Psychopharmacology (2002) 161:47–53
DOI 10.1007/s00213-002-1022-5

ORIGINAL INVESTIGATION

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Behavioral processes underlying the intake suppressive effects of melanocortin 3/4 receptor activation in the rat

Received: 14 June 2001 / Accepted: 10 January 2002 / Published online: 1 March 2002
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Abstract *Rationale:* Central application of MTII, a melanocortin 3/4 receptor agonist, reduces food intake. The behavioral mechanisms underlying the anorexia, however, have not been evaluated. *Objectives:* We examined the ingestive behavioral effects of MTII at the microstructural level using two complementary approaches. *Methods*: Rats were given daily 2-h sessions during which they drank 12.5% glucose solution; the time of occurrence of each lick event was recorded. We compared rats' glucose intake 30 min after the fourth ICV injection of 0.1, 0.33, and 1.0 nmol MTII or vehicle. The licking patterns were examined to discern effects on parameters related to taste processes and others related to post-ingestive inhibitory feedback. A second experiment directly analyzed the effect of MTII on motor performance by examining whether drug treated rats would, like controls, adjust licking output to maintain meal size when lick volume was shifted from 8 to 4 µl. *Results*: Meal size was reduced by MTII in a dose-dependent manner (20–50%) in both experiments. Rats treated with MTII compensated for decreased lick volume by substantially increasing the number of licks emitted. Licking parameters associated with taste evaluation were not significantly affected by MTII, whereas parameters associated with post-ingestive inhibition varied as a function of treatment. *Conclusions*: Results suggest that MTII reduces intake by amplifying post-ingestive feedback inhibition. That MTII-treated rats increase the number of licks emitted in response to the lick volume reduction discounts the suggestion that intake inhibition is secondary to disruption of motor performance.

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Keywords Melanocortin · Feeding behavior · Licking microstructure

Introduction

Substantial effects of central melanocortin 3/4 receptor (MC3/4-R) ligand administration on daily food intake have been reported (Grill et al. 1998; Murphy et al. 2000). A recent study indicates that these food intake effects are largely due to changes in the size of meals throughout the day (Moran et al. 2001). The adjustments in the pattern of ongoing ingestive behavior that underlie the meal size effects, however, have yet to be described. Here, we address the intake suppressive effects of fourth ICV administration of the MC3/4-R agonist, MTII, with particular attention to the pattern of licking during a glucose meal initiated 30 min after injection. Such ingestive-microstructural analyses often allow inferences about drug action on two processes that co-determine the size of the meal – the rat's evaluation of the taste properties of the stimulus and of the accumulating post-ingestive load (Davis and Levine 1977; Davis and Smith 1992). The approach may also help determine whether the effects of the treatment on intake reflect a disruption of motor performance as opposed to a primary action on physiological mechanisms of intake regulation.

In the first experiment, we demonstrate that MTII causes a dose-related suppression of meal size, and evaluate treatment effects on licking parameters associated with taste and post-ingestive influences on ingestion. The animal's evaluation of the taste properties of the stimulus may be reflected in the licking rate or pattern during the early part of the meal (e.g. 30–60 s) when the impact of post-ingestive feedback is minimal. For example, initial lick rate increases with sucrose concentration, presumably in keeping with the direct relationship between concentration and palatability (Davis and Levine 1977; Spector and Smith 1984). The change in the pattern of licking as meals progress is associated with intake-inhibitory feedback from the increasing post-inges-

tive load. Thus, the slope of decline in the minute-by-minute lick rate function should increase with the nutritive density of the test fluid (Davis and Levine 1977; Davis and Smith 1988). Lick microstructure analysis is a useful reductionistic tool for the evaluation of drug effects (e.g. Schneider et al. 1990; Asin et al. 1992), providing that there are no serious concerns about primary motor performance effects of the agents in question.

The possibility that motor disturbances underlie intake suppression is an important consideration for most anorexic treatments. Some treatments induce obvious effects such as ataxia [as observed for several minutes after MTII injection (unpublished observation)], or stereotopies that can affect the likelihood that a meal will be initiated. Performance deficits may be more subtle, but may affect meals in ways that can be difficult to discern. Some treatments (e.g. fluoxetine; CART) affect the frequency of licking within bursts (Asin et al. 1992; Aja et al. 2001), indicating an action on the central pattern generator (Weisenfeld et al. 1977) believed to underlie the licking rhythm. There are aspects of motor performance relevant to the interpretation of meal size reductions, however, that cannot be addressed with standard intake testing paradigms. For example, a treatment may induce fatigue, which could limit the number of licks the rat can emit or the amount of time it can maintain an ingestive posture at the drinking spout. Here, we apply a specialized experimental protocol to explore the possibility that the intake effect of MTII is secondary to a disruption of performance.

In this study, rats ingested glucose solution during short-term tests across which the rat acquires either 8 or 4 µl with each spout-lick. The behavioral adaptations to the lick volume constraint in the untreated rat (Kaplan et al. 2001) can be taken as a baseline against which to evaluate the effects of MTII treatment. Normally, rats accurately compensate for the halving of lick volume by doubling the number of licks emitted during the meal; meal size thereby remains stable across conditions. This dramatic increase in lick count is accomplished by a substantial increase in the duration of licking bursts and a reduction in the inter-burst interval, with no salient change in the number of bursts emitted in the meal or in the within-burst lick frequency. Independently of the drug's effect on meal size, we can evaluate whether the response to lick volume manipulation resembles that observed under vehicle conditions. If MTII limits the number of licks that an animal can emit, then the rat will fail to defend meal size when lick volume is reduced across tests from 8 to 4 µl. If, on the other hand, the pattern of behavioral adaptation in drug-treated rats resembles that under control conditions at the whole-meal level (i.e. lick doubling; intake defense), and also with respect to the internal organization of the meal (i.e. increased burst duration, decreased inter-burst-intervals), then a compelling case can be made against the suggestion that the intake suppressive effects of MTII arise from a performance deficit. Such results would be consistent with a role for MC3/4-R in the normal physiology of intake control.

Materials and methods

Subjects

Naive male Sprague-Dawley rats (Charles River) were housed in hanging stainless steel cages in a vivarium under a 12:12-h light dark cycle. Pelleted food (Purina) and water were available ad libitum. The experimental protocols used conform to institutional standards of animal care and the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

Surgery

Each rat received a guide cannula (Plastics One, 22-G), implanted 2.0 mm above the fourth ventricle, under ketamine (90 mg/kg) and xylazine (15 mg/kg IM) anesthesia. Fourth ventricle cannula placement was 2.5 mm anterior to occipital suture, on midline, and 4.5 mm below dura. The cannula was cemented to four jeweler's screws attached to the skull, and closed with an obturator. Rats recovered for at least 5 days while daily food intake and body weight was recorded. ICV cannula placement was evaluated after recovery from surgery by measuring a sympathetically mediated increase in plasma glucose after ICV injection of 210 µg of 5-thio-D-glucose in 3 µl of saline (Ritter et al. 1981). Only rats that showed at least a doubling of plasma glucose level in response to this treatment were used in experiments.

Although the fourth ventricular site of administration was arbitrary, there may be reason to suggest that the results obtained here would be generalizable to effects from other injection sites. We and others have shown that effects of MTII on 2-, 4- and 24-h cumulative pellet intake are comparable after fourth ventricle and lateral ICV administration, and similarly comparable after delivery to the paraventricular nucleus of the hypothalamus (Giraudo et al. 1998) and the dorsal vagal complex (Williams et al. 2000). The same may be true of MTII effects on lick microstructure, but it remains possible that there are differences depending on site of administration.

Habituation training

Rats were trained to drink 12.5% glucose from bottles hung on home cages for 120 min daily for approximately 10 days. They then entered a habituation phase in which they ingested glucose in the test cages (see Apparatus) during daily 120-min sessions. When session intake stabilized, rats received one to three test sessions 30 min prior to which saline (3 µl) was delivered ICV in the fourth ventricle to adapt them to the injection procedure.

Apparatus

The test chamber consisted of six individually hanging wire mesh cages, with metal spouts mounted on the front of each cage. With each spout lick, a circuit was completed that passed less than 50 µA through the rat. All licks were registered and their times of occurrence stored for off-line analyses. Six reservoirs held fluid at a constant pressure of 4.5 psi. Tygon tubing carried fluid from each reservoir through a filter to a two-way solenoid valve calibrated to open for a specified duration each time a lick was registered. For experiment 1, the valve opened for a period calibrated to allow approximately 6 µl of fluid to flow through a tube to the tip of the metal spout. For experiment 2, the valve was calibrated to open for a duration that would allow either 4 or 8 µl of fluid to flow down to the spout. Glucose intake (ml) was calculated as the weight difference between the reservoir content before and after the session, corrected for the g/ml ratio of the test stimulus.

Drug preparation and delivery

MTII is the modal treatment for the evaluation of the feeding effects of CNS MC-R stimulation (e.g. Grill et al. 1998; Murphy et

al. 2000; Hohmann et al. 2000; Moran et al. 2001). Like the endogenous ligand aMSH, MTII is not selective among MC3/4-Rs (Bednarek et al. 1999), but compared to aMSH, it has considerably higher affinity for these receptors (Schiöth et al. 1997). Both receptors have been implicated in the regulation of energy balance (Marsh et al. 1999; Butler et al. 2000).

MTII (Phoenix Pharmaceuticals, Belmont, Calif., USA) was prepared by dissolving the peptide in saline. Injections were made through 23G stainless steel tubing that extended 2 mm below the guide cannula. A 3-µl volume of drug or vehicle was injected over 3 min using a Hamilton microsyringe. The injector was left in place for another minute before removal. In experiment 1, saline and three doses of MTII (0.1 nmol, 0.33 nmol, or 1 nmol) were given, chosen on the basis of their effectiveness at decreasing solid food intake (Grill et al. 1998). In experiment 2, a 1-nmol dose of MTII was given, with saline as the vehicle. In both experiments, session starting times for each rat were staggered so that injections took place exactly 30 min before testing began.

Experimental procedures

Naive rats in experiment 1 (n=15) and experiment 2 (n=7) were given 120-min glucose intake test sessions daily at the same time during their light cycle. All animals were tested under each injection condition, with conditions run 3 or 4 days apart. Sessions (no injection) were run on each intervening day. Injection conditions in experiment 1 (saline, 0.1 nmol, 0.33 nmol, 1 nmol MTII) were presented in counterbalanced order across rats. In experiment 2, three rats received MTII and vehicle sessions at 4 µl/lick and again at 8 µl/lick, with the remaining subjects receiving their lick volume conditions in reverse order. MTII and vehicle sessions were presented in counterbalanced order across rats. In transition between lick volume conditions, one extra session (no injection) was run at the new lick volume. Home cage food and water intake was measured throughout all experiments by weighing food hoppers and water bottles each day. Body weights were also taken daily.

Statistical analyses

The files containing the lick event-time data were analyzed with a custom program to derive information about the principal meal of the session, which was defined as having ended after 5 min of no licking. (The meal was generally begun shortly after presentation of the spout. A later ingestion bout, usually of small size, when observed, was represented in the "total session intake" parameter, but not analyzed separately.) The licking parameters derived from the program included meal duration, number of bursts [with "burst" defined as a series of at least two licks separated by less than 1 s (Spector et al. 1998)], meal-average burst duration, mean within-burst inter-lick interval, meal-average licking rate (licks/min), and number of licks in the first minute of the meal. For experiment 1, these parameters as well as glucose intake, overnight pellet intake, and body weight change were analyzed separately as a function of MTII dose (0, 0.1, 0.33, 1 nmol) via one-way repeated measures ANOVA. In addition, repeated measures ANOVA was used to examine lick rate over the first 15 min of the meal across the four drug conditions. The 15-min limit for the analysis was chosen because that period includes data for the entire meal in 75% of cases. For experiment 2, the same parameters were analyzed via two-way (lick volume [4.8 µl]×MTII dose [0,1 nmol]) repeated-measures ANOVA. Where appropriate, pair-wise post hoc comparisons were done by Tukey's HSD method.

Results

Experiment 1: MTII licking dose response

Meal size was reduced by MTII in a dose-related manner [F(3,42)=15.36, P<0.001; see Fig. 1]. A similar pattern of results was obtained for total session intake [means: vehicle 18.69 ml; 0.1 nmol MTII 13.49 ml; 0.33 nmol MTII 14.58 ml; 1.0 nmol MTII 12.07 ml; F(3,42)=10.52, P<0.001], and for 24-h solid food intake [means: vehicle 22.41 g; 0.1 nmol MTII 11.01 g; 0.33 nmol MTII 10.21 g; 1.0 nmol MTII 6.95 g; F(3,33)=50.18, P<0.001] and body weight change over the 24 h following injection [means: vehicle –7.35 g; 0.1 nmol MTII –19.92 g; 0.33 nmol MTII –20.97 g; 1.0 nmol MTII –21.72 g; F(3,42)=10.10, P<0.001].

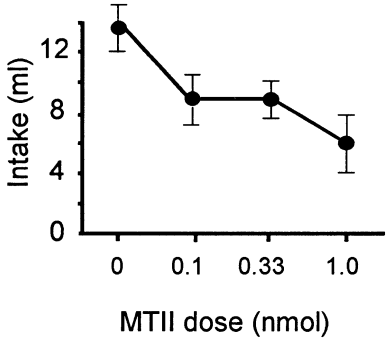

Fig. 1 Glucose intake (mean±SEM) in the primary meal of the session. Intake suppression was greatest at the 1.0-nmol dose (against vehicle baseline), with significant but smaller reductions at the 0.33- and 0.1-nmol doses (P<0.05)

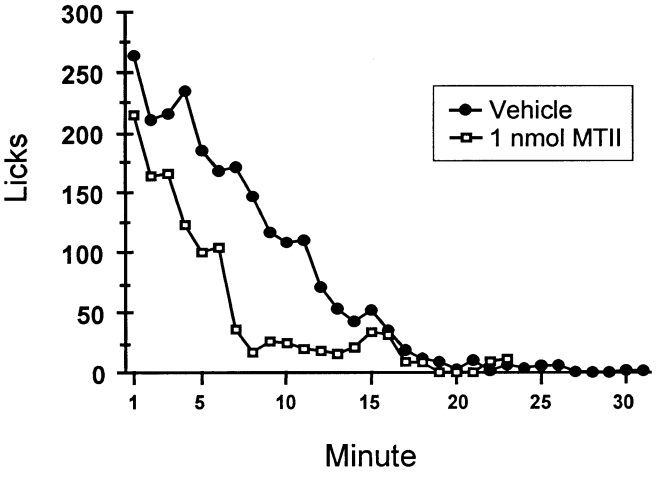

Fig. 2 Group curves showing the average number of licks per minute of the session. Only the curves for vehicle and highest-dose MTII conditions are presented, in order to reduce visual noise. The group curves for the intermediate MTII dose conditions, in general, fell between the vehicle and high-dose curves

Figure 2 shows group curves for average number of licks per minute after vehicle and after the 1-nmol MTII treatment. The trend toward a reduced initial (first-min) lick rate under MTII (Fig. 2; see also Table 1) approached, but did not achieve, statistical significance. The ANOVA examining lick rate over the first 15 min of the meal across the four drug conditions revealed main effects of dose [F(3,42)=11.15, P<0.001] and minute

Table 1 Means (standard errors) and main effects of the drug on licking parameters examined in Experiment 1

	Dose of MTII				
	0.0 nmol	0.1 nmol	0.33 nmol	1.0 nmol	Main effect; $F(3,42)$
Licks in the first minute	263.67 (12.64)[d]	235.93 (15.39)	239.40 (16.92)	214.60 (15.97)[a]	2.41 $P<0.08$
Licks in meal	2154.87 (212.00)[d]	1808.20 (219.43)[d]	1636.93 (185.10)	1147.33 (172.96)[a,b]	7.17 $P<0.001$
Meal duration (s)	813.67 (90.63)	680.51 (105.41)	678.84 (119.15)	504.50 (91.35)	2.09 $P=0.12$
Average ingestion rate (ml/min)	1.16 (0.12)	1.06 (0.12)	1.05 (0.11)	0.95 (0.11)	0.76 $P=0.52$
Inter-lick interval (ms)	167.91 (2.38)[c]	164.13 (3.03)	158.16 (2.63)[a]	165.51 (3.30)	3.95 $P<0.02$
Number of bursts	42.27 (7.71)[d]	29.47 (4.0)	28.20 (4.15)	23.47 (3.20)[a]	4.43 $P<0.01$
Meal-average burst duration (s)	12.93 (1.34)[d]	12.46 (1.44)	11.12 (1.21)	9.17 (0.89)[a]	4.04 $P<0.02$
Average duration of the first three bursts (s)	12.97 (3.15)	9.85 (1.55)	13.37 (4.71)	10.40 (2.04)	0.39 $P=0.76$
Time to completion of first three bursts (s)	44.59 (9.74)	39.90 (5.98)	47.98 (14.33)	38.98 (6.88)	0.21 $P=0.89$
Average burst duration excluding first three (s)	13.22 (1.53)[d]	13.02 (1.54)[d]	11.09 (1.15)	8.79 (0.73)[a,b]	5.68 $P<0.01$

[a] Differs significantly from vehicle
[b] Differs significantly from 0.1 nmol MTII
[c] Differs significantly from 0.33 nmol MTII
[d] Differs significantly from 1.0 nmol MTII

[$F(14,196)=35.22$, $P<0.001$]. A significant two-way interaction was obtained [$F(42,588)=1.62$, $P<0.01$].

The group curves give the impression that MTII substantially reduced meal duration relative to that under the vehicle condition (Table 1). Overall, meal duration was reduced in proportion to the meal size effect, but the effect of MTII dose on this parameter did not achieve statistical significance (Table 1). There was, however, a significant correlation between change in meal duration (drug minus vehicle values for all doses) and change in meal size ($r=0.35$, $P<0.05$). Average ingestion rate over the course of the meal was not affected by MTII (Table 1).

MTII yielded a particular pattern of effects on lick microstructure. The within-burst inter-lick interval was significantly affected by MTII (Table 1). This effect, however, was due to a small difference (<5%) between intervals at the 0.33 nmol dose compared to those under the other conditions, which did not differ from each other. Burst number decreased as a function of MTII dose (Table 1) in proportion to the overall meal size and meal duration effects. The meal-average burst duration was significantly lowered by MTII (Table 1), an effect that was accentuated by excluding the first three bursts of the meal (Table 1). The average duration of the first three bursts of the meal, and the average time it took rats to complete three bursts (mean across conditions=42.86 s), were not affected by MTII treatment (Table 1).

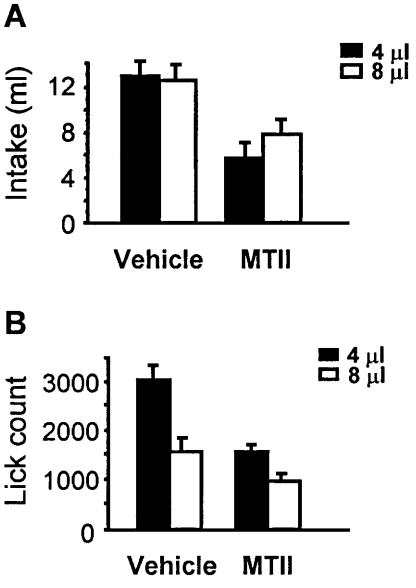

Fig. 3 **A** Glucose intake (mean±SEM) after vehicle or 1 nmol MTII treatment, under the 4-µl and 8-µl lick volume conditions. **B** Lick count (mean±SEM) after vehicle or 1 nmol MTII treatment, under the 4-µl and 8-µl lick volume conditions

Experiment 2: MTII licking with lick volume manipulation

The overall meal size effect of 1.0 nmol MTII [$F(1,7)=14.67$, $P<0.01$; see Fig. 3] was consistent with that obtained from the dose-response analysis. The lick

Table 2 Means (standard errors), main effects of drug and lick volume, and interaction effects for parameters examined in Experiment 1

	4 µl		8 µl		Effects; $F(1,7)$		
	Vehicle	1.0 nmol MTII	Vehicle	1.0 nmol MTII	Drug	Lick volume	Interaction
Licks in the first minute	270.25 (21.72)	254.75 (35.82)	239.50 (15.35)	186.25 (29.20)	2.48 P=0.16	2.28 P=0.17	0.70 P=0.43
Meal duration (s)	692.67 (73.82)	445.95 (84.82)	537.92 (52.98)	441.01 (92.12)	5.86 P<0.05	1.36 P=0.28	0.64 P=0.45
Average ingestion rate (ml/min)	1.12 (0.07)	0.75 (0.13)	1.39 (0.08)	1.25 (0.23)	4.03 P=0.08	4.91 P=0.06	0.70 P=0.43
Inter-lick interval (ms)	151.38 (5.12)	149.34 (6.15)	161.13 (5.54)	172.34 (10.18)	0.36 P=0.57	12.26 P<0.01	0.62 P=0.46
Number of bursts	24.38 (3.09)	16.88 (2.46)	30.38 (3.68)	24.63 (3.96)	8.54 P<0.05	2.78 P=0.14	0.19 P=0.68
Meal-average burst duration (s)	20.65 (2.41)	14.67 (2.86)	9.48 (1.54)	8.50 (0.95)	5.05 P=0.06	13.70 P<0.01	1.89 P=0.21

volume manipulation was without effect on meal size. Importantly, the two-factor (lick volume×drug) interaction term also was not significant. For meal size to have been unaffected when lick volume was reduced from 8 to 4 µl, the number of licks should have increased dramatically. That this was the case is clear from inspection of Fig. 3B, and from the significant effect of lick volume on the number of licks emitted during the meal [$F(1,7)=64.19$, $P<0.001$]. The lack of a significant two-factor interaction indicates that the compensatory increases in lick counts were obtained for both vehicle and MTII conditions.

There was general agreement between this and the previous experiment with respect to MTII action across the set of licking parameters evaluated (see Table 2). The results contrasted as a matter of degree for two parameters. In both experiments there was a prominent trend toward reduction in meal duration after drug treatment, which achieved statistical significance only in the present experiment. The MTII effect on burst duration was robust in experiment 1 (Table 1), whereas here the trend was only marginally significant ($P=0.06$; see Table 2).

The licking parameters that were and were not affected by the lick volume manipulation are listed in Table 2. The profile agreed with that described previously (Kaplan et al. 2001), but the important point here is the lack of any significant interaction for these parameters between lick volume and drug treatment factors.

Discussion

The present study examined the microstructure of licking in order to draw inferences about the behavioral processes through which MTII suppresses intake. In the first experiment, we evaluated the lick pattern early and later in the meal to discern drug effects on taste processes and on inhibitory feedback arising from the accumulation of the post-ingestive load. Our results implicate post-ingestive negative feedback as a target of MTII action. In the second experiment, we applied a specialized paradigm in which lick volume was varied, in order to examine the possibility that the intake suppression observed reflects a performance deficit, as opposed to MTII action on intake regulatory processes. We saw that after MTII injection, rats showed the same pattern of adaptation to the lick volume constraint as they did under vehicle conditions. The similarities were evident in the macrostructure (total intake, number of licks emitted) as well as the microstructure (burst-pause pattern) of the meal, and led us to reject the suggestion that the intake effects of MTII are secondary to disruption of motor performance.

In classical lick pattern analysis, licking behavior at the beginning of the meal, before inhibitory feedback from the load is pronounced, is taken to be informative about taste evaluation processes. The most commonly presented read-out of taste evaluation is lick rate during the initial minute of the meal, which tends to decline in response to treatments that decrease stimulus palatability (Davis and Smith 1988). There was a modest decrease in initial lick rate after the highest dose of MTII, although the trend did not reach statistical significance. It has been suggested that burst duration is a more sensitive measure of taste evaluation. Spector and colleagues (1998) have shown that the sucrose concentration response function for initial lick rate reaches asymptote at low concentrations, while burst duration continues to increase with concentration. We did report a decrease in the average burst duration in meals after MTII treatment which, taken at face value, might indicate a drug effect on taste processing. It can be argued, however, that average burst duration over the entire meal is not an appropriate measure, and that one should examine bursts occurring early in the meal (again, before post-ingestive inhibition becomes pronounced) in order to draw conclusions about effects on taste evaluation. Here we looked

at the average duration of the first three bursts of the meal, and found that this parameter was not affected by MTII treatment. On balance, we find little support for a reliable MTII effect on taste processes.

A case can be made for MTII action on the satiation process; that is, the drug may amplify peripheral signals associated with the ingested load or modify the central assessment of the cumulative intake magnitude. The first such suggestion may be gleaned from group curves for lick rate over the course of the meal, which show that intake declines more rapidly in the MTII conditions. This impression was captured in the significant interaction between drug condition and time from the beginning of the meal. Divergence in lick rate curves early in the meal represents the contribution of all subjects in all conditions. But further divergence of the group curves reflects, in large part, the difference across conditions in the number of rats that have already ended their meals. A main effect of MTII on meal duration, however, was not obtained in experiment 1 (the effect was significant in experiment 2), although there was a significant correlation between MTII effects on meal duration and meal size. This relationship between intake and meal duration effects, itself, can be taken as evidence for drug effects in satiation processes. Further support for the case that MTII amplifies post-ingestive feedback is derived from an examination of the duration of licking bursts. Spector and colleagues (1998) showed that burst duration decreased over progressive segments of sucrose meals, indicating a link between satiation and burst duration. It is interesting in this light that the effect of MTII on meal-average burst duration was seated in later portions of the meal. These points taken together offer support for the hypothesis that MTII influences intake by amplification of the satiation process.

The results of experiment 2 provide no support for the suggestion that intake effects of MTII reflect a disruption of motor performance. Under MTII, rats substantially *increased* the number of licks emitted during the meal when lick volume was reduced from 8 µl to 4 µl. The increase, in fact, was of appreciable magnitude such that there was no significant difference between the size of meals after MTII treatment ingested under the two lick volume conditions. This pattern of results was observed under vehicle conditions, and conformed to that described previously for otherwise untreated rats (Kaplan et al. 2001). Evaluation of burst/pause patterning adjustments in response to the lick volume challenge extends the parallel between the behavioral profiles of MTII and vehicle treatments. For both drug and vehicle conditions, the dramatic increase in licks emitted with reduction of lick volume was mediated by an increase in the duration of licking bursts (and corresponding increase in number of licks/burst), with no significant change in the number of bursts in the meal. Thus, MTII did not interfere with rats' ability to dynamically alter their licking behavior as an adaptive response to the lick volume constraint.

The two lick-testing approaches in this study yielded complementary results. The principal parameters affected by MTII in the dose-response study (i.e. intake, number of licks, burst duration, burst number, and meal duration and/or correlation between change in intake and in meal duration), in general, were similarly influenced by the drug in experiment 2. Two of these parameters, number of licks emitted and the duration of licking bursts, were also profoundly affected by the lick volume manipulation. The effects of MTII and lick volume on these two parameters were independent as evidenced by the lack of significant two-factor interactions for these treatments.

Perhaps the most revealing finding about the action of MTII concerns the meal size outcomes of experiment 2. With no drug treatment, rats demonstrate considerable flexibility in the licking pattern to compensate for lick volume adjustment such that meal size remains stable across conditions. The same proves to be the case for rats treated with MTII. This result suggests that the primary effect of MTII is a reduction in meal size to a level that the rat, if challenged, appears to defend via compensatory behavioral adjustments.

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