a single bottle test, which demonstrated that pimozide treatment decreased intake of low concentrations of sucrose (0.7 %, 2.4%), but did not affect higher sucrose concentrations (>7.0%).

An alternate interpretation to the results obtained using these preference tests is that pimozide could be increasing postingestive satiety thereby reducing intake, rather than reducing the palatability of the sweet solution (Geary & Smith, 1985; Towell, Muscat & Willner, 1987). Postingestive consequences can be minimized using a sham feeding

preparation in which ingested solutions drain out of a chronically-implanted gastric fistula. Although not all satiety cues are completely eliminated (ie. increases in blood glucose), these have not been found to affect intake (Sclafani & Nissenbaum, 1985). The contribution of oral factors alone in the control of ingestion can be evaluated using this preparation (Weingarten & Watson, 1982; Weingarten, 1993).

Using the sham feeding technique, Geary & Smith (1985) compared the effect of pimozide (0.25 mg/kg) and reducing the concentration of sucrose on sham intake. Animals sham fed a range of sucrose solutions (5%-40%) after injections of pimozide or vehicle. Pimozide reduced sham intake in a manner similar to that seen when the sucrose concentration is decreased; for example, animals sham feeding 20% sucrose pretreated with pimozide drank the same amount as animals sham feeding 5% sucrose pretreated with vehicle. Latency to begin feeding as well as the temporal pattern of intake throughout the 30 minute testing session did not differ for pimozide and vehicle-treated animals indicating that intake suppression was not produced by motor deficits. These results suggest that dopaminergic activity plays a role in the positive reinforcing effect of sham fed sucrose (Geary & Smith, 1985). Weingarten, Duong & Elston (1996) have also demonstrated, using a curve shift paradigm, that pimozide treatment shifted the sucrose concentration-sham intake curves to the right, indicating that pimozide altered the sucrose solutions' hedonic value.

Subsequent investigations have attempted to examine whether there is a differential role of D1 and D2 receptor subtypes in these effects. Pimozide and haloperidol are preferential D2 antagonists but have been shown to block D1 as well as D2 receptors in vitro (Seeman, 1980). Many studies have used more selective D2 receptor and D1 receptor antagonists to examine the pharmacological specificity of

dopamine blockade on sucrose sham feeding. Schneider and associates (Schneider, 1989; Schneider, Davis, Watson & Smith, 1990; Schneider, Gibbs & Smith, 1986; Schneider, Greenberg & Smith, 1988), demonstrated that both the selective D2 receptor antagonist (-)raclopride and the selective D1 receptor antagonist SCH23390 both inhibit sham feeding of a 10% sucrose solution and a 100% corn oil solution (Weatherford, Greenberg, Gibbs & Smith, 1990). Raclopride and SCH23390 have also been found to decrease a preference for sucrose over water in a two-bottle preference test (Muscat & Willner, 1989). These results suggest that both D1 and D2 receptors play an important role in the orosensory control of sucrose intake (Schneider, 1989).

All of the aforementioned studies have used systemic injections of dopamine antagonists thus providing little information about which dopaminergic systems mediate these effects. Duong & Weingarten (1993) found that domperidone, a peripheral D2 antagonist, had no effect on real or sham feeding of sucrose solutions, indicating that peripheral D2 receptors are not involved. Moreover, Schneider and associates (Schneider, Davis, Rauhofer, Gibbs & Smith, 1990; Smith & Schneider, 1988), compared ICV infusion to intraperitoneal injections of sultopride (central D2 antagonist),and found that ICV infusions were 30 times more potent in reducing sucrose sham feeding. Similar results have also been obtained with raclopride and SCH23390 (Schneider, Watson & Smith, 1992). These findings suggests that dopamine antagonists are acting on central dopamine receptor populations, however, these experiments do not isolate the specific dopaminergic pathways involved.

Many ex vivo and in vivo studies have attempted to identify the discrete dopaminergic sites within the brain which mediate feeding and palatability (Smith, 1995). Biggio, Porceddu, Fratta and Gessa, (1977) report increases in DA utilization within the

brain measured one hour after a meal. Heffner and associates (Heffmer, Hartmen and Seiden, 1980; Heffner, Vosman & Seiden, 1984) measured DA metabolism in several DA terminal fields and found that one hour after consumption of a meal, dopamine metabolism was increased in the nucleus accumbens, amygdala and hypothalamus. These changes were not correlated to the onset or termination of feeding thus providing no information about the nature of the involvement of DA activity and feeding. They also found that intragastric infusions of food failed to alter the DOPAC/DA ratio in the hypothalamus and nucleus accumbens suggesting that oral stimulation was needed to alter dopamine metabolism within these two structures.

Results from these ex vivo studies suggest that two main dopaminergic pathways may be involved; the mesolimbic and hypothalamic DA systems. The cell bodies of the mesolimbic system originate within the ventral tegmental area (VTA) which extend to several limbic forebrain structures including the nucleus accumbens (NA), amygdala, hippocampus, septum, bed nucleus of stria terminalis and prefrontal cortex (Blackburn, Pfaus & Phillips, 1992; Fibiger & Phillips, 1986). Extensive research has focused on the role of mesolimbic DA function in the mediation of drug reward, intracranial self-stimulation, food reward and incentive motivation (Fibiger, & Phillips, 1986; Wise & Rompre, 1989).

Another smaller, yet distinct DA pathway is the incertohypothalamic system. It is divided into two projections: the caudal segment originates in the zona incerta and projects to the dorsomedial and anterior hypothalamus, while the rostral protion originates in the periventricular region and projects to the medial preoptic and suprechiasmatic nuclei (Bjorklund, Lindvall & Nobin, 1975; Moore, 1987). Lookingland and Moore (1984) have demonstrated that the incertohypothalamic DA neurons are regulated by dopamine

receptor-mediated mechanisms but the function of this DA system is relatively unknown.

In vivo microdialysis studies implicate both mesolimbic and hypothalamic dopaminergic sites in the control of feeding. Yoshida et al. (1992) found that extracellular levels of dopamine increased significantly within the nucleus accumbens and ventral tegmental area in response to feeding, with elevations continuing 20-60 minutes following meal termination. Similar results have been reported for the prefrontal cortex (D'Angio & Scatton, 1989; Hernandez & Hoebel, 1990), rostromedial hypothalamus (Orosco & Nicolaidis, 1992), and striatum (Inoue, Kirike, Okuno, Ito, Fujisaki, Matsui& Kawakita, 1993; Radhakishun, vanRee & Westerink, 1988). Church, Justice and Neill, (1987) and Hernandez and Hoebel (1988) also demonstrated increases in dopamine release in the nucleus accumbens during operant responding for food.

This neurochemical evidence suggests that several different DA innervated sites may be involved in feeding. Furthermore, several aspects of feeding (ie, appetitive, consummatory, postingestive) have been linked to dopaminergic activity, however few studies have directly examined which DA sites mediate the hedonic aspects of feeding. Blackburn, Phillips, Jakubovic and Fibiger (1986), measured changes in DA metabolism in tissue samples from the nucleus accumbens and striatum 1 hour after the consumption of food pellets, liquid diet or a palatable saccharin solution. They report increased HVA/DA ratios in both structures in response to the consumption of food pellets while no changes were seen in the DOPAC/DA ratios. Both HVA and DOPAC/DA ratios were increased when the animals consumed a liquid diet while no changes in DA metabolism were seen with consumption of the non-nutritive saccharin solution. These results indicate that increases in dopaminergic activity associated with feeding vary with the type of food consumed (liquid diet, pellets). Also, the results with saccharin suggest that the increase

in DA metabolism seen with the other types of food may be due to postingestive consequences rather than an effect on the rewarding aspects of feeding (Blackburn et al, 1986). Schneider and associates (Schneider, Sikorsky, Rauhofer, Davis & Smith, 1993; Schneider, Sikorsky, Rauhofer, Davis & Smith, 1992) provide preliminary evidence that nucleus accumbens dopaminergic activity may be involved in sucrose sham feeding. They report that bilateral infusions of raclopride and SCH23390 into the nucleus accumbens inhibited sham feeding of a 30% sucrose solution indicating that nucleus accumbens dopamine may mediate palatability.

Although role of dopamine in feeding and palatability has typically been focused on the mesolimbic dopaminergic systems, some evidence suggests that hypothalamic dopamine may be involved in palatability (Heffner, et al, 1984; Smith, Bourbonais, Jerome & Simansky, 1987). Smith, Bourbonais, Jerome & Simansky (1987) utilized the sham feeding preparation (which eliminates postingestive consequences) to assess the effect of palatability on DA metabolism in regional DA sites. They report that DOPAC/DA ratios were increased within the hypothalamus when animals were allowed to sham feed a sucrose solution for 9 minutes. Higher concentrations of sucrose produced higher DOPAC/DA ratios. Dopamine metabolism did not increase in any of the other sites examined which included the nucleus accumbens, striatum, prefrontal cortex and olfactory tubercles. This experiment provides the first evidence that hypothalamic DA activity is involved in the orosensory control of sucrose intake (Smith, et al, 1987)

The purpose of the present research project was to identify the specific dopaminergic site(s) within the brain that mediate palatability. Few studies have been conducted which examine the function of the incertohypothalamic dopamine pathway. A review of the neurochemical evidence suggests that the hypothalamus may be involved in

the consumption of palatable sucrose solutions (Smith, et al, 1987), and that hypothalamic dopamine metabolism is increased during feeding (Heffner, et al, 1980; Orosco & Nicolaidis, 1992). The majority of the literature has focused on the role of the mesolimbic dopamine pathway in mediating reward and feeding (Blackburn, et al, 1986; Phillips & Fibiger, 1986). It is possible that the incertohypothalamic dopamine system may also be involved in some of the behavioural effects which have typically been attributed to the mesolimbic dopamine pathway (Blackburn, et al, 1992). In order to assess the relative contribution of mesolimbic and hypothalamic dopaminergic activity in the hedonic aspects of feeding, the preferential D2 dopamine antagonist haloperidol was infused into the hypothalamus and nucleus accumbens and its effects on the sham feeding of sucrose solutions was measured.

EXPERIMENT 1

The purpose of the present experiment was to examine the role of hypothalamic and nucleus accumbens dopamine in the control of sucrose sham feeding. A pilot experiment was conducted in order to determine which doses of the preferential D2 antagonist, haloperidol, were to be used and the placement coordinates for the of brain cannulas. Appendix A outlines this experiment which demonstrated that a 5ug dose of haloperidol bilaterally infused into the hypothalamus produced a level of suppression similar to that of a 10 ug dose. Also, significant motor impairment was observed with the 10 ug dose but not with 5 ug HP. Therefore, a range of haloperidol doses lower than 10 ug, (1.25-7.5 ug), were bilaterally infused into the HT and NA in order to obtain a dose-reponse curve. The effects of these infusions on the sham feeding of a 15% sucrose solution were measured.

GENERAL METHODS

Subjects

Sixteen male Sprague-Dawley rats weighing 250-300 gms at the beginning of the experiment, were individually housed in plastic cages. The animals were given ad libitum access to Purina rat show and water unless otherwise specified in the experimental protocol and were maintained on a 12 hour light/dark cycle.

Drug

Haloperidol, a preferential D2 antagonist, (Sigma Chemical Co.) was dissolved in a 3% tartaric acid vehicle, and the vehicle and drug solutions were adjusted to pH 4.6 with NaOH.

Cannula surgeries

An indwelling stainless steel gastric cannula and bilateral brain cannulas were implanted into each animal during two separate surgical sessions in which the rats were anaesthetized using ketamine (75 mg/kg) and xylazine (10mg/kg) and given an analgesic buprenorphine (Temgesic, .006mg/rat)

Brain cannulas

The guide cannulas were constructed from stainless steel hypodermic tubing (.028" OD x .016" ID, 2.1 cm long), each affixed with a hub of silastic glue 0.5 cm from the top. The two rods were then secured together using dental acrylic. Dummy cannulas were constructed from stainless steel hypotube rods (.014" OD x .007" ID, 2.3 cm long). The animals were secured in a stereotaxic apparatus, a midline incision was made on the scalp and a bilateral was lowered into the specified structure using coordinates from the Paxinos & Watson (1986) stereotaxic atlas: HT, .3mm posterior, 11mm lateral and 8.5 mm ventral to bregma; NA, 2.0mm anterior, 1.5 mm lateral and 6.8 mm ventral to bregma. Skull screws were placed in the four quadrants surrounding bregma and the cannulas secured to the skull using dental acrylic forming a headcap. The animals were given 7 days to recover before gastric cannula surgery.

Gastric cannulas

A 5 cm midline incision was made in the abdomen and the stomach exposed. Two sets of purse string sutures (0.5 cm diameter) were made (5-0 silk), and the cannula inserted into the stomach via a hole made within the sutures. The cannula was then

secured within the stomach by fastening the purse string sutures. A hole was then made through the muscle and skin and the cannula was passed through the aperture and secured to the skin using purse string sutures. The abdominal muscles was sutured with catgut and silk sutures closed the skin on the abdomen. A topical antibacterial ointment (furacin) was applied to sutured areas to prevent infection. The animals were given 14 days to recover from surgery.

Training

Training and testing took place in Plexiglas cages (20.5 cm x 10 cm x 10 cm) suspended on 20 cm stilts. Collection trays were placed underneath the cages. The sucrose solution was contained in graduated tubes and delivered via a spout which extended into the cage.

The animals were maintained on an 18 hour food deprivation schedule. Before being placed into the cages, the animals' cannulas were opened and rinsed with tap water and a 15 cm drainage tube was attached to the cannula which allowed solutions to easily drain from the cannula. The animals were allowed to sham feed (SF) a sucrose solution for 30 minutes during training and testing periods. Following SF training and testing the stomachs were rinsed again and cannulas closed. Purina rat chow was returned to the animals cages 1 hour after training/testing. The animals were trained to SF sucrose until intakes were stable (12 days). The animals were habituated to the infusion procedure during the last 3 days of training. The internal cannulas were inserted and attached to the syringe with the infusion pump turned on but the syringe was not connected to the pump.

Testing

A mixed-factor design was utilized with the between-subject factor of infusion site (HT, N=8 and NA, N=8), and the within-subject factor of drug condition (1.25, 2.5, 5, 7.5 ug HP or 3% lactic acid vehicle). All animals received each drug condition with each animal serving as its own control. The order of drug infusions was counterbalanced across subjects. In order to prevent the problem of drug remaining in the guide cannula after infusion, two vehicle trials were conducted between successive drug trials and the data from the second vehicle trial was used in the data analysis (Vehicle, Vehicle, HP, day off). One day without infusions followed each drug day to allow clearing of the drug from the animals' system. Half the animals from each group received the HP doses in ascending order while the other half received the doses in descending order.

The dependent variable measured was the total amount (mls) of sucrose solution sham fed during the 30 minute testing. Cumulative intake was also recorded every minute for the first 5 minutes and then at 5 min intervals for the 30 min test. High doses of haloperidol have been demonstrated to impair motor behaviour therefore it was important to determine whether any decreases in intake seen are due to haloperidol's effect on motor behaviour or on the reinforcing properties of sucrose. Observations of general motor behaviour, particularly any standing behaviour (ie. standing for short periods of time without exploration or grooming) and latency to begin feeding (sec) were measured. The behaviours exhibited by the animals when not feeding (eg. grooming, exploratory behaviour) were also monitored.

On testing days, the animals were infused bilaterally (using Hamilton micro-syringe) with either 1.0 ul 3% lactic acid or 1.0 ul Haloperidol (1.25, 2.5, 5, 7.5 ug/side) with 0.5 ul infused into each cannula at a rate of 0.5ul/18s. The internal cannula was left

in place for two minutes following the infusion to help ensure that the drug did not diffuse up the cannula shaft. The infusion pump was checked before and after the actual test infusion to ensure the pump was indeed working; if the pump was working immediately before and after the test infusion it was assumed that the drug was infused into the brain. The animals were returned to the testing cages, and 10 minutes following the infusion behavioural testing began. The gastric contents were rinsed and animals were allowed to sham feed a sucrose solution for 30 minutes.

Histology

At the end of testing the animals were sacrificed using intraperitoneal injection of chloral hydrate (350 mg/ml) and perfused transcardially via a 16 gauge needle placed in the left ventricle, with .15M saline followed by 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde and sectioned at 40u in coronal plane. Brain slices were mounted on slides and stained with cresyl violet for placement verification.

Statistical Analysis

Results were analyzed with dependent sample t-tests and analyses of variance (ANOVA) in order to compare the amount of sucrose consumed (ml) after HP and vehicle infusions for the sham feed groups. Newman-Keuls multiple comparisons tests were used to detect which groups differed. Latency to begin feeding (seconds) was analyzed using analysis of variance and mean standing time was calculated in order to assess whether there were any differences between vehicle and haloperidol trials. Percent suppression (formula:1-(drug intake/vehicle intake)x100%) by which haloperidol decreased sham feeding relative to vehicle conditions was also calculated.

The vehicle data used in the sham intake ANOVA and used for calculating the percent suppression was the mean of 4 separate vehicle days (ie. each day was the vehicle day which immediately preceeded each of 4 drug days). Since the animals' sham intake under vehicle treatment fluctuated slightly over the two week testing period, the mean seemed to be the most appropriate measure of the animals' intake rather than using the data from the very first vehicle day alone. The data was analyzed using both the mean of 4 vehicle days and using the data from the first vehicle day alone and the results were the same, therefore the data presented here utilized the mean vehicle day.

RESULTS

Histology

Two animals from the nucleus accumbens group lost their gastric cannulas before testing was completed and one animal had inaccurate placement; the data for these three animals was not included in the analyses. The remaining five animals had placements located in the target area. All eight hypothalamus animals also had good placement located within the specified site. Photomicrographs of brain sections showing good cannula placements for NA and HT are in Appendix C.

Statistical Analysis

Figure 1 depicts the mean amount (mls/30 minutes) of 15% sucrose sham fed following haloperidol and vehicle infusions into the nucleus accumbens (N=5) and hypothalamus (N=8). A mixed-factor ANOVA with the between-subject factor of infusion site (NA, HT) and the within-group factor of drug condition revealed that haloperidol produced a dose-dependent suppression of sham feeding for both NA and HT

infusions ($F(4,44)=13.5$, $p<.001$). The main effect for injeciton site as well as the interaction were non-significant ($F(1,11)=93.5$, $p<.68$).

Multiple comparisons between doses for the HT group revealed that the 2.5, 5 and 7.5 ug doses of haloperidol significantly suppressed sham intake compared to vehicle and to the 1.25 ug dose (all $P<.05$). No significant differences in sham feeding were found between the 2.5, 5 and 7.5 ug doses of haloperidol.

For NA infusions, multiple comparisons showed that all four doses of haloperidol signifcantly inhibited sham feeding when compared to vehicle (all $p< .05$). No significant difference in suppression was found between the 1.25 and 2.5 ug doses while both the 5 and 7.5 ug doses produced more suppression of sham feeding than the two lowest doses ($p< .01$). This is clearly illustrated in Figure 2 which shows the percent suppression by which haloperidol decreased sham feeding relative to vehicle for the two injection sites. Statistical analyses revealed a dose-dependent increse in suppression of sham feeding for both the NA and HT ($F(3,33)= 8.13$, $p<.001$). The 5 and 7.5 ug doses of haloperidol produced similar levels of suppression for both HT and NA suggesting that the 5 ug dose may produce an asumptotic level of inhibition. While there was a trend for increased suppression with higher doses of haloperidol with HT infusions, multiple comparisons tests revealed that no significant differences in percent suppression were obtained with the 2.5, 5 and 7.5 ug dose.

Although the level of suppression of sham intake with all doses of haloperidol was similar for both hypothalamic and nucleus accumbens infusions, it is possible that the pattern of intake leading to this suppression could be different for the two sites. In order to address this question, cummulative intake during the 30 minute testing session was recorded every 5 minutes. Figures 3 A-D depict mean cummulative sham intakes for HT

and NA groups with each dose of haloperidol presented separately. The sham feeding data was assessed using mixed-factor ANOVAs calculated for each haloperidol dose separatly. These analyses showed that the temporal pattern of intake was similar for HT and NA animals for all four doses (all F values non-significant).

Latency to eat and standing time were observed in order to assess whether the doses of haloperidol utilized produced motor deficits. A mixed-factor ANOVA showed that there was no difference in latency to begin feeding between drug and vehicle conditions for any dose of haloperidol for both the NA and HT infusions ($F(4,44)=0.46, p<.76$). Several animals showed standing behaviour (ie. standing stationary without grooming, exploring or drinking) when given the 7.5 ug dose of haloperidol, The mean standing time was 2.6 minutes for the three NA rats who exhibited this behaviour and 40 seconds for the three animals in the HT group. These results indicate that suppression of feeding with the 7.5 ug dose may be partly due to motor impairment. No standing behaviour was observed during any vehicle trial or with any other dose of haloperidol for both infusion sites.

Anatomical Controls

The second experiment outlined in Appendix B demonstrated that infusions of 5 ug haloperidol into an area adjacent to the nucleus accumbens (within the anterior olfactory nucleus) did not produce suppression of sham feeding.

Figure 1 Effects of haloperidol (1.25, 2.5, 5 and 7.5ug) and vehicle infusions into the nucleus accumbens (N=5) and hypothalamus (N=8)on sham feeding of a 15% sucrose solution. Data are group mean intakes (mls) for 30 minute test. Vertical bars represent 1 SEM.

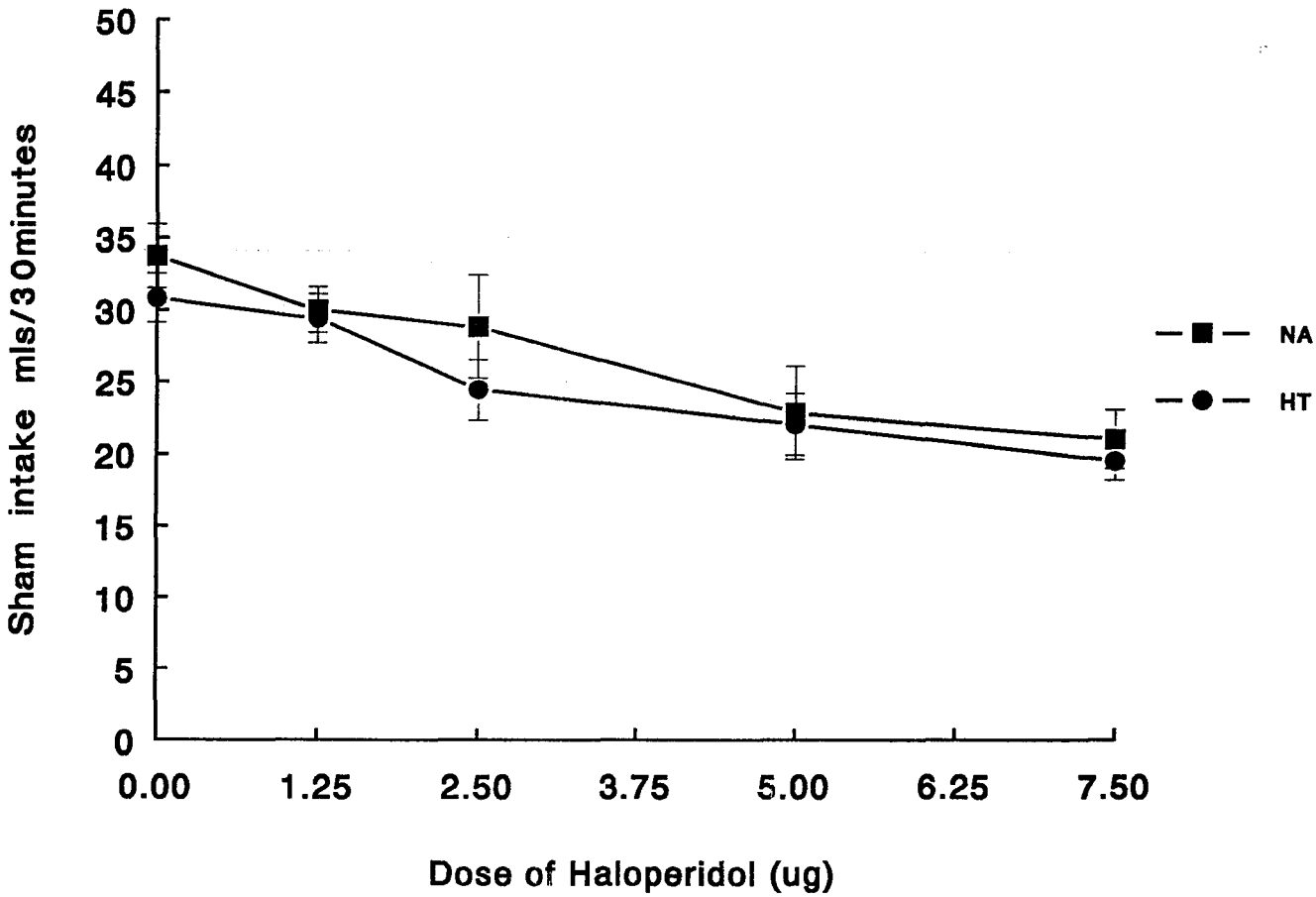

Sham intake mls/30minutes
50
45
40
35
30
25
20
15
10
5
0
0.00
1.25
2.50
3.75
5.00
6.25
7.50
Dose of Haloperidol (ug)
NA
HT

Figure 2 Effects of haloperidol (1.25, 2.5, 5, 7.5 ug) infusions into the nucleus accumbens and hypothalamus on percent suppression of sham feeding relative to vehicle. Vertical bars represent 1 SEM.

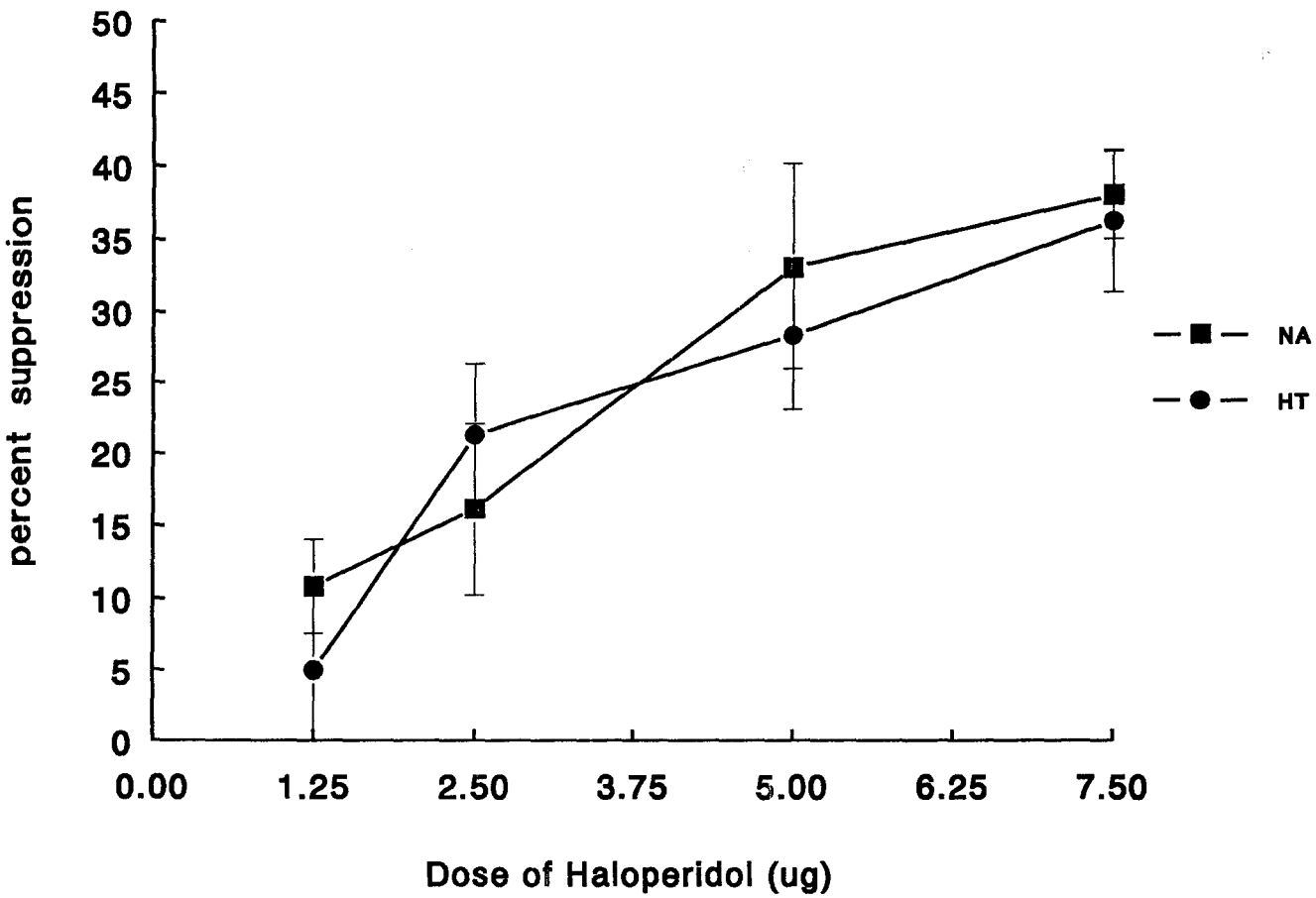

50
45
40
35
30
25
20
15
10
5
0
percent suppression
0.00
1.25
2.50
3.75
5.00
6.25
7.50
Dose of Haloperidol (ug)
NA
HT

Figure 3A and B Effects of haloperidol A)1.25 ug infusions and B) 2.5 ug infusions into the hypothalamus and nucleus accumbens on sucrose sham intake. Data are mean cumulative sham intake from 0-30 minutes after presentation of sucrose. Vertical bars represent 1 SEM.

Figure 3A, 1.25 ug haloperidol

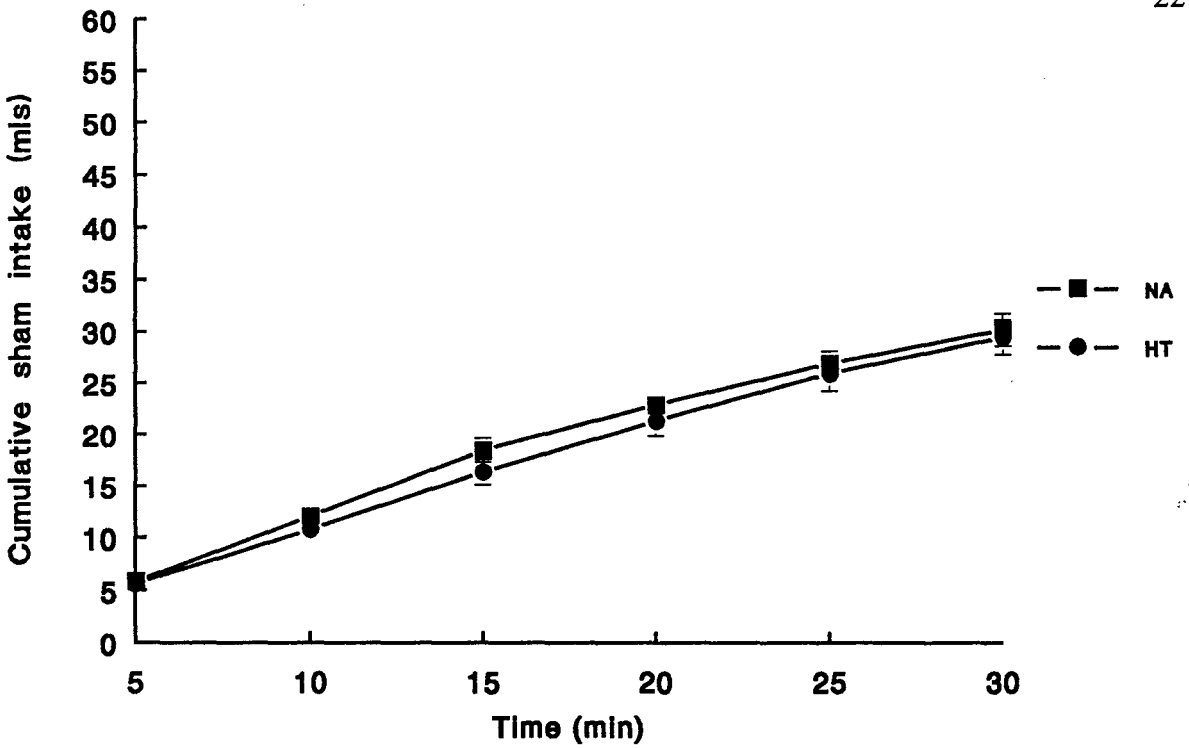


Figure 3B, 2.5 ug haloperidol

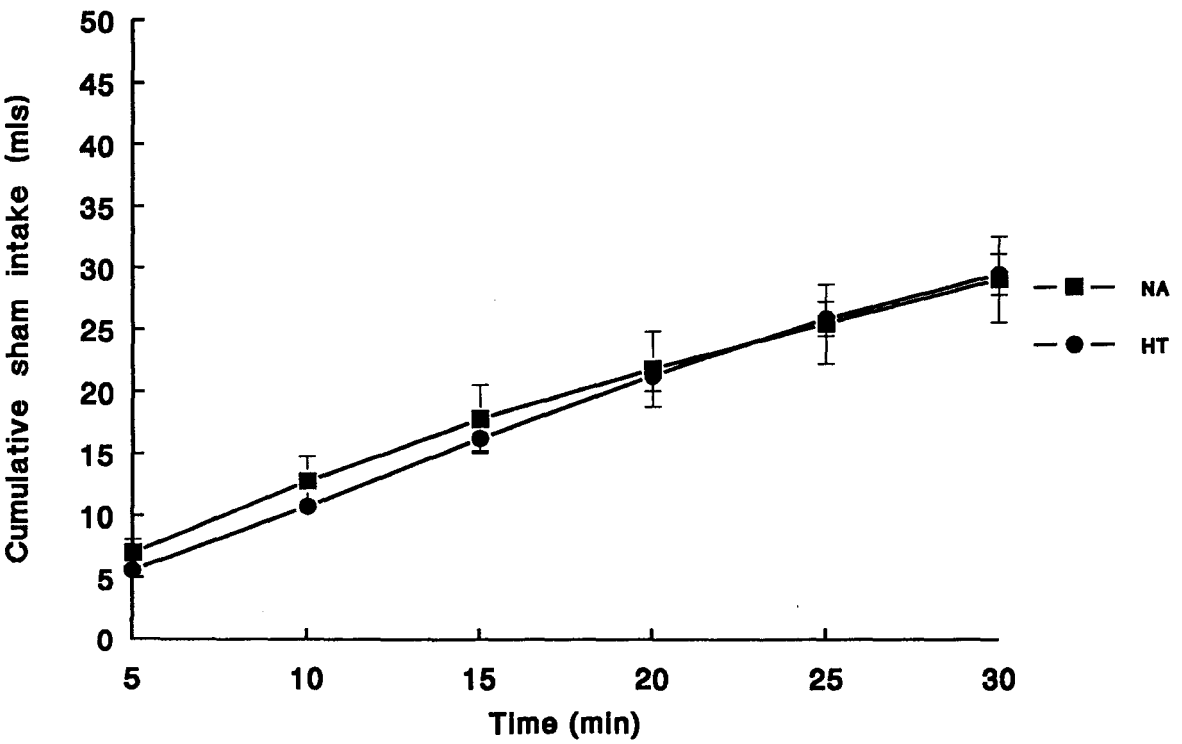


Figure 3C and D Effects of haloperidol C) 5 ug infusions and D) 7.5 ug infusions into the hypothalamus and nucleus accumbens on sham intake. Data are mean cumulative sham intake for 0-30 minutes after presentation of sucrose.

Figure 3C, 5 ug haloperidol

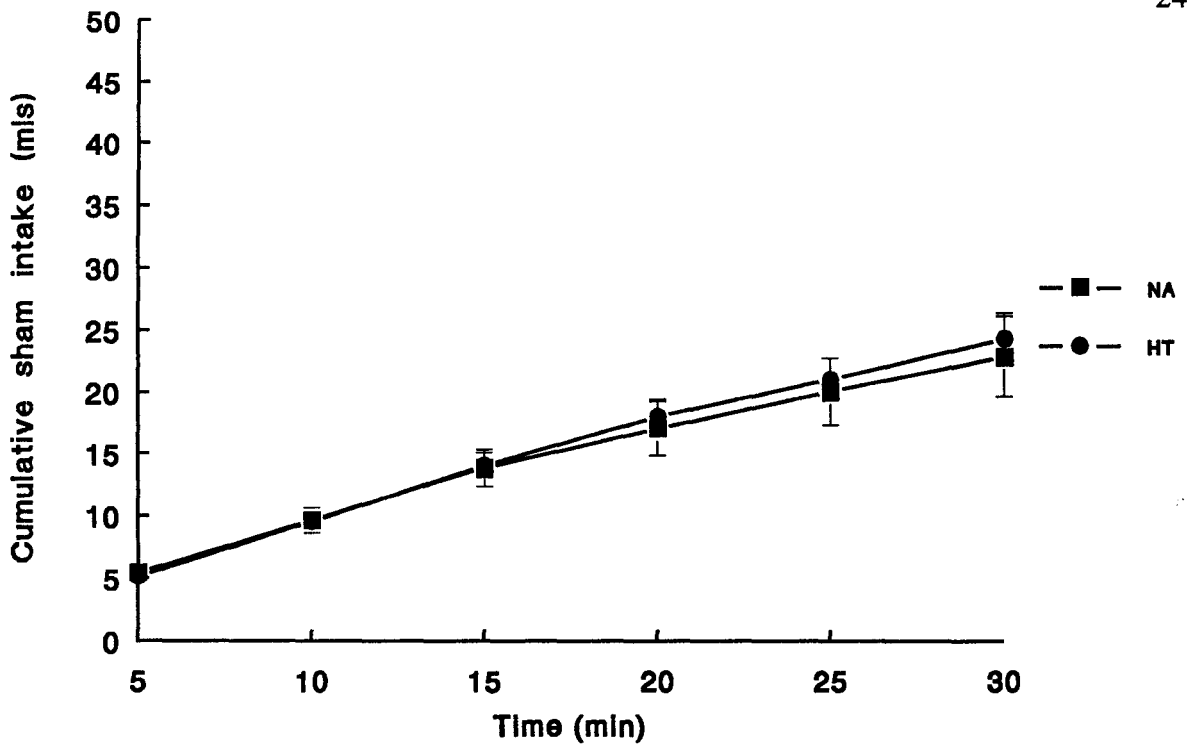
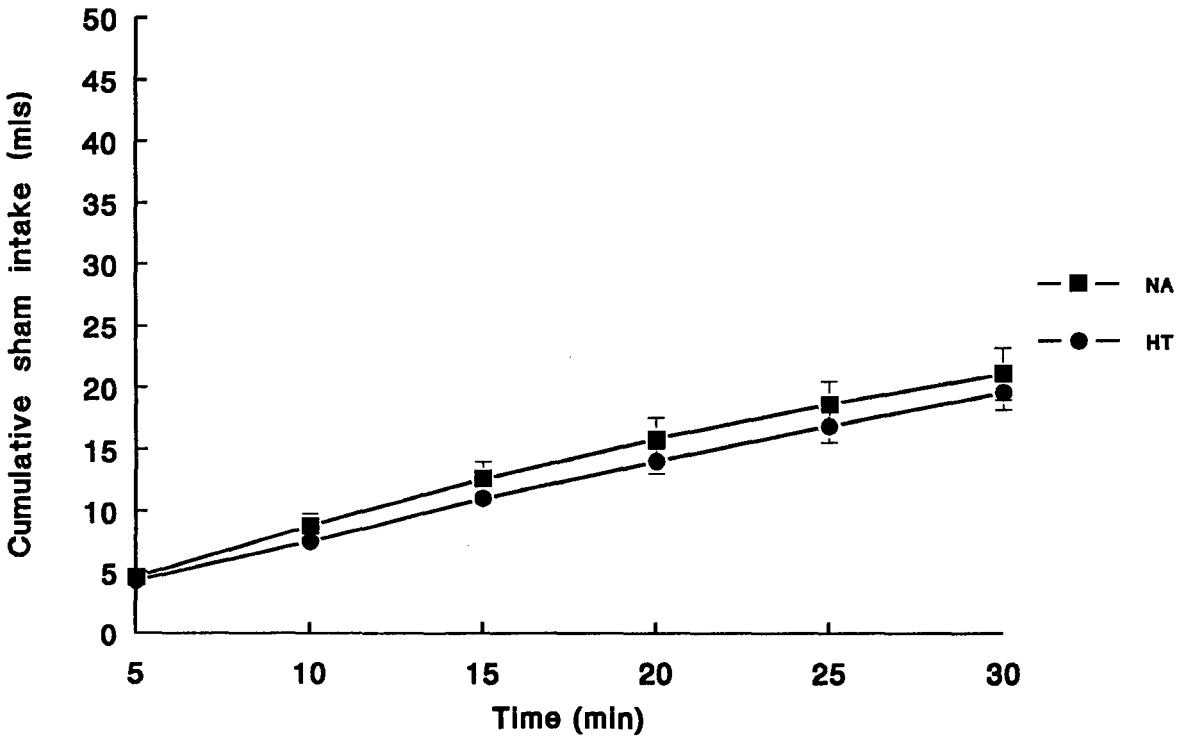


Figure 3D, 7.5 ug haloperidol



DISCUSSION

The present study has demonstrated that hypothalamic and nucleus accumbens infusions of the preferential dopamine antagonist, haloperidol, dose-dependently suppressed sucrose sham feeding. These results suggest that dopaminergic transmission within the hypothalamus and nucleus accumbens may play a role in the mediation of the hedonics of feeding. Our data supports a previous report by Smith et al (1987), demonstrating increased DOPAC/DA ratios within the hypothalamus during sucrose sham feeding. As well, the results we obtained with the blockade of NA dopamine receptors confirm previous preliminary findings by Schneider, et al (1993), demonstrating that infusions of DA antagonists (raclopride and SCH 233690) into the NA inhibited sucrose sham feeding. These results are also in accordance with in vivo microdialysis studies which show that extracellular DA levels increase within the nucleus accumbens and hypothalamus during feeding (Hernandez & Hoebel, 1988; Orosco & Nicolaidis, 1992; Yoshida, et al, 1992). In order to further substantiate the role of NA and HT dopamine in mediating palatability, in vivo microdyialysis studies are needed to measure level of DA release from these two structures during sucrose sham feeding.

Schneider, et al (1993), have also provided preliminary evidence that both D1 and D2 DA receptors are involved in the nucleus accumbens effect on sucrose sham feeding. A similar assessment examining the differential role of D1 and D2 receptors within the hypothalamus is also needed.

An alternative hypothesis which could explain the inhibition of sham feeding produced by dopamine antagonists is that these drugs have been found to produce motor impairment at certain doses (Morley, Bradshaw & Szabadi, 1984). In order to assess whether intake was affected by motor deficits, latency to eat and standing behaviour (ie.

standing stationary without exploring, licking or grooming) were observed. While no dfferences in latency to begin feeding were observed between vehicle and drug-treated animals, the highest doses used (7.5 and 10 ug) caused sedation in several of the animals (ie.standing). The 2.5 and 5 ug doses of haloperidol also significantly suppressed sham feeding in both HT and NA animals but no sedation or motor impairment were observed.

All doses of haloperidol utilized in this experiment produced similar levels of suppression of sham intake for both NA and HT infusions. Moreover, mean cumulative intake over the 30 minutes testing period was similar for both infusions sites for vehicle and all drug doses. It was also demonstrated in a pilot study that infusions of haloperidol into a site adjacent to the nulceus accumbens (ie.anterior olfactory nucleus) did not suppress sham feeding indicating that the drug effect can be localized within the nucleus accumbens and providing a necessary anatomical control within the experiment. These results suggest that two separate dopaminergic pathways are involved in the control of sham feeding palatable sucrose solutions. It has yet to be determined whether the dopaminergic activty of these two sites functions indepedently to produce suppression of sham intake or whether they interact to produce this effect.

Several studies have demonstrated that DA projections in the brain do not function in isolation; they are in fact highly interconnected and interdependent (Louilot, Le Moal & Simon, 1989). Louilot and associates (1989) demonstrated that infusions of DA agonists and antagonists into the medial prefrontal cortex and lateral septum altered neuronal activity within the nucleus accumbens (eg. sulpiride infusions into PFC increased extracellular DOPAC in the nucleus accumbens) and other similar demonstration have shown the functional interdependence between mesoamygdala and mesoaccumbens DA projections (Simon, Taghouti, Goslan, Studler, Louilot, Herve, Glowinski, tassin & Le

Moal, 1988). Therefore, it seem reasonable to suggest that several DA sites may be involved in mediating the effects of palatability. Other DA mediated structures within the mesolimbic system could also be involved such as the prefrontal cortex, amygdala, ventral tegmental area.

While most of literature has focused on the role of nucleus accumbens and mesolimbic DA activity in food reward and hedonics, this thesis provides evidence that another DA pathway may be involved in mediating the hedonic aspects of ingestion. A question which follows from this demonstration is whether HT DA activity is primarily involved in palatability or whether this dopaminergic site is also involved in other behvioural effects such as operant responding for food reward or place preferences conditioned using food reward.

It would also be interesting to assess whether DA blockade of HT dopamine receptors would interfere with palatability using other paradigms which have been used to measure palatability, such as preference tests, taste reactivity and brief exposure tests. Obtaining similar effects across several different paradigms would strengthen our hypothesis.

Although this research has focused on the function of one neurotransmitter, it is unlikely that HT and NA dopamine are functioning alone to produce these effects. The brain is highly interconnected system, and research endeavours should aim to address this complexity. A future challenge would be to address the question of what other factors (eg. other neurotransmitters, neuropeptides etc.) interact with HT and NA dopaminergic activity to control palatability.

APPENDIX A

Pilot Study

A pilot study was conducted in order to determine what doses of haloperidol and what concentration of sucrose to use. Haloperidol (5ug and 10ug) was bilaterally microinjected into the hypothalamus and the effects of this dopamine blockade on the sham feeding of 30% and 15% sucrose solutions was assessed.

METHOD

Subjects included 6 male Sprague-Dawley rats weighing 250-300 grams at the beginning of the experiment. Bilateral brain cannulas were implanted within the hypothalamus using the following stereotaxic coordinates: 2.3 mm posterior, 1.0 mm lateral and 8.5 mm ventral to bregma.

A within-subject design was utilized with all animals receiving each sucrose concentration (15% and 30% sucrose), and each drug condition (5ug, 10 ug haloperidol or 3% lactic acid vehicle), and serving as its own control. The order of drug infusions was counterbalanced, with half the animals receiving drug infusions on the first testing day and the other half receiving vehicle infusions on the first day. All training and testing procedures were identical to those utilized in the main experiment.

RESULTS

Histology

Cannula placement was verified histologically and revealed that one animal had placement which was located within the 3rd ventricle, therefore the data for this animal was removed from the analysis. All other cannula placements were within the

hypothalamus in the specified site.

Statistical Analysis

Figure 1 depicts the mean amount (mls/30 min) of sucrose solution comsumed by sham feedind (N=5) animals during Tests 1,2,3. The intake data was analyzed using dependent-sample t-tests. The 10ug dose of haloperidol produced a significant decrease in sham intake (Test 1,t (4)=-3,p<.04) of 30% sucrose solution. Motor sedation was observed in several animals with this high dose (ie. periods of standing). Infusions of 5ug HP into the hypothalamus also produced significant suppression of sham feeding of 15% (Test 2 approached significance, t(3)=-2.7,p<.07) and 30% sucrose solutions (Test 3, t(4)=-2.9,p>.04). Observations of the animals during testing revealed no obvious signs of motor impairment or sedation (ie. no standing behaviour was observed) with the 5 ug dose of haloperidol.

Also, no differences in latency to begin feeding were found on any test (t-tests all NS). Animals began drinking almost immediately when placed in the test chamber and drank almost continuously throughout the testing session.

Figure 2 shows the percent suppression by which haloperidol inhibited sham feeding relative to vehicle for tests 1, 2 and 3. Statistical analysis using dependent t-tests showed that there was no significant difference in percent suppression of sham feeding with different doses of HP (5 and 10 ug) or with different sucrose concentrations.

Conclusion

This experiment provides preliminary evidence that infusions of haloperidol into the hypothalamus suppresses sham intake of sucrose solutions. It was also determined

that a high dose of 10 ug HP produced significant motor impairment while the 5ug dose of HP did not. No further reduction in sham feeding was seen with the 10ug dose indicating that the 5ug dose may produce an asymptotic level of suppression of sham intake for 30% sucrose solution.

Figure 4 Effects of haloperidol (5 and 10ug) and vehicle infusions into the hypothalamus on sham feeding of 15% and 30% sucrose solutions. Data are group mean intakes (mls) for 30 minute test (Test 1, n=5; Test 2, n=4; Test 3, n=5). Vertical bars represent 1 SEM.

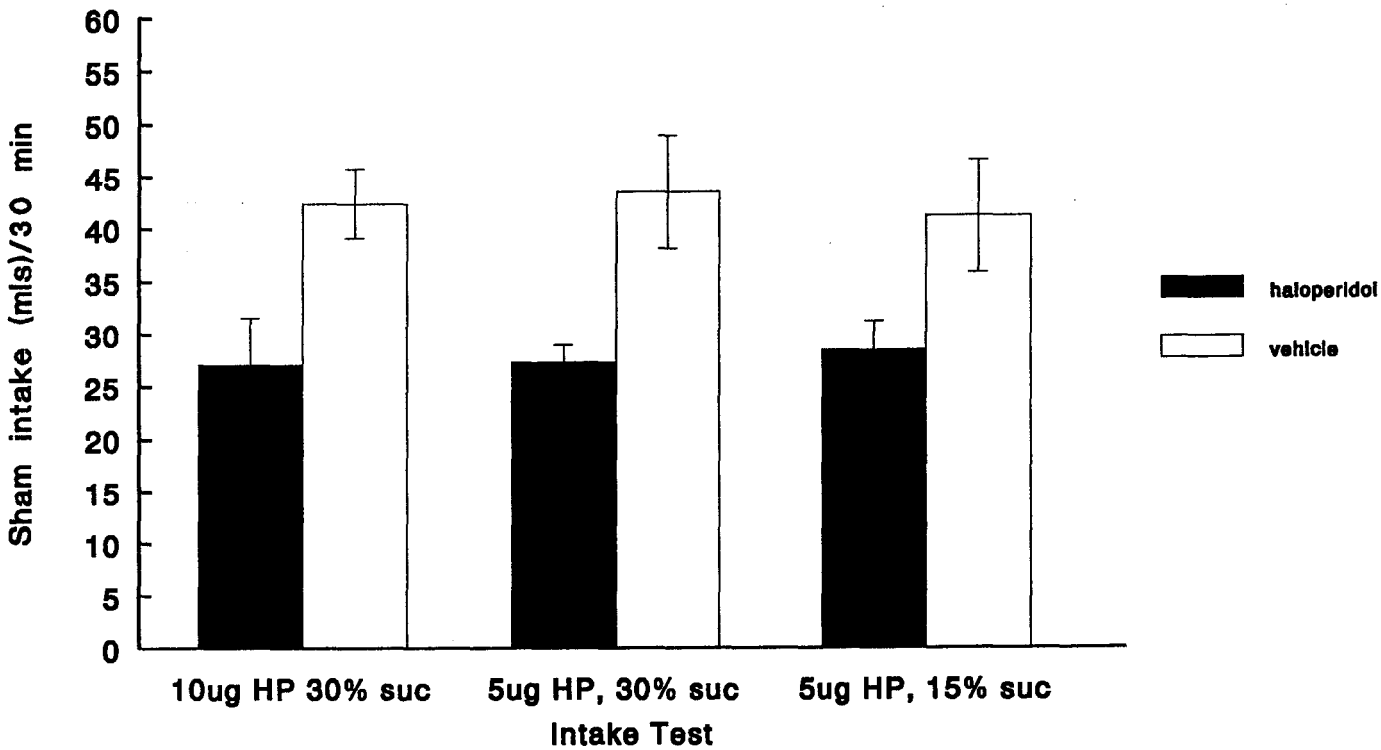
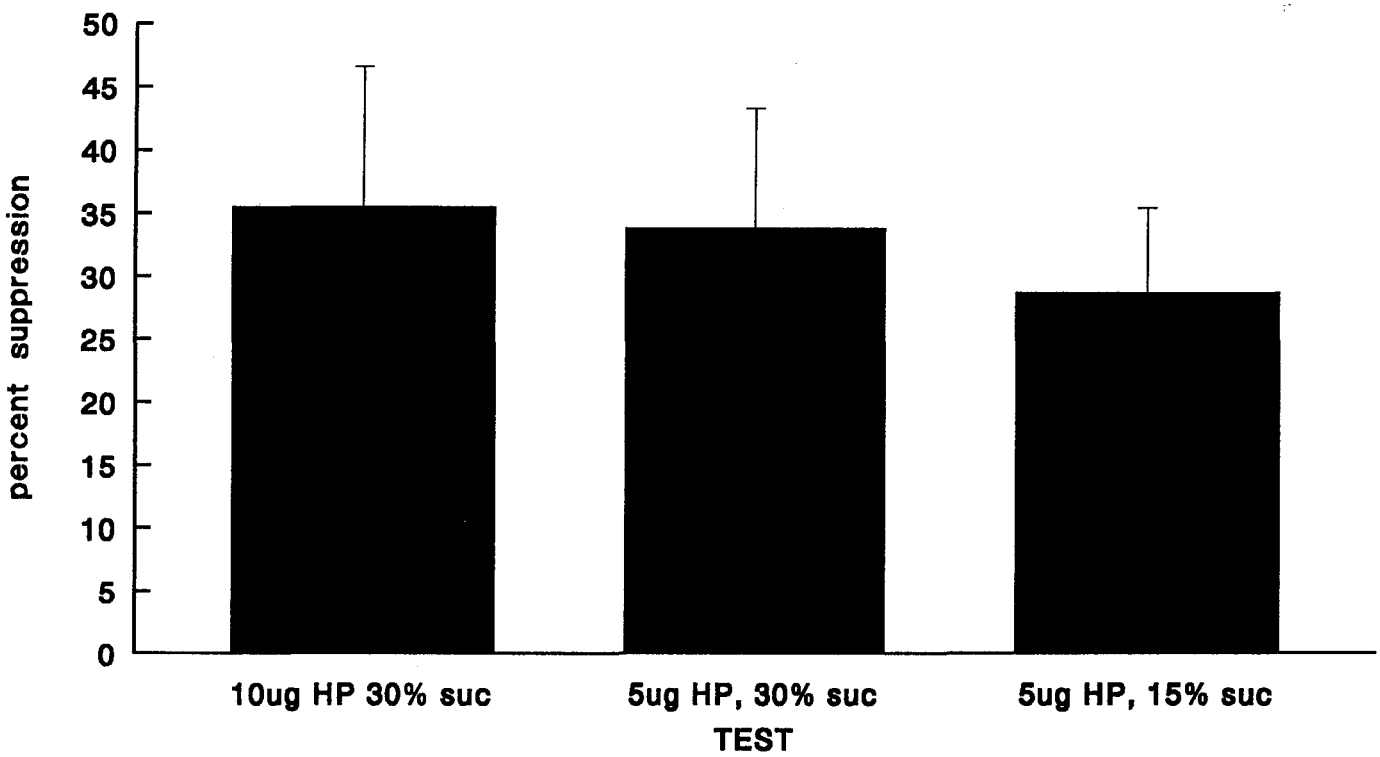

Sham intake (mls)/30 min
60
55
50
45
40
35
30
25
20
15
10
5
0
10ug HP 30% suc
5ug HP, 30% suc
5ug HP, 15% suc
Intake Test
haloperidol
vehicle

Figure 5 Effects of haloperidol (5 and 10ug) infusions into the hypothalamus on the percent suppression of sham feeding relative to vehicle. Vertical bars represent 1 SEM.


50
45
40
35
30
25
20
15
10
5
0
percent suppression
10ug HP 30% suc
5ug HP, 30% suc
5ug HP, 15% suc
TEST

APPENDIX B

Anatomical Control

A second pilot experiment was conducted with infusions of haloperidol (5ug) into the nucleus accumbens (N=5). Procedures and testing were identical to those outlined in the General Methods section. Cannula placement was inaccurate and located adjacent to the nucleus accumbens within the anterior olfactory nucleus.

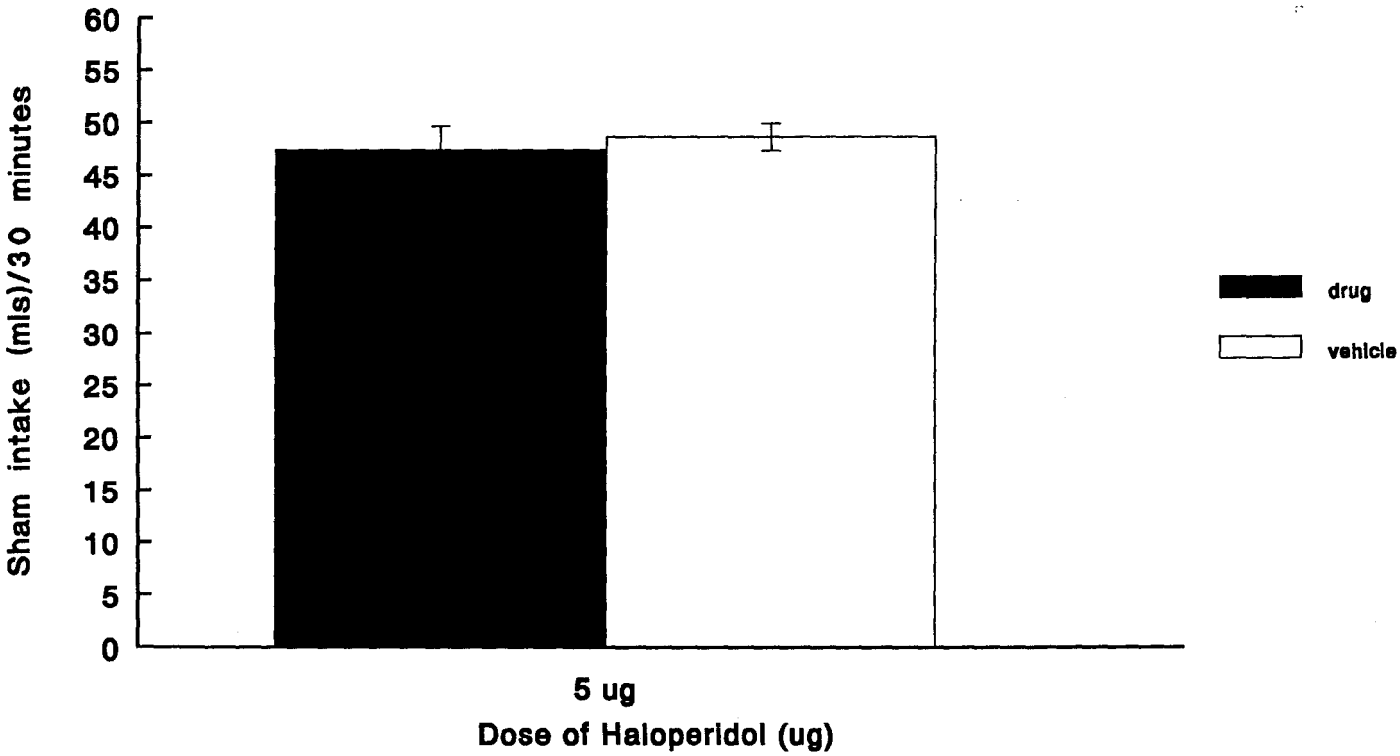
RESULTS

Anterior Olfactory nucleus Figure 9 shows the mean sucrose intake (mls/30 min) of sham feeding animals pretreated with 5 ug/side haloperidol or 3% lactic acid vehicle. Statistical analyses revealed that infusions of haloperidol into the area just anterior to the nucleus accumbens had no effect on sham feeding of a 15% sucrose solution, ($t(4)= -.57, p<0.7$ N.S.).

Conclusion

Infusions of haloperidol (5 ug) into the area just anterior to the nucleus accumbens does not suppress sham feeding. This negative effect provides a necessary anatomical control for this thesis.

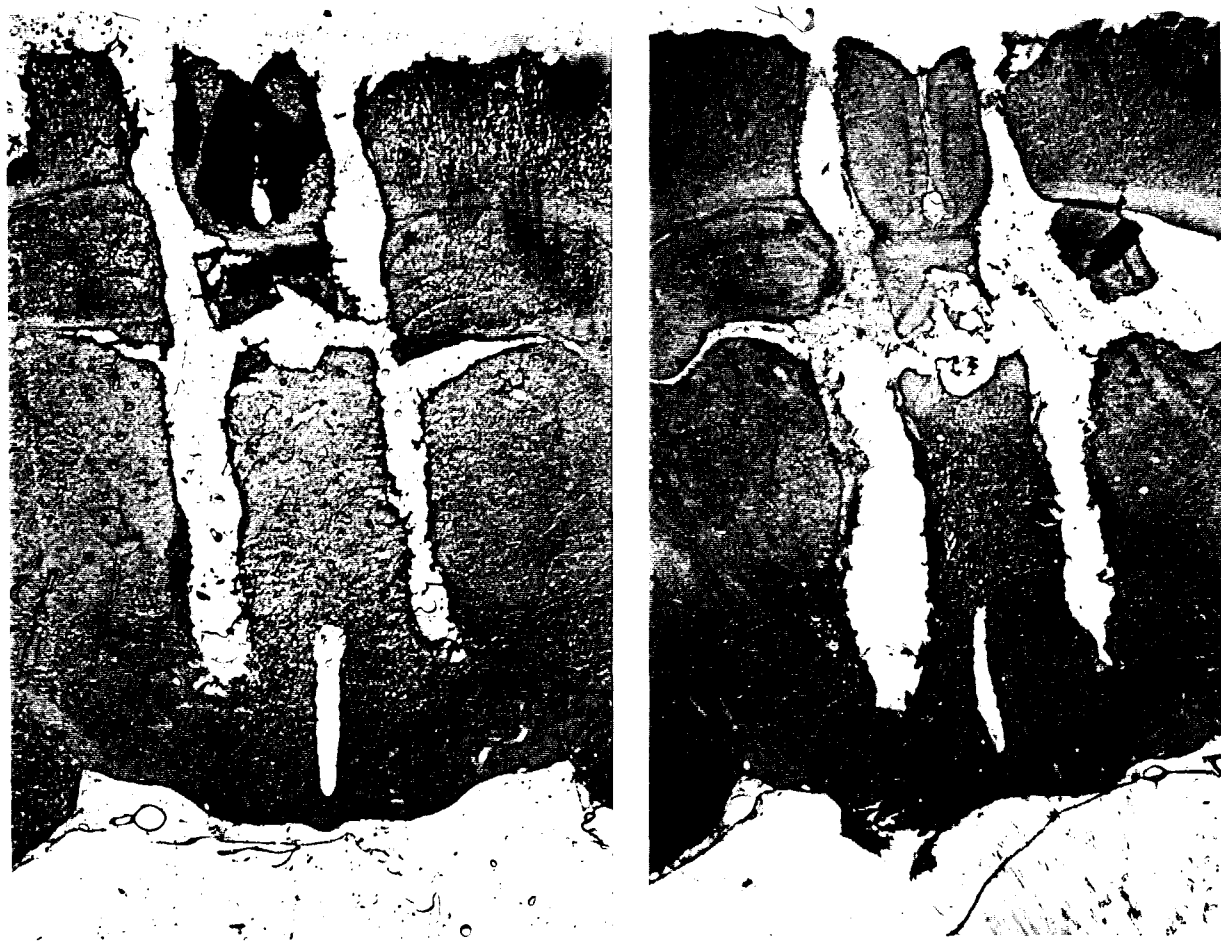
Figure 6 Effects of haloperidol (5 ug) and vehicle infusions into the anterior olfactory nucleus (N=5) on sham feeding of a 15% sucrose solution. Data are group mean intakes for 30 minute test. Vertical bars represent 1 SEM.


Sham intake (mls)/30 minutes
60
55
50
45
40
35
30
25
20
15
10
5
0
5 ug
Dose of Haloperidol (ug)
drug
vehicle

APPENDIX C

Figure 7. Photomicrographs of brain sections showing cannula placements.

A)Hypothalamus



B) Nucleus Accumbens

C) Control - Anterior Olfactory Nucleus

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