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The role of the hypothalamic melanocortin system in behavioral appetitive processes

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Abstract

Much evidence suggests that the hypothalamic melanocortin (MC) system plays an important role in the control of food intake. However, investigations of the potential behavioral mechanisms have been limited to measures of aversion. The purpose of the present experiment was to assess whether other behavioral consequences of administration of MC peptides were similar to those produced by 0- or 24-h food deprivation, respectively. Rats were first trained while food deprived that a tone predicted the delivery of peanut oil. They then received exposure to oil under food deprivation, satiation, intra-third-cerebroventricular (i3vt) infusion of MTII (a potent MC agonist) or SHU-9119 (a potent MC antagonist). All rats were then tested during extinction for levels of responding to the tone under food satiation. Previous results demonstrated that sated exposure reduces subsequent test responding to the tone. During the present extinction test, rats that received sated exposure exhibited reduced responding to the tone, relative to rats that received deprived exposure. Unlike satiation, rats that received exposure after MTII exhibited continued high levels of responding to the tone. Further, rats that received SHU-9119 exhibited a small reduction in responding. These data suggest that MTII and SHU-9119 do not influence intake via the same mechanisms as hunger and food satiation, respectively.

Keywords: Melanocortins; Food intake; Energy regulation; Behavior; Behavioral processes; Postingestive

1. Introduction

Neurons that express melanocortin (MC) peptides and their receptors are located in the arcuate nucleus (ARC) of the hypothalamus and project to other hypothalamic structures known to play a role energy regulation, including the paraventricular nucleus (PVN) and the lateral hypothalamus (Grill et al., 1998). Interestingly, the MCs comprise one of the few CNS systems with both an endogenous agonist and antagonist. Arcuate neurons synthesize and release alpha-melanocyte-stimulating hormone (α-MSH), the endogenous MC3/4 receptor agonist associated with decreased food intake (Adan et al., 1994; Tsujii and Bray, 1989). A separate group of ARC neurons synthesize and release agouti-related peptide (AgRP), the endogenous MC3/4 receptor antagonist associated with increased food intake (Fong et al., 1997; Ollmann et al., 1997). A growing body of evidence links the activity of these critical populations of neurons to the control of food intake and body weight.

The first type of evidence that supports the hypothesis that the central MC system plays an important role in food intake and energy balance is the finding that intra-third-cerebroventricular (i3vt) infusions of the peptides elicit potent changes in food intake. Infusion of MC agonists, such as the endogenous α-MSH (Tsujii and Bray, 1989) or the synthetic MTII, causes significant reductions in food intake and body weight (Fan et al., 1997; Thiele et al., 1998). Additionally, intracerebroventricular infusion of MC antagonists, such as the endogenous AgRP or the synthetic SHU-9119, cause profound and longlasting increases in food intake (Fan et al., 1997; Hagan et al., 2000).

Second, ARC neurons that express mRNA for the MC precursor molecule, proopiomelanocortin (POMC) also contain receptors for the adiposity hormone, leptin (Cheung et al., 1997). Indeed, changes in body weight or energy

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balance lead to significant changes in the hypothalamic expression of the MC precursor. Fasting leads to a significant decrease in the expression of POMC mRNA in the ARC (Mizuno et al., 1998; Schwartz et al., 1997). Further, the central MC system contributes to the anorexic effect of leptin. For example, administration of MC antagonists, in doses that do not affect food intake alone, can block the anorexia normally seen after an infusion of leptin (Seeley et al., 1997). Collectively, these data suggest that the central MC system is regulated by energy balance and mediates the effects of leptin to alter food intake.

Finally, mice with targeted disruption of the MC system are profoundly obese. For example, targeted disruption of the MC4R leads to profound increases in food intake and body weight (Huszar et al., 1997). Supporting the suggestion that MC4R is the important receptor involved in the MC's regulation of energy balance, these mice are insensitive to the anorexic effects of i3vt infusion of the MC3/4R agonist MTII (Marsh et al., 1999). Taken together, these results strongly implicate the central MC system in the control of food intake.

The goal of the present research is to assess whether MC agonists and antagonists induce states that function in ways similar to the states associated with food repletion and food depletion. To pursue our hypothesis, we utilized a paradigm that allowed us to determine if infusing MC peptides into the brain had effects on conditioned appetitive behavior similar to those produced by periods of ad libitum feeding (i.e., "satiety") or by periods of 24-h food deprivation (i.e., "hunger"). Recent research has demonstrated that animals must learn about the consequences of eating food when satiated before satiation can exert a suppressive effect on appetitive behavior (Benoit et al., 2000; Davidson and Benoit, 1998; Dickinson and Balleine, 1994). In these types of studies, rats are trained when food deprived to approach a food cup or make another response to receive food. In a subsequent exposure phase, the rats are removed from the original training situation and given the opportunity to eat more of the same food that was used in training. The critical manipulation in these studies is whether the rats are food sated or food deprived during this exposure phase. In a final test phase, the rats are returned to the original training situation where the tendency to perform appetitive responses is assessed during extinction (i.e., no food is delivered) when all rats are food sated.

Appetitive performance during satiated testing depends on whether or not the rats had been food sated or food deprived when they ate the food during the prior exposure phase (Balleine, 1992; Benoit et al., 2000). Specifically, satiation exerts little or no suppressive effect on appetitive behavior during testing unless the rats had consumed the training food when sated during the prior exposure phase. These data are consistent with the hypothesis that before satiation can exert a suppressive effect on intake rats must learn that the consequences of eating food when sated are different from the consequences when hungry.

The present study used this research design to investigate whether prior experience with eating following i3vt infusion of the MC agonist MTII in hungry rats would have the same effects on test phase appetitive responding as prior experience with eating when sated (i.e., when under 0-h food deprivation). This study also examined whether or not i3vt infusion of the MC antagonist SHU-9119 in food-sated rats would affect test phase responding in the same manner as prior experience with eating when hungry (i.e., when deprived of food for 24 h).

2. Methods

Table 1 details the experimental design. Briefly, we first trained food-deprived rats that an auditory cue signaled the delivery of a small amount of peanut oil. We then gave different groups of rats exposure in a different apparatus to peanut oil. Group Satiated (SAT) was fed adlib in the homecage for 24 h prior to receiving the opportunity to eat the peanut oil whereas Group Deprived (DEP) was food deprived for 24 h prior to this type of exposure. Groups MTII and SHU were of most interest. Group MTII was food deprived for 24 h prior to exposure sessions and was also given an infusion of MTII that was expected to produce a strong suppression of intake. Group SHU was fed adlib for 24 h prior to the exposure phase, but prior to that phase was also infused with a dose of SHU-9119 that was expected to elicit robust food intake. All groups were then food sated and returned to the original training apparatus where the capacity of the auditory cue to evoke appetitive conditioned responses was tested in extinction.

Based on previous findings, appetitive responding during satiated testing should be more suppressed for Group SAT

Table 1
Experimental design

	Training				
Group	One magazine	Eight sessions	i3vt	Oil exposure (two sessions)	Test (one session)
SAT	Noyes Pellets	T → Oil	surgery	0-h deprived	T-
DEP	Noyes Pellets	T → Oil	surgery	24-h deprived	T-
MTII	Noyes Pellets	T → Oil	surgery	i3vt MTII	T-
SHU	Noyes Pellets	T → Oil	surgery	i3vt SHU-9119	T-

T = 10-s tone. Oil = 0.3 ml peanut oil. During habituation session, neither tone nor oil was delivered. MTII was administered when rats were food deprived for 24-h. SHU-9119 was administered when rats were food deprived for 0-h. Exposure session length = 15 min. All other session lengths = 30 min.

than for Group DEP (Benoit et al., 2000). In addition, if MTII influences behavior via the same mechanisms as satiation, then test phase responding for Group MTII should also be more suppressed than for Group DEP. Furthermore, if infusion of SHU-9119 interferes with satiation, then test phase appetitive responding should be less suppressed for rats in Group SHU than for rats in Group SAT. Single doses of the synthetic peptides were chosen, which were expected to produce potent changes in food intake, and thus interoceptive sensory consequences.

2.1. Subjects

Subjects were 26 naive male Sprague–Dawley albino rats (Harlan Sprague–Dawley, Indianapolis, IN). The rats were approximately 90 days old and weighed 250–300 g on arrival at the laboratory. Subjects were housed individually in stainless-steel cages and maintained on a 12-h light/dark cycle with lights on at 7:00 AM. Water was available ad lib in the homecage (except where noted). Food availability (Purina Rat Chow 5001) was as described below. Four rats died between the end of conditioning and the beginning of the extinction test. All data from these animals were discarded.

2.2. Apparatus

All conditioning and testing procedures were conducted in four identical conditioning chambers, constructed of aluminum end walls and clear Plexiglas sides, measuring $21.6 \times 21.6 \times 27.9$ cm. A grid of 0.48 cm in diameter stainless-steel bars, spaced 1.9 cm apart, served as the floor of each chamber. A food cup was located on one end wall of each chamber. The tone CS was produced by a Radio Shack Piezo Alerting Buzzer (Catalog No. 273-068) located outside each chamber by the end wall with the food cup. The room in which the chambers were located was dark during conditioning sessions. All experimental events were controlled by computers located in an adjoining room.

Changes in appetitive behavior (behavior directed toward the food cup) were monitored by a computer-controlled infrared monitoring system. Sixteen electronic beams (an ENV 256C infrared Photobeam Controller and D16-712 Photobeam Input, Med Associates, East Fairfield, VT) lined each chamber from side wall to side wall. Interruptions of these beams were monitored, and data were analyzed by a software developed in the laboratory for the measurement of appetitive behavior. The amount of appetitive behavior was defined as the percentage of time the beam directly in front of the food cup was broken. The computers and relay panels controlling the beams were located in an adjoining room.

Exposures were conducted in four identical opaque plastic tubs with wire tops measuring $45.7 \times 36.8 \times 21.6$ cm. The room in which the tubs were located was light during exposure sessions.

2.3. Procedure

2.3.1. Surgery

Animals were anesthetized with 3 ml/kg equithesin and implanted with a 21-ga stainless-steel guide cannulas aimed at the third ventricle (2.2 mm posterior to bregma, 7.5 mm ventral to dura). The guide cannula was cemented to jewelers screws attached to the skull and an obturator was inserted into the cannula. All animals received 0.15 ml Baytril (enrofloxacin, 2.27%) prophylactically. Animals were allowed to recover for 18 days, during which they were handled and body weight measurements were taken daily.

2.3.2. Pretraining procedures

Following recovery from surgery, subjects were gradually reduced to 85% of their free-feeding body weight over 7 days. This was accomplished by restricting daily chow intake by 40–50%. They then received daily chow sufficient to maintain at this weight for the following two weeks. Subjects were weighed immediately before being placed in the conditioning apparatus and received their daily ration of chow approximately 15 min after being returned to the homecage. Experimental sessions began at approximately 3:00 PM.

2.3.3. Magazine training

All subjects received one 10-min session in which two 45-mg Noyes (Lancaster, NH) plain pellets were delivered per minute. No behavioral measures were taken during this session.

2.3.4. Pavlovian training

All subjects received one 30-min conditioning session per day for 8 consecutive days. Each session contained 15 conditioning trials lasting 20 s each. For the first 10 s (pre-CS period) of each trial, no CS was presented. However, appetitive behavior was recorded by the infrared monitoring system. In the second 10 s (CS period) of each trial, the tone was presented and appetitive behavior was again measured. All trials terminated with the delivery of one 0.15 ml drop of Planters 100% peanut oil. The mean intertrial interval (ITI) was 120 s. Subjects were run in squads of four. Following the eighth session, all subjects were returned to ad lib feeding.

2.3.5. Verification of cannula patency

Animals were divided randomly into two groups. On Posttraining Days 2 and 3, water bottles were removed from all animals cages and weighed. Four hours after removal, on Day 2, Group 1 animals ($n = 18$) were given intracerebroventricular injections of 10 ng angiotensin-II (AII). Immediately following injection, water bottles were replaced, then removed and reweighed after 1 h. Animals in Group 2 ($n = 8$) received no injection, their water bottles were replaced after the 4-h deprivation, then weighed again

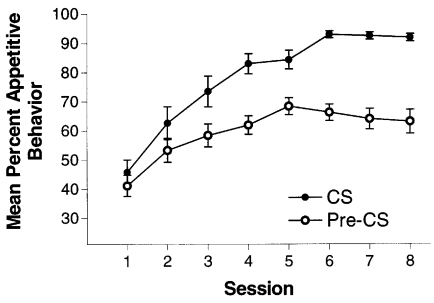

Fig. 1. Mean percent appetitive behavior in blocks of 15 trials. Open circles represent data for pre-CS periods while filled circles represent data for CS periods. By the end of acquisition, rats exhibited more appetitive behavior during the CS than pre-CS period.

after 1 h. On Day 3, Group 2 animals received AII injections and eight randomly selected animals from Group 1 were treated as controls. Animals that drank more following AII administration than the mean amount consumed by the control group on that same day were considered to have patent cannulas.

2.3.6. US exposure

Twenty-four animals met the cannula patency criterion and were divided equally into four groups matched on body weight and mean percent appetitive behavior during the CS periods of the final conditioning session. The two animals that did not meet this criterion were assigned to the DEP and SAT conditions. Twenty-four hours prior to exposure sessions food was removed from the cages of animals in groups DEP and MTII. On Posttraining Day 7, animals were given intracerebroventricular injections of either 1.0 nmol MTII ($n=6$), 0.75 nmol SHU-9119 ($n=6$), or physiological saline (DEP, $n=7$; SAT, $n=7$). MTII and SHU-9119 (Phoenix Pharmaceuticals, Mountain View, CA) doses were prepared using sterile physiological saline and kept frozen until use. A 2-μl volume was loaded into a Hamilton microsyringe and injected over a 1-min period. The injector was withdrawn 1 min later. One hour after injection, animals were placed in the exposure apparatus. A small glass dish containing 4.5 ml Planters 100% peanut oil was placed in one corner of the tub. After 15 min, animals were removed from the apparatus and the amount of peanut oil consumed was measured. This procedure was repeated when homecage food intake no longer differed between groups (Posttraining Day 11).

2.3.7. Food intake measurement

Immediately following exposure sessions, all animals were given a preweighed amount of chow in the homecage and paper was placed beneath the cage to collect spillage. Remaining chow and spillage were weighed at 1, 25, and 49 h after return to the homecage.

2.3.8. Extinction testing

On Posttraining Day 15, all animals received one 30-min test session with fifteen 10-s presentations of the tone CS. No US was presented during this session. Appetitive behavior was measured during pre-CS and CS periods.

3. Results

3.1. Training

Fig. 1 depicts mean percent appetitive behavior during pre-CS and CS periods for all animals across eight 15-trial training sessions. Appetitive behavior increased across sessions and, by the end of training, was higher during CS periods than pre-CS periods. A $2 \times 8 \times 4$ mixed ANOVA was conducted on percent appetitive behavior using Period (pre-CS vs. CS) and Session (1–8) as within-subjects variables and Group (SAT vs. DEP vs. MTII vs. SHU) as a dummy between-subjects variable. This analysis yielded significant main effects of Period [$F(1,17)=62.26$, $P<.01$] and of Session [$F(7,119)=19.66$, $P<.01$], confirming that responding was higher during CS than pre-CS periods and that responding increased over sessions. In addition, there was a significant Period × Session interaction [$F(7,119)=14.89$, $P<.01$], indicating that the difference between CS and pre-CS responding was greater at the end of training. The dummy variable of Group did not yield a reliable main effect, nor did it interact with any other variables, indicating that acquisition of responding to the tone was similar among groups that would be treated differently in subsequent phases of the experiment (largest $F(21,119)=1.05$, $P>.1$].

3.2. Intake tests

3.2.1. Exposure sessions

Mean peanut oil consumption data for each group is depicted in Fig. 2. It appears that animals in Group MTII had a somewhat reduced intake and animals in Group DEP had a somewhat elevated intake relative to other groups. A one-way ANOVA confirmed this finding, yielding a significant between-groups difference [$F(3,18)=3.57$, $P<.05$]. A post hoc Newman–Keuls test indicated a reliable difference between the DEP and MTII groups ($P<.05$). This indicates that animals given MTII consumed

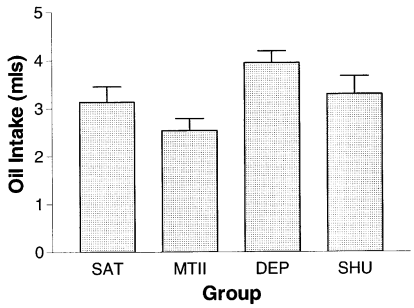

Fig. 2. Mean amount (ml) peanut oil consumed during the exposure sessions.

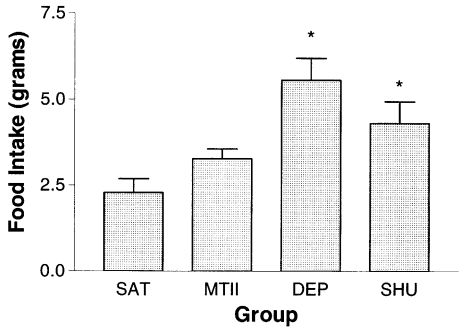

Fig. 3. Mean amount (g) homecage food intake during first 1-h tests, immediately following exposure sessions. Groups DEP and SHU had more food intake than Groups SAT and MTII.

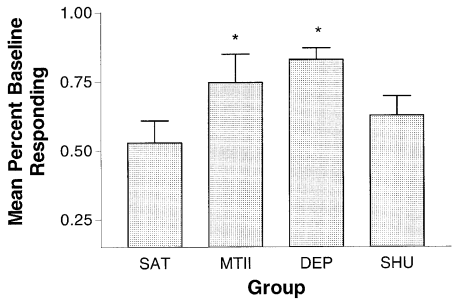

Fig. 5. Mean percent baseline responding (final training session/test session average). Responding was higher in Groups MTII and DEP than in Group SAT.

significantly less peanut oil than animals equally food deprived, but given a saline injection, while animals fed ad lib consumed similar amounts of peanut oil regardless of whether they were treated with SHU or saline. Furthermore, deprivation state alone did not affect intake in the exposure session, as there was no reliable difference between animals in the SAT and DEP groups.

3.2.2. Homecage intake

Fig. 3 depicts that homecage food intake during the 1-h period following the exposure session was lower for animals in the SAT and MTII groups and higher for animals in the DEP and SHU groups. A significant main effect of Group [$F(3,18) = 6.44$, $P < .01$] was found using a one-way ANOVA. A post hoc Newman–Keuls test confirmed that MTII-treated deprived animals consumed reliably less food than their saline-treated deprived controls ($P < .05$), while SHU-treated satiated animals consumed reliably more food than their saline-treated satiated controls animals ($P < .05$). In addition, for animals given saline injections, deprived animals ate more than satiated animals ($P < .01$).

Fig. 4 depicts mean food intake for the next 24-h period (beginning approximately 3-h postinjection). It appears that intake in MTII-treated animals remained low and intake in SHU-treated animals remained elevated relative to animals in both saline-treated conditions. Again, a one-way ANOVA confirmed this observation, yielding a significant main effect of Group [$F(3,18) = 18.84$]. Post hoc Newman–Keuls analysis of this effect indicated that, in fact, animals treated with MTII did reliably decrease intake relative to animals in all other groups, while SHU-treated animals increased intake relative to all other groups ($P < .01$). The effect of the MTII and SHU treatments on food intake appear to have faded by the following 24-h period (beginning approximately 27 h postinjection), as depicted in Fig. 4. A one-way ANOVA revealed no reliable differences in food intake between groups at the end of this period [largest $F(3,18) = 0.661$, $P > .5$].

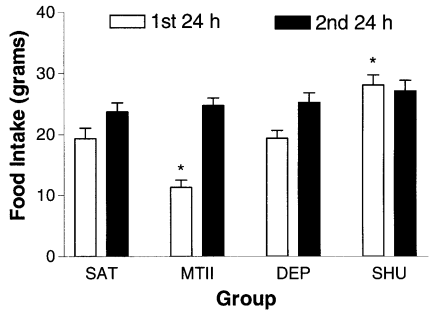

Fig. 4. Mean amount (g) food intake during 1st 24-h following exposure sessions (open bars) and 2nd 24-h period following exposure sessions. During the first 24 h following infusion and exposure, Group MTII had less food intake than all other groups. Group SHU had greater food intake than all other groups.

3.3. Extinction test

Fig. 5 displays the mean percent of baseline responding during the first six extinction trials, where baseline is defined as responding during the last six trials of training. It appears that the SAT group reduced responding to the tone during the test relative to Groups DEP, MTII, and SHU. Separate between-groups ANOVAs confirmed this observation, yielding significant differences between the SAT and DEP groups [$F(1,9) = 6.36$, $P < .05$], and the SAT and MTII groups [$F(1,9) = 5.20$, $P < .05$], but no significant difference between Groups DEP and SHU.

4. Discussion

Consistent with previous reports, i3vt administration of synthetic nonselective MC peptides caused significant changes in food intake. In the present experiment, administration of 1.0 nmol MTII caused a significant reduction in both 1- and 24-h food intake measures, as well as in peanut oil intake during exposure sessions. Conversely, administration of 0.75 nmol SHU-9119 led to an increase in each of the three intake measures in the present experiment. Indeed, for intake measures, administration of MTII and SHU-9119 was followed by patterns of data that closely mimicked those seen after satiation and deprivation, respectively. These data are consistent with the hypothesis that the

mechanisms underlying the effects of MTII and SHU-9119 on intake are similar to those underlying food satiation and deprivation, respectively.

In contrast, the effects of experience with eating food following infusion of MTII on subsequent test phase appetitive responding, differed sharply from those of experience with eating food when sated. Replicating previous results (Benoit et al., 2000; Davidson and Benoit, 1998), this experiment found that, relative to exposure under deprivation, exposure to peanut oil under satiation led to decreased appetitive responding during satiated testing. However, infusion of MTII in hungry rats during the exposure phase, failed to decrease later test phase appetitive responding relative to rats that were food deprived and infused with saline during the exposure phase. That is, the effects of exposure after i3vt MTII were more like those of food deprivation than those of food satiation. These results differ from previous work with other anorectic peptides in similar behavioral paradigms. For example, cholecystokinin (CCK) has been found to support incentive learning similar to that supported by food satiation (Balleine and Dickinson, 1994; Balleine et al., 1995).

With respect to exposure under SHU-9119, the results from the extinction test were equivocal, with the level of responding for Group SHU between that of Groups SAT and DEP. However, the absolute levels of responding in Group SHU were closer to that of Group SAT than Group DEP. One possible explanation for the failure to find a statistically significant difference between Group SHU and Group SAT is that the 0.75-nmol dose of SHU-9119 employed in this experiment may have been too low to interfere strongly with the effects of food satiation during the exposure phase. Thus, animals that received exposure after i3vt SHU-9119 would have incomplete blockade of the effects of food satiation, thereby preventing differences between Groups SHU and SAT from emerging during the test phase. This seems unlikely, however, as the dose of SHU-9119 was chosen for its ability to augment food intake similar to that seen after 24-h food deprivation. Indeed, both single doses of the synthetic peptides were chosen because they were expected to produce maximal changes in food intake in approximately 24 h. Further, our previous work suggests that other orexigenic peptides may elicit interoceptive sensory signals unlike either food deprivation or repletion. Neuropeptide-Y was found to elicit potent, discriminative sensory stimuli that generalized to neither 0- nor 24-h food deprivation (Seeley et al., 1995).

The present experiment is the first to examine the behavioral mechanisms through which the central MC system might alter food intake. It is highly likely that the phenomenon of food satiation involves a number of distinct processes or mechanisms. For example, relative to food deprivation, satiation appears to induce distinct interoceptive stimuli (Davidson, 1993). Furthermore, satiation may suppress eating by altering either, or both, the orosensory or postingestive consequences of food intake. The present findings indicate that although MTII strongly suppressed food intake, MTII apparently did not mimic completely all of the effects of food satiation.

The underlying hypothesis for the present experiment is that for satiation to suppress appetitive behavior, animals must learn by experience that the consequences of eating food are different when they are satiated compared to when hungry (Davidson and Benoit, 1998; Dickinson and Balleine, 1994). The present result indicates that rats infused with MTII prior to eating did not acquire this knowledge, even though their intake was suppressed at the time of exposure and for many hours thereafter. It may be that MTII reduced intake by some nonspecific means such as producing malaise or lethargy. These effects could not have influenced appetitive behavior in the test phase, because testing took place long after the effects of MTII on intake had disappeared. However, casual observation of the rats given MTII in this study provided no evidence of decreased nonspecific behavioral activity. On the other hand, previous research has indicated that eating food following MTII infusion results in a mild taste aversion (Thiele et al., 1998). However, other studies suggest that conditioning a taste aversion to either a peanut oil or a sucrose US, also reduces subsequent responding to an auditory CS that is associated with that particular US (Davidson et al., 1997). In the present experiment, rats that ate peanut oil following infusion of MTII, did not exhibit less test phase responding than saline controls to a tone that had been paired with the delivery of peanut oil during original training.

Another possibility is that MTII reduces food intake by altering the orosensory or postingestive consequences of intake without inducing interoceptive signals like those produced by food satiation. If this were the case, the rats in Group MTII would reduce intake during the exposure phase, but would not have the opportunity to learn that interoceptive satiety cues signal changes in the consequences of intake. In the absence of this learning, the introduction of satiety cues during the test phase would not predict a change in the consequences of intake and therefore would not reduce the performance of appetitive conditioned responses during subsequent testing when food was not available.

It is also possible that MTII infusions and food satiation give rise to similar interoceptive satiety cues, but differ in terms of their effects on the orosensory properties of food. For example, satiation may suppress intake by altering the intragastric consequences of eating without changing the orosensory properties of food. If MTII alters the orosensory properties of food, the rats might not recognize that the food presented in the exposure phase was the same as the food that was associated with the tone during training. Therefore, during the exposure phase even though MTII could have produced both interoceptive sensory signals and postingestive consequences like those produced by satiety, the food given during the exposure phase might not have been perceived as the same as the same food that was

paired with the tone during training. Under these circumstances, the animals would not have had the opportunity to learn that consequences of eating the food associated with tone were reduced in value or otherwise altered (Davidson et al., 2000) by satiation. In the absence of this learning, test phase appetitive responding to the tone that signaled the food would remain high. It should be possible to design experiments that will enable us to evaluate these different alternatives.

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