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Role for dopamine-3 receptor in the hyperphagia of an unanticipated high-fat meal in rats

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Abstract

Behavioral studies have indicated that midbrain dopamine projections arising in the ventral tegmental area and substantia nigra play a central role in integrating violations of expectancy in reward-related paradigms. The present study was designed to assess violations of dietary expectation and the role the dopamine-3 receptor plays in integrating reward-related food intake in violations of expectancy. Two groups of rats were conditioned to a meal-feeding schedule (3 h of access to food per day) in which they received either standard rodent chow or a preferable, high-fat diet. Animals either received the diet they had access to during the training period (no contrast) or the opposite diet (negative and positive contrast). As predicted, animals in the positive contrast condition were hyperphagic compared to no contrast animals. Animals in the negative contrast (high fat to chow) condition were hypophagic compared to no contrast animals. A dopamine agonist specific to the dopamine three receptor, ((±)-7-Hydroxy-dipropylaminotetralin HBr) and the dopamine-2 receptor antagonist raclopride were administered in equimolar doses peripherally to assess the involvement of the dopamine receptor subtypes in the violation of expectancy food intake effects. 7-Hydroxy-dipropylaminotetralin HBr blocked the hyperphagia associated with positive contrast and did not disrupt intake in the negative contrast or no contrast paradigm. Raclopride was ineffective at disrupting food intake. These results support the hypothesis that the dopamine-3 receptor is involved in the hyperphagia of an unexpected high fat meal.
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1. Introduction

A large body of evidence supports a role for dopamine signaling in food reward paradigms (Bassareo and Di Chara, 1997; Gambarana et al., 2003; Sotak et al., 2005; Verty et al., 2004). A variety of genetic, functional, and pharmacological studies have sought to determine the exact nature of how dopamine might exert influence over the control of food reward. In particular, mice unable to synthesize dopamine display drastically reduced food intakes and bodyweights, and require exogenous dopamine to prevent starvation (Zhou and Palmiter, 1995). Selective lesions of dopamine neurons in the mesolimbic system using 6-hydroxy dopamine (6-OHDA) lead to decreased food hoarding behavior (Kelley and Stinus, 1985), decrease the number of crossings of an electrified grid to obtain food (Papp and Bal, 1987), and lead to decreased feeding strategy efficiency (Salamone et al., 1990). Pharmacological manipulation of dopamine signaling within the striatum has been shown to attenuate feeding behavior and these studies have focused primarily on action at the dopamine-1 (D1) or dopamine-2 (D2) receptor subtypes (Salamone et al., 1990; Smith and Schneider, 1988; Terry and Katz, 1992; Hsiao and Smith, 1995; Lutz et al., 2001). The dopamine 3 receptor (D3R) is relatively new to the food intake field and only a few reports have focused on a potential role for it in the regulation of food reward. Prior studies from this lab have shown that pharmacological disruption of the D3R with the agonist ((±)-7-Hydroxy-dipropylaminotetralin HBr) (7-OHDPAT) attenuates the feeding response in 21 h food deprived animals (McQuade et al., 2003). The activation of the D3R is hypothesized to oppose D1/D2R activation both at the cellular as well as behavioral level (Ridray et al., 1998; Richtand et al., 2001).

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Table 1
Experimental protocol for experiment 1 in which animals were maintained on a 21 h restricted access protocol of standard rodent chow (CH) or a high fat diet (HF) for four consecutive weeks and prior to the initial violation of dietary expectancy test session

Conditioning phase (30 days)	Violation	No contrast group	Contrast group
HF animals ($n=10$)	HF or CH	HF/HF animals ($n=5$)	HF/CH animals ($n=5$)
CH animals ($n=10$)	CH or HF	CH/CH animals ($n=5$)	CH/HF animals ($n=5$)

In the present study, we sought to determine the role of the D3R in particular when an unexpected food reward is encountered for the first time. We focused on the D3R specifically because prior reports from our lab have demonstrated that disruption of signaling at this receptor blocks the rebound hyperphagia typically observed after food deprivation. To assess this hypothesis, we utilized a behavioral protocol in which violations of diet expectancy occurred. Animals were trained to anticipate access to either standard lab chow or a preferred, high-fat diet (HF). Food intake was then assessed when animals were given what they anticipated (no contrast) or the opposite diet (dietary contrast). Contrast conditions were labeled as either "positive" (receiving the HF diet when expecting chow) or "negative" (receiving chow when expecting the HF diet). One previous study utilized this paradigm to examine the effects of pharmacological inhibition of the D2 receptor on violation of reward expectancy (Roitman et al., 2001). This study reported that pharmacological inhibition of the D2 receptor with the D2-specific antagonist raclopride attenuated hyperphagia associated with positive contrast with no apparent effect on food intake in non conditioned animals (Roitman et al., 2001). Presumably, the hyperphagia associated with the positive contrast is DA-mediated and occurs as a result of elevated DA release by midbrain DA neurons in response to a novel more palatable food source. We therefore hypothesized that violation of expectancy inducing positive-contrast hyperphagia would be accompanied by a similar pattern of increasing midbrain dopamine upon encountering a novel more palatable food source. In keeping with this hypothetical framework, we further hypothesized that the positive contrast hyperphagia would be attenuated by 7-OH-DPAT administration, and that this effect would be restricted to the overeating normally observed in the positive contrast group, and without effect in the negative contrast, and no-contrast conditions.

To assess the potential role of the D3-R in mediating the altered ingestive responses when rats are exposed to dietary contrasts, we administered the dopamine-3 receptor (D3-R) agonist, 7-OH-DPAT and the selective D2-R antagonist, raclopride to animals undergoing a no contrast, positive contrast, or negative contrast situation.

2. Methods

2.1. Animals

Male Long–Evans rats (Harlan, IN) weighing 200–250 g were housed individually in a vivarium with a 12:12 light/dark schedule ($n=10$/group). The temperature of the room was maintained at 25 °C. All animals had *ad libitum* access to water and were placed on a 21-h food restriction, meal-feeding schedule. At lights out, animals received 3-h access to either pelleted rodent chow (Teklad, 3.41 kcal/g, 0.51 kcal/g from fat) or a HF diet (Dyets, inc., Bethlehem, PA, 4.41 kcal/g, 1.71 kcal/g from fat).

2.2. Drugs

The D3-R agonist, 7-OH-DPAT ((±)-7-Hydroxy-dipropylaminotetralin HBr), and the D2-R antagonist, raclopride (RBI Co., MA), were dissolved in sterile 0.9% NaCl.

3. Procedures

3.1. Experiment 1 (positive and negative contrasts)

Animals were maintained on chow or the HF restricted meal-feeding schedule for 1 month prior to the start of the experiment. On test days, rats received access to food at the scheduled time. However, on each test day, one half of the rats received the previously trained diet (no contrast). The remaining rats received the opposite diet used in training (contrast) (Table 1). That is, rats that had previously received chow received HF and HF trained rats received chow. Food intake was measured for 3 h.

3.2. Experiment 2 (subcutaneous 7-OH-DPAT and raclopride)

Animals were maintained on chow or the HF restricted meal-feeding schedule for 1 month. At the conclusion of experiment 1, the same group of animals (high fat (HF) meal fed $n=10$, chow (CH) meal fed $n=10$) were used in a second experiment to observe effects of D3 activation/D2 receptor inhibition on violation of diet expectancy. To accomplish this, after the initial violation in experiment 1, each group of animals CH and HF were continued on the 21 h restricted access protocol and tested once per week for a total of 14 weeks. To minimize predictions in dietary expectancy animals from both groups were randomly assigned to either the positive or negative contrast groups for each subsequent test session and each test session was

Table 2
Experimental protocol for experiment 2 in which animals were maintained on a 21 h restricted access protocol of standard rodent chow (CH) or a high fat diet (HF) for eight consecutive weeks during which they received one of three doses of 7-OH-DPAT, raclopride, or saline prior to a positive, negative, or no contrast violation of dietary expectancy

Violation groups	Week 1, 3, 5, 7 Saline	Week 1, 3, 5, 7 Raclopride (0, 38, 100 μg)	Week 2, 4, 6, 8 Saline	Week 2, 4, 6 ,8 7-OH-DPAT (0, 10, 25, 50 μg)
No contrast	HF/HF ($n=5$)	HF/HF ($n=5$)	HF/HF ($n=5$)	HF/HF ($n=5$)
	CH/CH ($n=5$)	CH/CH ($n=5$)	CH/CH ($n=5$)	CH/CH ($n=5$)
Contrast	HF/CH ($n=5$)	HF/CH ($n=5$)	HF/CH ($n=5$)	HF/CH ($n=5$)
	CH/HF ($n=5$)	CH/HF ($n=5$)	CH/HF ($n=5$)	CH/HF ($n=5$)

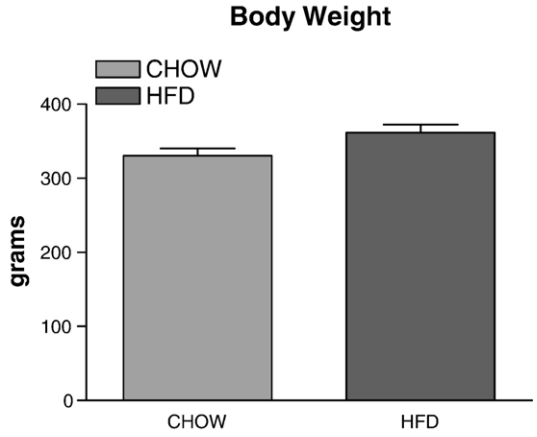

Fig. 1. Mean body weights from animals maintained on a 21 h restricted access protocol of standard rodent chow (CHOW) or a high fat diet (HFD) for four consecutive weeks and prior to any experimental manipulation.

randomized relative to the day of the week in which testing occurred. Each group then received the diet switch protocol as in Experiment 1 after receiving s.c. injection of 7-OH-DPAT, raclopride or saline (Table 2). Doses of 7-OH-DPAT were 0, 10, 25 or 50 μg/kg, s.c. in counterbalanced order. Injection of drug or saline preceded lights out and replacement of food by 15 min. Food intake was monitored for 3 h post injection. Doses of raclopride used in this experiment were 0, 38, 100 μg/kg, s.c. The 38-μg/kg dose of raclopride was chosen because it is an equimolar dose to 25 μg/kg 7-OH-DPAT. The 100 μg/kg of racloride was chosen because it has been demonstrated to be a sub-threshold dose for reducing food intake (Baker et al., 2001; Lutz et al., 2001). For both experiments 1 and 2 animals were first given an injection of saline, and then the following day given one of three doses of either 7-OH-DPAT or raclopride. Following each test session the animals resumed there standard meal feeding protocol receiving either chow or high fat diet for 3 h each day. Animals were tested in this fashion every other week for a total of eight total sessions occurring over 14 weeks (Table 2).

3.3. Statistical analysis

Body weight data was analyzed using a one-way analysis of variance (ANOVA) with diet as a factor. Food intake data were analyzed using repeated-measures analysis of variance (ANOVA) with factors of diet, drug and time when applicable. Drug administration was performed as a within subjects design in every experiment. When appropriate, post-hoc analyses were performed using the LSD test.

4. Results

4.1. Experiment 1 (positive and negative contrasts)

Rats maintained on the 21 h restricted access protocol to either chow or high fat diet for 1 month prior to testing did not differ in body weight at the conclusion of the 4 week conditioning period (Fig. 1). Rats that were accustomed to consuming chow, but instead received access to the preferred HF diet, were acutely hyperphagic in that they consumed more food and more total calories than they did when given the anticipated chow (positive contrast). In contrast, animals anticipating the HF diet but receiving chow were hypophagic (negative contrast). These data are depicted in Fig. 1. A three-way ANOVA (training diet × test diet × time) supported these conclusions ($F_{(2,32)}=6.89$, $P<0.05$). A two-way (training diet × test diet) ANOVA revealed that at each time point assessed, 30 min ($F_{(1,16)}=134.9$, $P<0.05$), 60 min ($F_{(1,16)}=44.1$, $P<0.05$) and 180 min ($F_{(1,16)}=72.6$, $P<0.05$), animals were hyperphagic faced with the positive diet contrast and hypophagic faced with the negative diet contrast.

4.2. Experiment 2 (subcutaneous 7-OH-DPAT and raclopride)

The D3-R agonist, 7-OH-DPAT (10, 25 and 50 μg/kg), was administered s.c. in the 3 conditions (no, positive, and negative contrast) and food intake was measured at 30, 60, 120, and 180 min. First, we replicated the previous finding that a positive contrast results in hyperphagia while a negative contrast results in hypophagia (Fig. 2). Second, 7-OH-DPAT attenuated the hyperphagia of positive contrast, but had little effect on any other condition (Fig. 2 summarizes these data). Because the only reliable effects were observed in the middle dose of 7-OH-DPAT, only these doses are represented graphically for 7-OH-DPAT and raclopride at the 60 min time interval, while the remaining doses

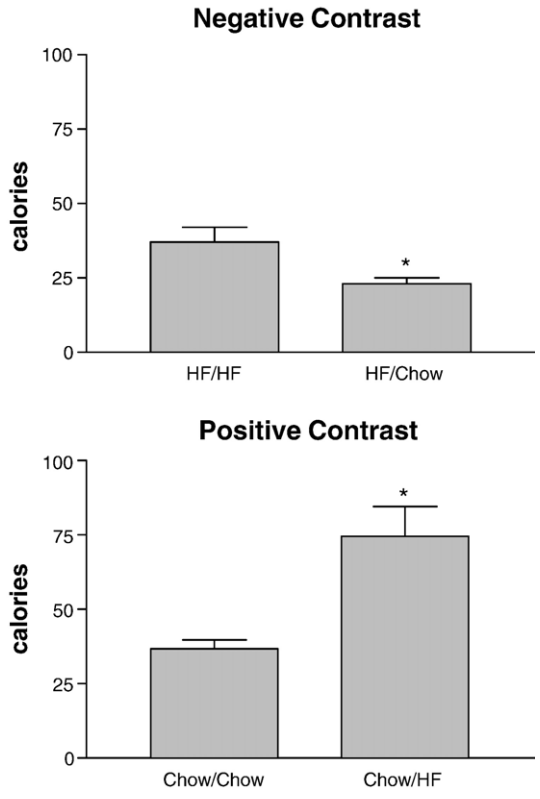

Fig. 2. Mean caloric intake at 60-min following a negative (upper panel) violation and a positive (lower panel) violation of diet expectancy. "*" = $P<0.05$.

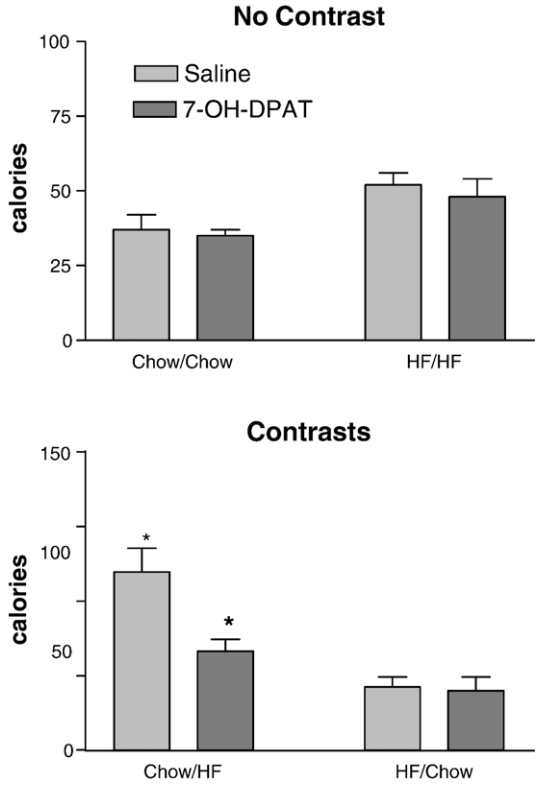

Fig. 3. Mean caloric intake at 60-min following i.p. administration of saline or 25 μg/kg 7-OH-DPAT. Upper panel depicts data from rats without a diet contrast (Chow/Chow and HF/HF). Lower panel depicts data from rats with a diet contrast (Chow/HF and HF/Chow). "*"=$P<0.05$.

for each compound and each time point are simply mentioned in the text. In the second part of the experiment, raclopride (38 μg/kg and 100 μg/kg) was administered to no, positive, and negative contrast groups. Unlike 7-OH-DPAT, raclopride was not effective to systematically change food intake in any condition (no, positive or negative contrast). Fig. 3 summarizes these results.

For effects of treatment with 7-OH-DPAT, two-way ANOVAs (diet × drug) and one-way ANOVAs (drug) were performed with respect to no contrast or contrast at each time point. The only significant result obtained with the 10 μg/kg dose was a main effect of drug at 180 min in the no contrast animals ($F_{(1,8)}=7.45$, $P<0.05$). Two-way ANOVA (diet × switch) indicated violation of diet expectancy reliably increased intake (positive contrast) and decreased intake (negative contrast) relative to the no contrast ($F_{(1,16)}=66.5$, $P<0.05$). Two-way ANOVAs (diet × drug) revealed 25 μg/kg reliably reduced intake in positive contrast animals but not in negative contrast animals at 30 and 60 min ($F_{(1,8)}=8.38$, $P<0.05$ and $F_{(1,8)}=6.51$, $P<0.05$, respectively). There was also a main effect of drug at 30 and 60 min ($F_{(1,8)}=10.2$, $P<0.05$ and $F_{(1,8)}=8.31$, $P<0.05$, respectively). Furthermore, there were no interactions in no-contrast animals at any time point. Two-way ANOVA (diet × switch) indicated violation of diet expectancy reliably increased intake (positive contrast) and decreased intake (negative contrast) relative to the no contrast ($F_{(1,16)}=97.7$, $P<0.05$). Two-way ANOVAs (diet × drug) revealed 50 μg/kg reliably reduced intake in positive contrast animals but not in negative contrast animals at 30 and 60 min ($F_{(1,8)}=49.4$, $P<0.05$ and $F_{(1,8)}=3.52$, $P=0.09$, respectively). There was also a main effect of drug at 30 and 60 min ($F_{(1,8)}=35.3$, $P<0.05$ and $F_{(1,8)}=11.4$, $P<0.05$, respectively). Unlike the lower doses, this dose of 7-OH-DPAT did yield a main effect of drug in no contrast animals at 30, 60 an 180 min ($F_{(1,8)}=14.3$, $P<0.05$, $F_{(1,8)}=3.74$, $P<0.05$ and $F_{(1,8)}=6.98$, $P<0.05$, respectively). Two-way ANOVA (diet × switch) indicated violating diet expectancy reliably increased intake (positive contrast) and decreased intake (negative contrast) relative to the no contrast ($F_{(1,16)}=80.8$, $P<0.05$).

Similar analyses for the effects of raclopride were performed. The only reliable finding with the 38 μg/kg dose of raclopride was a main effect of drug at 180 min in positive contrast animals ($F_{(1,8)}=6.25$), $P<0.05$). Two-way ANOVA (diet × switch) indicated violation of diet expectancy reliably increased intake (positive contrast) and decreased intake (negative contrast) relative to the no contrast ($F_{(1,16)}=24.2$, $P<0.05$). Two-way ANOVAs performed on the data from the higher dose of raclopride indicated there were no reliable findings. There were reliable main effects of drug at 30 and 60 min in the no contrast and positive/negative contrast animals ($F_{(1,8)}=21.8$, $P<0.05$ and $F_{(1,8)}=16.0$, $P<0.05$, respectively). There was also a main effect of drug in no contrast animals at 60 min ($F_{(1,8)}=35.2$, $P<0.05$). Two-way ANOVA (diet × switch) indicated violation of diet expectancy reliably

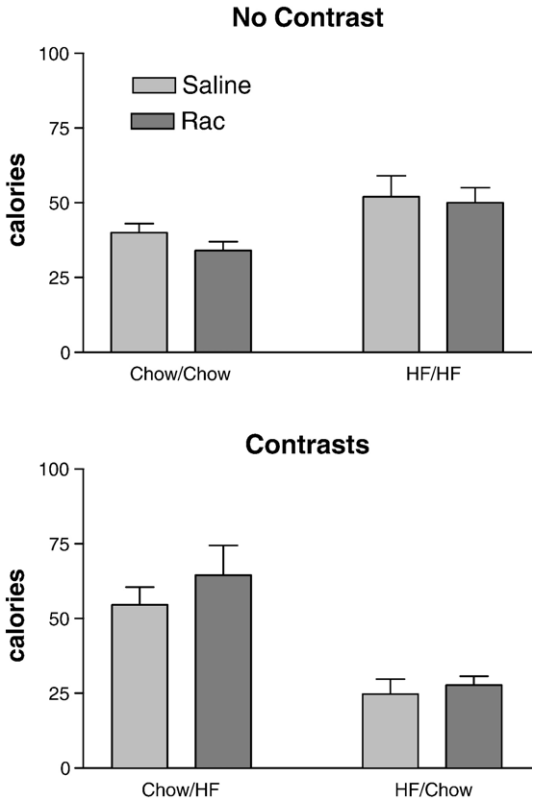

Fig. 4. Mean caloric intake at 60-min following i.p. administration of saline or 38 μg/kg raclopride. Upper panel depicts data from rats without a diet contrast (Chow/Chow and HF/HF). Lower panel depicts data from rats with a diet contrast (Chow/HF and HF/Chow).

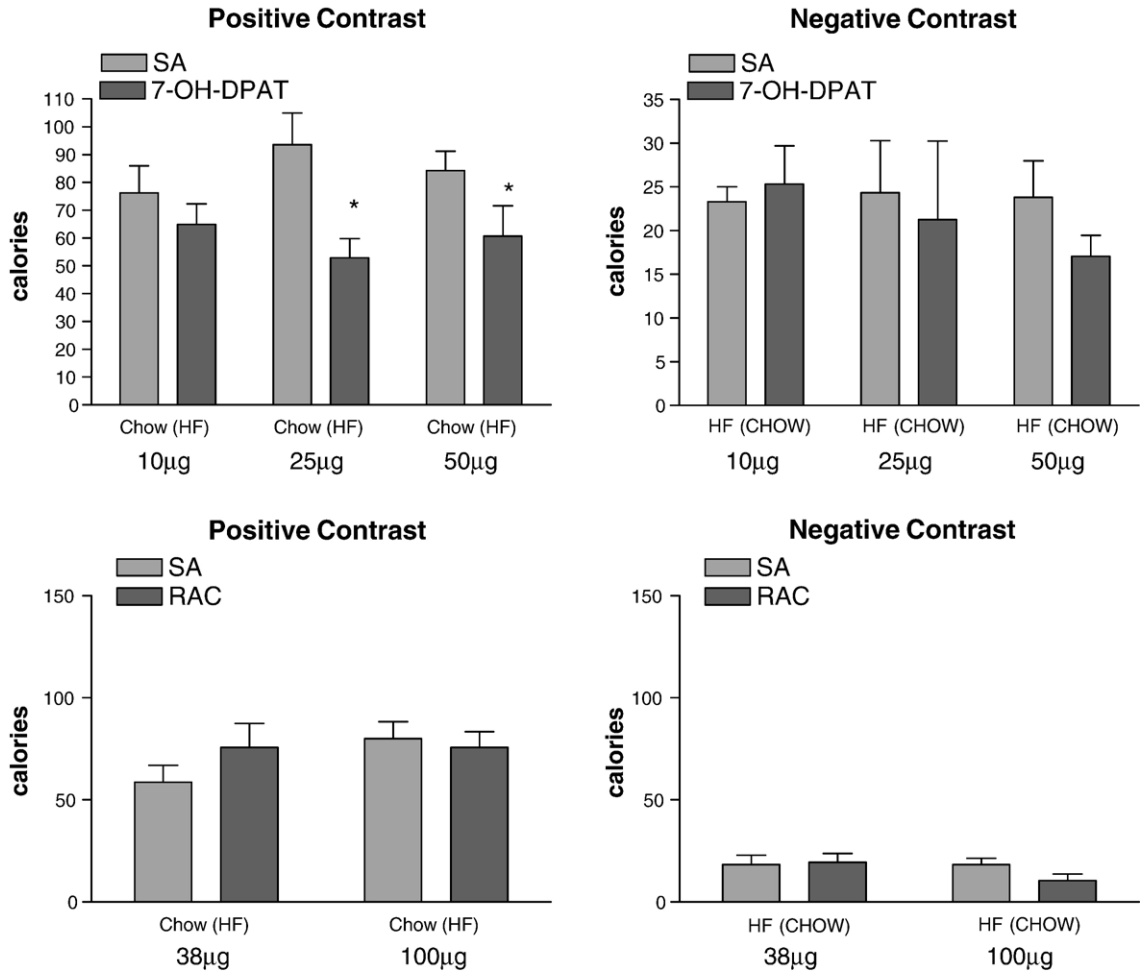

Fig. 5. Mean caloric intake at 60-min following i.p. administration of saline or 7-OH-DPAT over a range of doses from 10–50 µg/kg or 38–100 µg/kg of raclopride. Upper panel depicts a positive violation and the lower panel depicts a negative violation of diet expectancy. "*"=$P<0.05$.

increased intake (positive contrast) and decreased intake (negative contrast) relative to the no contrast ($F_{(1,16)}=137, P<0.05$) (Figs. 4 and 5).

5. Discussion

The experiments in the present study utilized a violation of diet expectancy protocol to assess food intake when a preferred HF diet is given to animals anticipating access to standard rodent chow (positive contrast). In addition, food intake was assessed when standard rodent chow was given to animals anticipating access to the preferred HF diet (negative contrast). Access to diets was scheduled at lights-out each day, which provided rats with predictive temporal and visual cues. This protocol relies on conditioning the animals to anticipate a particular non-novel food stimulus, which is acquired over a prolonged period of time. With subsequent exposure to this type of "meal-feeding" protocol, animals learn to anticipate the delivery of the food stimulus on a daily basis (Woods and Strubbe, 1994). Based on the theoretical framework outlined by Shultz (1998). One would expect striatal dopamine to increase directly before the daily access to the food each day, and this pre-meal dopamine signal should subside upon consummation of the food. However, when the animals are presented with a novel, more palatable food source rather than the animals had never been allowed access to (positive contrast), an additional "novelty induced" dopamine signal should occur, and supersede the pre-meal anticipation surge (Kiyatkin and Gratton, 1994; Roitman et al., 2004). Thus, this protocol enables a unique "conditioned novelty" effect towards food reward.

In the first experiment we used this behavioral protocol to assess food intake following a no, positive, and negative diet contrast independent of any dopaminergic manipulation. Animals that had access to chow during the conditioning phase and received the preferred, HF diet on the test day displayed profound increases in food intake compared to animals that received a predicted diet. This effect on food intake in the positive contrast group was detectable for the entire 8 weeks of testing in this experiment. After each test session the animals resumed their normal meal restriction protocol of chow or high fat for 3 h a day, thus it is difficult to determine how long the positive contrast would have endured if the animals were not retrained between the test sessions. Prior reports using this violation protocol also failed to mention the duration of these effects (Roitman et al.,

2001), thus it is unclear how long the effect of hyperphagia observed in the positive contrast group would persist without retraining. Animals that had access to a HF diet during the conditioning phase and received chow on the test day had reduced intake compared to animals that received a predicted diet. Presumably, the hyperphagia associated with the positive contrast is DA-mediated and occurs as a result of elevated DA release by midbrain DA neurons in response to a novel more palatable food source. In addition, the hypophagia associated with the negative contrast reflects a depression in DA release mediated by midbrain DA neurons. Further, the food intake responses observed in these experiments reflect similar results obtained using spatial and temporal violation models (Roitman et al., 2001).

In the second experiment we assessed the ability of the D3-R agonist, 7-OH-DPAT, to disrupt food intake resulting from a violation of diet expectancy. Three doses of 7-OH-DPAT were assessed to rule out effects not specific to signaling at the D3 receptor (for review see Richtand et al., 2001). The lowest dose of 7-OH-DPAT (10 μg/kg) was ineffective at reducing food intake in any of the diet models. The two higher doses (25 and 50 μg/kg) attenuated food intake selectively in the positive contrast condition. However, 7-OH-DPAT had no effect in the no contrast or negative contrast conditions. 7-OH-DPAT has been suggested to activate the D2-R (Levant et al., 1996; Xu et al., 1999) indicating our results may be mediated by the D2-R and not the D3-R. Therefore, we administered raclopride, the specific D2-R antagonist, at an equimolar dose. Raclopride was chosen because locomotor data indicates the D2-R and D3-R have opposing functions on behavior despite being members of the same dopamine receptor family. Thus, if the results obtained with the D3-R agonist were mediated by the D2-R, raclopride should have similar effects on intake as 7-OH-DPAT. Raclopride had no effect in the no, positive and negative contrast conditions. Furthermore, the higher dose of raclopride only caused reductions in intake in the no contrast conditions, suggestive of non-specific behavioral deficits. A previous study by Roitman and colleagues (Roitman et al., 2001) reported that inhibition of the D2 receptor attenuated the hyperphagia associated with violations of dietary expectancy that were specific to positive contrast only. Additionally, raclopride did not block the effects of hyperphagia associated with 24 h food restriction. In that study, animals were able to anticipate food reward using two separate modalities to predict food exposure, time of day, and spatial location of food exposure. Both predictors reliably lead to hyperphagia in the positive contrast situation, and inhibition of the D2 receptor with raclopride inhibited this effect. In the present study, systemic injection of a similar dose of raclopride was without effect. However, it should be noted that in this study, an additional novelty component was present that was not present in the previously described study. In our study, high fat diet was included in the positive contrast situation, which represents a novel food source to these animals. The animals in this study did expect food, but did not expect the more preferable, high fat food. Therefore, the effects reported by Roitman and colleagues could be specific to an unanticipated feeding bout rather than an unexpected reward per se.

These data support the contention that the primary effects of 7-OH-DPAT are mediated by its actions at the D3-R. Furthermore, this report is one of the few examples which implicate the D3 receptor in the context of food reward. Prior studies from this lab have reported that activation of the D3 receptor inhibits consumption of a high fat diet in animals that had undergone a 21 h period of food restriction. This result was interpreted to suggest that animals conditioned to expect a meal with high caloric value (high fat) are reliant upon D1/D2 signaling, as D3 activation is hypothesized to functionally oppose D1/D2 activation, to consume such a meal following acute caloric restriction (McQuade et al., 2003). However, this is the first account to our knowledge in which the D3 receptor has been shown to alter the consumption of an unexpected reward. Given the fractionation of the anhedonia hypothesis of dopamine reward, these data must be interpreted carefully and at least a few alternative explanations are possible: 1) Increased feeding induced by the introduction of the novel high-fat food source is attenuated by the D3-R activity and thus positive contrast hyperphagia is a dopamine dependent phenomenon, 2) the increased feeding observed with a preferred highly palatable food is actually an opioid dependent process and the initiation of this process is driven by dopamine activity, and is thus susceptible to dopamine manipulations. To consider this phenomenon as purely dopamine dependent is inline with Schultz' (Schultz and Dickinson, 2000) model, thus, this effect is specific to the positive contrast group only because this group is the only one in which increased dopamine accompanies the presentation of a novel more palatable food source, the 7-OH-DPAT should have little effect in the no contrast and negative contrast conditions because DA neurons in the midbrain are relatively inactive and thus DA signaling is difficult to disrupt. Additionally, over the last few years it has been suggested that dopamine efflux in the striatum does not actually encode reward, but works together with alternative systems to regulate rewarding behaviors (Salamone and Correa, 2002; Salamone et al., 2005). In keeping within this hypothesis, dopamine signaling is important for responding to conditioned stimuli associated with food reward, but is not necessary for consumption of the food itself. Thus, it is possible that activation of the D3 receptor opposes normal dopamine signaling, and this mimics attenuated responses towards food conditioned stimuli observed with D1/D2 antagonism (Salamone et al., 2005). Additionally, it is possible that inhibition of the D3 receptor in the perifornical hypothalamus (PFH) mimics the anorectic effects of D1/D2 receptor activation at this level of the brain (Leibowitz et al., 1986). In this way, attenuation of signaling at the D3 receptor, which normally opposes D1/D2 function, could actually tip the balance in favor of occupancy and activation of the D1/D2 receptor subtype which lead to suppression of food intake. Nevertheless, the present approach of using systemic injections makes it difficult to do more than speculate as to which brain region, nucleus accumbens/striatum or hypothalamus is responsible for this dopamine dependent effect on positive contrast. Additionally, neither in the present study nor in the previous study by Roitman and colleagues was D1 receptor antagonists administered to animals undergoing positive or

negative contrast (Roitman et al., 2001). Although D1 activity was beyond the scope of the current study, the lack of this experimental group is a significant caveat in these types of studies and thus it remains unclear if D1 signaling is necessary for this phenomenon.

However, it is possible that the hyperphagia seen in the positive contrast situation is primarily driven by opioid signaling and thus signaling through the hypothesized inhibitory D3 receptor subtype alters the hedonic properties of the high fat diet. Indeed, several studies have reported a role for opioids in food reward (Apfelbaum and Mandenoff, 1981; Berridge, 1996; Cooper and Kirkham, 1993; Yeomans et al., 1997; Levine and Billington, 2004). These reports and others have lead to the suggestion that opioids regulate the hedonic properties of food reward while dopamine mediates the motivation to consume rewarding foods (Kelley et al., 2005). In this study the animals were presented with the high fat food and thus did not have to work to obtain the food. Therefore, it seems unlikely that a disruption in the motor effort needed to obtain the high fat diet could lead to diminished consumption in the positive contrast protocol. However, given that the high fat food is a novel food source in the positive contrast situation, and that dopamine efflux is known to occur with novelty (Bassareo and Di Chiara, 1999; Bassareo et al., 2002) it is possible that D3R activity opposes this effect. Perhaps a more plausible explanation for this result is a combination of the two: the hyperphagia observed in the positive contrast situation is primarily a novelty driven phenomenon, and given the decreased ability to respond to conditioned stimuli when dopamine signaling is disrupted, the effects of D3 receptor agonists are additive towards disrupting this "conditioned novelty" effect.

Taken together, these results suggest that D3-R signaling is involved in positive contrast hyperphagia observed with a novel palatable food source. In particular, these findings indicate that D3-R activity is reduced under normal circumstances when an unanticipated positive dietary contrast is encountered. This is the first report that identifies a potential role for D3 signaling in food reward, and thus expands the hypothetical framework of dopamine signaling during food reward. Nevertheless, future studies are warranted to define the neural substrates responsive to D3 activation, and also to understand better how this receptor subtype may interact with the opioid system to mediate the rewarding properties of food.

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