Effects of melanin-concentrating hormone on licking microstructure and brief-access taste responses

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Recent studies have shown that intracerebroventricular and brief-access taste responses. Effects of melanin-concentrating hormone on licking microstructure and brief-access taste responses. Am J Physiol Regul Integr Comp Physiol 291: R1265–R1274, 2006. First published June 8, 2006; doi:10.1152/ajpregu.00143.2006.—The effects of intracerebroventricular application of melanin-concentrating hormone (MCH) on licking for sucrose, quinine hydrochloride (QHCl), and water solutions were evaluated in two experiments. In experiment 1, rats received 90-min access to sucrose and water solutions after MCH or vehicle microinjection to the third ventricle (3V). MCH increased intake largely through increases in the rate of licking early in the meal and in the mean duration of lick bursts, suggesting an effect on gustatory evaluation. Therefore, in experiment 2, brief access tests were used with a series of sucrose and QHCl concentrations to behaviorally isolate the effects of intracerebroventricular MCH on gustatory evaluation. MCH uniformly increased licking for all sucrose solutions, water, and weak concentrations of QHCl; however, it had no effect on licking for the strongest concentrations of QHCl, which were generally avoided under control conditions. Thus MCH did not produce nonspecific increases in oromotor activity, nor did it change the perceived intensity of the tastants. We conclude that MCH enhanced the gain of responses to normally accepted stimuli at a phase of processing after initial gustatory detection and after the decision to accept or reject the taste stimulus. A comparison of 3V NPY and MCH effects on licking microstructure indicated that these two peptides increased intake via dichotomous behavioral processes; although NPY suppressed measures associated with inhibitory feedback from the gut, MCH appeared instead to enhance measures associated with hedonic taste evaluation.

Gustatory; sucrose; rat; feeding

MCH-immunoreactive fibers have been identified in brain nuclei associated with control of food intake and/or metabolism, including the insular cortex, bed nucleus of the stria terminalis, nucleus accumbens, paraventricular hypothalamic nucleus, median eminence, central nucleus of the amygdala, parabrachial nucleus, nucleus of the solitary tract (NST), dorsal motor nucleus of the vagus (DMX), and ventral medulla (7, 28, 61, 62, 63).

Although the orexigenic effects of MCH are well established, the behavioral specificity of these actions remains unclear. MCH has been demonstrated to potentiate ingestion of a broad range of comestibles; in addition to increasing low- and high-fat chow intake, MCH administration has been shown to increase intake of water, sucrose, and sucrose-quinine, sucrose-saccharin, and ethanol-saccharin mixtures (8, 22, 34, 41). However, it remains unclear whether MCH stimulates ingestion as a result of increases in thirst, gustatory evaluation, preference conditioning, appetitive motivation, reductions in inhibitory post-igestive feedback, or combinations thereof. A meal pattern analysis by Kowalski et al. (34) determined that MCH treatment affected meal size rather than meal number, which suggests that MCH effects are exerted on the processes that control the progress of meals (e.g., taste evaluation or satiation) rather than foraging behavior or other meal-initiating factors. A treatment effect of MCH on meal size, however, does not reveal which sensory or motor processes are specifically affected by MCH: increases in meal size could be due to enhancement of gustatory evaluation, a reciprocal effect on inhibitory gut feedback (satiation), some combination of both, and/or nonspecific motor effects. Thus the present study sought to further clarify the effects of MCH on the behavioral processes underlying ingestion of a meal. We performed a detailed microstructural analysis of licking responses for sucrose, quinine hydrochloride (QHCl), and water solutions after MCH or vehicle injections in either longer-term (90 min) tests or brief-access (20 s) tests.

Licking microstructure analysis permits assessment of treatment effects on taste evaluation and inhibitory feedback from the gut (12, 14, 15, 49). Taste evaluation is commonly inferred from the avidity with which rats begin a meal: because very little is ingested early in the meal, behavioral activity at this phase is considered to be guided largely by orosensory evaluation of the tastant (e.g., 12, 14, 52, 60). The initial rate of licking in the meal (the first 1 to 3 min) increases systematically with increases in the concentration of palatable tastants, such as sucrose, and it is suppressed by bitter tastants or by the
formation of a conditioned taste aversion (4, 29, 52, 53). Mean burst size or duration also appears to reflect taste evaluation. During a meal, rats ingest in discontinuous bursts of licking at a constant frequency—and, similar to the initial lick rate—the mean size of these bursts also increases with more palatable tastants and is accordingly reduced with naturally or conditioned aversive stimuli (4, 29, 52, 53). Additionally, we also evaluated changes in the rate of ingestion observed over the course of the meals in MCH- and vehicle-treated rats. Once a meal is under way, inhibitory feedback from the gut effects changes in the rate of ingestion over longer time frames that incorporate the combined influence of bursts and the pauses between them. For example, a graded decline in the rate of licking over the course of the meal has been reported to vary as a function of the caloric and volumetric properties of the ingested load. Moreover, as the meal progresses, increasing the size of gastrointestinal loads produces an increasingly rapid decline in ingestion rate and a reduction in meal duration and the number of bursts in the meal (see Refs. 11–14, 17, 18, 20, 23, 44). Brief-access tests were also used to further isolate the effects of MCH on taste evaluation; in such tests, several concentrations of a taste stimulus are presented over very brief trials, allowing a concentration-licking function to be determined. The brief nature of the taste trials ensures that postigestive feedback influences are minimized, allowing the effects of MCH on orosensory processing to be revealed through curve shifts in the concentration-response function (e.g., 26, 51).

METHODS

Subjects

Albino male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 390 ± 18 g on the first day of the experiment were used. Rats were maintained individually in plastic tubs with wire lids on a 12:12 light-dark schedule in a temperature-controlled room. Food (Purina rat chow 5001, Ralston Purina, St. Louis, MO) and tap water were available ad libitum in the home cage, except where noted below. Rats were tested at the same time each day, between 6 and 8 h after lights on, in a separate test cage.

Surgery

Rats were anesthetized with a mixture of ketamine HCl (66 mg/kg) and xylazine HCl (6 mg/kg). A 22-gauge guide cannula (Plastics 1, Roanoke, VA) was stereotaxically implanted into the third ventricle (coordinates: AP: −2.3 mm, ML: 0 mm, DV: −8.5 mm relative to bregma), and fastened with dental acrylic and skull screws. The 28-gauge injection cannula extended 1 mm beyond the tip of the guide cannula, and an obturator cut flush to the guide tip was maintained in the guide at all other times. After surgical recovery, cannula placement was confirmed by assessing 30 min water intake after a cannula infusion of the dipsogen, ANG II (50 ng/μL; 2 μL/min). Rats that did not drink at least 5 ml were removed from the study. Concluding behavioral experiments, cannula placements were also confirmed by intracerebroventricular injection of India ink (5 μL) immediately after a lethal overdose of nembutal sodium (100 mg/kg). Rats were then transcardially perfused with isotonic saline followed by 10% formalin. The brain was removed, bisected middasagittally, and inspected for the rostrocaudal extent of ventricular ink perfusion. Data for rats with no ink perfusion through the third and fourth ventricles were discarded.

Apparatus

Experiment 1. Rats were taken from their home cages and tested in individual plastic tubs (48 × 25 × 15 cm). A drinking spout (3-mm orifice; Girton, Millville, PA) was introduced to the test chamber with the spout opening positioned 4 cm from the floor and 1–2 mm behind a slit (8 × 28 mm) in a metal plate attached to the front of the cage. A lickometer (DiLog Instruments, Tallahassee, FL) and PC computer were used to record licking; tongue contacts with the spout completed a circuit, which allowed the computer to record the time of each lick with 1-ms resolution. Files for each test session for each rat were saved for off-line analysis.

Experiment 2. A novel group of rats was tested daily in an automated lickometer referred to as the “Davis Rig” (Davis MS-160, DiLog Instruments, Tallahassee, FL). Unlike the single-bottle lickometer in experiment 1, the Davis Rig allows the presentation of up to 16 different taste stimuli (one at a time) within a single behavioral session, with a minimum interstimulus interval of 5 s (37, 50). Rats were placed in a plastic rectangular cage (30 × 14.5 × 18 cm) with a wire mesh floor and had access to a single sipper tube (when a computer-operated shutter was lifted) via an oval opening centered in the front wall of the test chamber. Spout licks were recorded by a microcomputer using a circuit similar to that of the lickometer used in experiment 1.

Procedures

Experiment 1. Rats (n = 15) were habituated in the test cage daily where they were free to ingest the middle concentration in the series, 0.1 M sucrose, for 90 min. Habituation training continued until session intakes stabilized and exceeded 5 ml per session (2–5 sessions). For the experiment, responses to 3V MCH and artificial cerebrospinal fluid (aCSF) injections were tested at each of three concentrations of sucrose (0.0 M, 0.1 M, and 1.0 M). Rats were exposed to the same taste concentration for 90 min over five consecutive test days, with intracerebroventricular injections on days 3 and 5 of each concentration block. Two rest days intervened between each 5-day concentration test block. Concentration blocks and drug order with those blocks were counterbalanced using a Latin square design. On drug test days, rats received a 5 μg/5 μl cannula injection (2 μl/min) of either rat MCH (American Peptide, Sunnyvale, CA; 2 nm) or the vehicle aCSF (Harvard Apparatus, Holliston, MA) 15 min before intake testing. Infusions were made using a 10-μl Hamilton syringe in a programmable syringe pump (KD Scientific model #100).

Experiment 2. Rats (n = 11) were habituated to the Davis Rig over daily sessions under 23.75-h water deprivation conditions. On sessions 1 and 2, rats were offered a single 15-min trial of distilled water (clock beginning with the first lick). On sessions 3 and 4, rats were offered water for 32 trials (20 s each trial) using eight bottles housed on a motorized track outside the test chamber 4 times each. Each trial began with the first lick. At the end of the first trial, the shutter closed for 5 s, while the next sipper tube was positioned. If a rat did not initiate the second trial within 20 s, the trial was terminated and the next trial was initiated. Stimulus bottles were weighed to the nearest 0.01 g before and after the session to monitor intake for each bottle. Training continued until each rat sampled water on each of the 32 trials (2 to 6 sessions).

Concluding habituation, the rats were exposed to two consecutive 4-day tastant blocks (sucrose and QHCl), in which licking for eight concentrations of sucrose (0, 15, 31, 62, 125, 250, 500, and 1,000 mM) and QHCl (0.0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, and 1 mM) was assessed. On days 2 and 4, we injected rats with aCSF or MCH, using parameters identical to experiment 1. The order of tastant blocks and drug order within those blocks were counterbalanced using a Latin square. Rats remained on water restriction for the QHCl testing block but not for the sucrose testing block (see Ref. 24). On each test day, rats sampled tastants in 2 descending and 2 ascending concentration series (see Ref. 51), in fully counterbalanced order.
**Data Analysis**

**Experiment 1.** Data were analyzed according to previously established analysis parameters, as follows (see Refs. 4, 31, 52 for details).

Total session intake (ml) was measured as the difference between pre- and post-test weights of the spout bottle (adjusted for the specific gravity of each solution). This value was then divided by the total number of licks in the session to yield the average lick volume (μl) for each test. Meal size (ml) was then calculated by multiplying the number of licks in the meal [first lick of the first burst to last lick of the last burst (4, 53)] by the average lick volume for that session. The end of the meal was defined by a pause in licking ≥ 10 min (52, 64).

Meal duration (min) was defined as the session time of the last lick in the meal minus the session time of the first lick in the meal. Average ingestion rate (licks/s) was calculated as the number of licks in the meal divided by meal duration in seconds. This value was then multiplied by 60 to provide the average lick rate per minute.

The temporal distribution of licking was analyzed using a variety of custom-made programs (4, 31). A licking burst was defined as two or more consecutive licks with no interlick interval (ILI) exceeding 1 s. Thus pauses greater than 1 s determined burst termination (52). Burst duration (s) was calculated by subtracting the session time of the first lick in the burst from the time of the last lick in that burst. Mean burst size (lick count) was calculated as the cumulative number of licks in all bursts in the meal divided by the number of bursts in the meal. To minimize artifact registrations due to nonlingual spout contacts, meal onset was defined as the first lick of the first burst containing at least three licks. Latency (s) was defined as the time between placement of the rat into the test cage and the onset of the first burst of licking. Initial lick rate (licks/min) was the number of licks in the first minute of the meal. The average duration (s) of the first three bursts in each meal was also calculated to provide a measure of initial ingestion rate (59).

ILI’s were analyzed in several ways. The average within-burst ILI (ms) was determined by averaging all ILIs (160 ms) was also commonly observed (4, 11, 52, 53), we evaluated the principal mode of the ILI distribution determined (53). Because a second distribution of ILIs with a mode below this cutoff (11), the average of ILIs all ILIs in a meal are multiplied by 60 to provide the average lick rate per minute.

**RESULTS**

**Table 1.** Licking measures across taskant and drug conditions

<table>
<thead>
<tr>
<th>Drug Condition</th>
<th>Water</th>
<th>0.1 M Sucrose</th>
<th>1.0 M Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tastant</td>
<td>aCSF</td>
<td>MCH</td>
<td>aCSF</td>
</tr>
<tr>
<td>Meals initiated</td>
<td>2.00 ± 0.24</td>
<td>2.07 ± 0.23</td>
<td>2.13 ± 0.22</td>
</tr>
<tr>
<td>Meal lick count</td>
<td>297.7 ± 0.79</td>
<td>483.8 ± 0.55</td>
<td>1830 ± 231</td>
</tr>
<tr>
<td>Lick volume, μl</td>
<td>6.06 ± 0.45</td>
<td>6.08 ± 0.55</td>
<td>6.69 ± 0.55</td>
</tr>
<tr>
<td>Meal duration, min</td>
<td>5.60 ± 1.37</td>
<td>6.18 ± 1.62</td>
<td>21.91 ± 4.22</td>
</tr>
<tr>
<td>Latency, s</td>
<td>43.71 ± 9.11</td>
<td>162.96 ± 124.76</td>
<td>16.61 ± 5.67</td>
</tr>
<tr>
<td>1st min lick rate</td>
<td>53.80 ± 23.37</td>
<td>129.80 ± 28.98</td>
<td>162.13 ± 21.53</td>
</tr>
<tr>
<td>1st 3 bursts, s/burst</td>
<td>13.16 ± 2.63</td>
<td>16.55 ± 2.93</td>
<td>16.14 ± 2.51</td>
</tr>
<tr>
<td>Mean lick rate, licks/min</td>
<td>126.37 ± 35.00</td>
<td>126.87 ± 28.22</td>
<td>114.64 ± 14.65</td>
</tr>
<tr>
<td>Burst count</td>
<td>9.00 ± 2.42</td>
<td>8.07 ± 1.03</td>
<td>32.27 ± 5.38</td>
</tr>
<tr>
<td>Mean burst size, licks</td>
<td>54.63 ± 11.49</td>
<td>70.31 ± 13.45</td>
<td>62.59 ± 6.32</td>
</tr>
<tr>
<td>Mean burst duration, s</td>
<td>7.09 ± 1.53</td>
<td>9.61 ± 1.81</td>
<td>9.47 ± 0.97</td>
</tr>
<tr>
<td>Pause time, %</td>
<td>80.86 ± 6.40</td>
<td>65.05 ± 6.80</td>
<td>69.70 ± 2.47</td>
</tr>
<tr>
<td>Mean pause duration, s</td>
<td>65.34 ± 30.03</td>
<td>44.73 ± 13.12</td>
<td>27.80 ± 10.5</td>
</tr>
<tr>
<td>Within-burst lick rate, licks/s</td>
<td>5.81 ± 0.29</td>
<td>5.90 ± 0.29</td>
<td>6.41 ± 0.28</td>
</tr>
<tr>
<td>Mean ILI (0–249 ms)</td>
<td>140.92 ± 2.08</td>
<td>144.02 ± 1.61</td>
<td>141.92 ± 1.14</td>
</tr>
<tr>
<td>Mean ILI (250–499 ms)</td>
<td>352.59 ± 7.78</td>
<td>334.13 ± 8.32</td>
<td>336.50 ± 10.40</td>
</tr>
<tr>
<td>Mean ILI (500–999 ms)</td>
<td>689.61 ± 18.58</td>
<td>700.22 ± 16.43</td>
<td>700.24 ± 11.25</td>
</tr>
<tr>
<td>ILI% (0–249 ms)</td>
<td>90.34 ± 2.67</td>
<td>93.79 ± 19.94</td>
<td>95.43 ± 0.54</td>
</tr>
<tr>
<td>ILI% (250–499 ms)</td>
<td>6.07 ± 2.05</td>
<td>3.30 ± 0.79</td>
<td>3.47 ± 0.34</td>
</tr>
<tr>
<td>ILI% (500–999 ms)</td>
<td>3.59 ± 0.77</td>
<td>2.92 ± 1.20</td>
<td>4.10 ± 0.16</td>
</tr>
</tbody>
</table>

Bold values indicate that P met the criterion for statistical significance, which was set at P < .05. Values for water, 0.1 M sucrose, and 1.0 M sucrose are given as means ± SE. ILI% indicates proportion of interlick interval (ILIs) in the range indicated relative to the total of all ILIs < 1 s.

Data were analyzed according to previously established analysis parameters, as follows (see Refs. 4, 31, 52 for details).
There was no significant tastant-drug interaction \( F(2,28) = 2.66, P = 0.09 \). Although meal duration varied significantly across concentration conditions, such that meals were longest at the 0.1 M sucrose condition \( F(2,28) = 16.34, P < 0.001 \), there was no significant effect of MCH on meal duration \( F(1,14) = 1.54, P = 0.24 \) and no significant interaction \( F(2,28) = 0.89, P = 0.42; \) see Fig. 2, top.

**Licking microstructure.** Although MCH treatment produced some very small changes in the pattern of licking within bursts, these changes made no contribution to its orexigenic effect. Analysis of ILIs \( 250 \) ms revealed a small but statistically significant 3% increase in the mean duration of ILIs, indicating slower licking for all tastants (Table 1). Analysis of the frequency distribution of ILIs within the bursts showed that MCH significantly increased the proportion of ILIs \( 250\) ms by \( 2\)% overall and commensurately reduced the proportion of ILIs in the range of \( 250–499 \) ms by \( 2\)% (see Table 1). Overall, these minor effects of MCH did not translate into any significant effect on the average rate of licking within bursts (Table 1) nor on the average ingestion rate (Table 1).

MCH did not augment intake via an increase in the number of bursts (Fig. 2, bottom). Rather, the mean burst duration was significantly increased under MCH treatment conditions (Fig. 3, bottom; Table 1). When bursts were characterized by burst size, the MCH-induced increase was similar in magnitude, although the statistic reached only a marginal level of significance \( P < 0.06; \) see Table 1). MCH significantly reduced the proportion of meal time expressed in pauses, suggesting that this neuropeptide increased the temporal “density” of licking during the meal, an effect consistent with the nonsignificant trend toward a reduction of mean pause duration under MCH conditions and the failure of MCH to significantly increase meal duration (Table 1; Fig. 2, top).

**Meal dynamics.** Under control conditions, the initial rate of licking increased with concentration in a monotonic function, as previously described (e.g., 52; Fig. 3, top). Under MCH conditions, the initial rate of licking was markedly and significantly increased for all concentrations (Figs. 3 and 4; Table 1). In accordance with this result, the average duration of the first three bursts in the meal was also robustly increased with MCH treatment. The increases produced by MCH were scaled across concentrations such that the shape of the concentration-response function was preserved (see Fig. 4). This observation is supported by the lack of any significant concentration \( \times \) drug interaction terms for the measures described above (Table 1).

MCH clearly increased avidity for the test solutions, but this effect did not persist throughout the meal. The group curves depicted in Fig. 3 suggest that MCH increased ingestion rates only early in the meal. However, such curves include attrition due to rats whose meals have already ended (see Ref. 31). To
control for attrition, we limited analysis to the first 5 min of 0.1 M and 1 M sucrose meals; periods in which all rats at 1 M sucrose and all but two (excluded from the statistical analysis) at 0.1 M sucrose were actively ingesting. MCH significantly increased the rate of licking for 1 M sucrose, as indicated by a significant main effect of drug \( F(1,14) = 4.94, P < 0.04 \). Overall, ingestion rate declined over these 5 min, as shown by a significant main effect of minute \( F(4,56) = 3.57, P < 0.01 \). Furthermore, the interaction term was statistically significant \( F(4,56) = 3.01, P < 0.03 \), indicating that the lick rate increase that was induced by MCH was lost by the 5th min, as lick rates were comparable across drug conditions by this time (see Fig. 4). The brief nature of MCH’s excitatory effect on ingestion rate was similarly observed for 0.1 M sucrose meals. Although the main effects did not achieve statistical significance \( P > 0.08 \), the interaction term was significant \( F(4,48) = 3.59, P < 0.01 \), supporting the observation that ingestion rates under MCH were higher than control conditions in the first minute but comparable to them by the 5th min (see Fig. 4).

To characterize the progress of meals through their middle and late phases, meals were divided into thirds and the lick rate for each meal third was evaluated. The MCH-induced increases in ingestion rate observed early in the meal quickly dissipated as meals progressed, as there were no differences in lick rate across drug groups \( F(1,14) = 1.65, P < 0.22 \), and there was no significant drug \( \times \) meal-third interaction \( F(2,28) = 0.15, P < 0.86 \). These results support the overall lack of effect of MCH on the average rate of ingestion for the entire meal (Table 1).

**Experiment 2**

**Sucrose.** As expected, increases in sucrose concentration yielded a monotonic function for lick rate \( F(7,63) = 47.49, P < 0.001 \); see Fig. 5, *top*. Overall, MCH significantly increased licking responses for sucrose, as indicated by a main effect of drug \( F(1,9) = 32.85, P < 0.001 \). Figure 5 indicates that MCH uniformly elevated licking for all tastant concentrations tested, including water, a conclusion supported by a nonsignificant drug \( \times \) concentration interaction term \( F(7,63) = 0.76, P < 0.62 \). All significant differences between MCH and control conditions were lost when sucrose responses were standardized to the water response [drug: \( F(1,9) = 0.74, P = 0.41 \], and although licking continued to increase with increases in concentration \( F(6,54) = 45.93, P < 0.001 \); see
Fig. 5, bottom], the interaction term was not significant here \( F(6,54) = 0.76, P = 0.60 \). Therefore, MCH clearly increased avidity for all of the tastants offered, but it failed to modify the shape of the concentration-response function.

**QHCl.** Under vehicle conditions, licking for low concentrations of QHCl (0.001 mM to 0.03 mM) was comparable to that for water, but for the three strongest concentrations (0.1 mM to 1 mM), licking declined as a negative exponential function (see Fig. 6, top), and a significant main effect for the concentration term was observed \( F(7,70) = 41.70, P < 0.001 \). MCH appeared to increase responses to water and some weak QHCl concentrations that were not avoided under control conditions (see Fig. 6, top), as suggested by a significant main effect for the drug term \( F(1,10) = 12.23, P < 0.006 \), although the drug × concentration interaction term was not significant \( F(7,70) = 1.69, P = 0.13 \). When QHCl licking responses were standardized to water, it was clear that MCH significantly increased responses to water \( t(10) = 2.68, P < 0.02; \) Fig. 6, bottom, inset) and that there was no significant main effect of MCH \( F(1,10) = 1.24, P = 0.29 \). Simply stated, although MCH increased licking for water, it did not increase licking for QHCl concentrations that were normally avoided.

**DISCUSSION**

In experiment 1, intracerebroventricular MCH (2 nM dose) moderately, but significantly, increased consumption of both water and sucrose. Intake was most prominently increased at the 0.1 M concentration (by 61%), with a comparable increase in water intake (60% increase) and a more moderate increase in meal size for the 1 M sucrose concentration (29% increase). MCH did not increase the number of meals initiated, which is consistent with the report that the MCH1 receptor antagonist T-226296 (34) significantly reduced average daily meal size...
 Investigators have previously reported that intracerebroventricular MCH increases water intake over 2-h and 4-h tests, although this apparent thirst effect is not due to an influence of MCH on plasma osmality or urinary excretion (8), nor is it diminished by pretreatment with the ANG II antagonist, saralasin (41). It is interesting to note that in previous studies, MCH did not increase water intake when offered simultaneously with preferred sucrose or glucose solutions in two-bottle tests (41). However, when water was offered alone (8), as in the present study, or cooffered with isopreferrred (5 mM) and less-preferred (10 mM) saccharin solutions (41), water intake was significantly increased. The effects of MCH on water consumption may therefore reflect an effect of the hormone to increase consumption of either the most preferred, or the only, tantstant available, if it is normally accepted. This conclusion was supported by the findings of Experiment 2 (discussed below).

Effects of MCH on licking microstructure. Aside from motoric side effects of a treatment, meal size in controlled tests varies as a function of the conflation of two separate but contributing factors: inhibitory feedback reflecting caloric and volumetric aspects of ingesta and excitatory influences based on orosensory evaluation of the tantstant. We explored the microstructural features of the meal to determine whether measures that typically covary with postigestive, gustatory, and/or motoric manipulations were affected by MCH treatment.

Measures of oromotor output. Areas that control oromotor functions, including the hypoglossal nucleus and reticular formation, are a major target of MCH projections to the caudal brainstem (42, 63). Analysis of the ILI distribution within bursts indicated that MCH did not significantly increase the average speed of licking within bursts (Table 1). Closer analysis revealed that MCH produced a small, significant 3% increase in the mean duration of ILIs < 250 ms (indicating a slower rate of licking), which was offset by comparatively small decreases in the proportions of longer ILIs (250–499 ms), resulting in no net effect on the average rate of licking within bursts. These longer intervals are commonly ascribed to missed lick contacts or brief oromotor responses such as gapes or tongue protrusions (11). We conclude that MCH showed no major effect on central mechanisms of lick pattern generation and that the small effects of the hormone on ILIs within bursts were negligible with regard to its orosensory effect.

Measures of postigestive feedback. Several studies have shown that gastrointestinal or physiological state treatments that tend to increase food intake, such as sham feeding via an open gastric fistula or food deprivation, are associated with a reduced rate of decline in ingestion rate, an increase in burst count, and prolonged meal duration (see Ref. 49; also 12–14, 17–19, 23, 44, 52). Although it significantly increased meal size, MCH produced no significant effect on any of these measures: while MCH increased ingestion rate early in meals, there was no difference in ingestion rate decline in the later meal phases, as indicated both by group curve (Fig. 4) and meal-third analyses.

The failure of MCH to affect licking measures associated with postigestive feedback is somewhat perplexing. Zheng et al. (63) recently reported extensive MCH-immunoreactive processes throughout viscerotopic areas of caudal NST and DMX, with some fibers in close apposition to NST cells that expressed c-Fos immunoreactivity after a nutritive gastric preload. Furthermore, MCH bath application suppressed brainstem slice responses of glutamergic vagal-afferent terminals in the caudal NST. Despite this strong support for a role of MCH in gastrointestinal processing, fourth-ventricular (4V) infusions of MCH (4 nM) failed to increase 2-h intake of water or rat chow (63). Although a more detailed behavioral analysis may have revealed more subtle effects of 4V MCH on feeding, those results combined with the present study suggest that intracerebroventricular MCH fails to modulate vagal- and MCH-sensitive neurons in the caudal brainstem that directly influence ingestive behavior.

Measures of gustatory evaluation. In experiment 1, licking measures commonly associated with gustatory evaluation were significantly enhanced by MCH. The largest overall effect of MCH was its increase of the initial rate of licking for water and both sucrose solutions tested (Fig. 4). Consistent with this result, the mean duration of the first three bursts in the meal was also significantly increased by MCH. This excitatory effect was, however, short-lived, as ingestion rate increases were not sustained into later phases of the meal, nor were meals prolonged by MCH treatment.

Interestingly, the increases in initial lick rate and burst duration induced by MCH were not limited to sucrose: consistent with previous reports, avidity for water was also increased (see Fig. 3). Therefore, in experiment 2, we used a series of brief-access tests of licking for sucrose, water, and QHCl to evaluate whether these increases were nonspecific. If MCH increased ingestive behavior without regard to the hedonic value of a tantstant, then we would expect MCH to increase licking for all tantstants, be they palatable, vapid, or aversive. Consistent with experiment 1, brief-access responses to all sucrose concentrations and to water were increased; importantly however, MCH failed to increase licking for aversive QHCl, an effect that clearly could not have been limited by a ceiling effect on oromotor output (see Fig. 6). It may be concluded then, that MCH-induced increases in licking were limited to normally accepted tantstants, with no effect on solutions that were avoided under control conditions.

The failure of MCH to affect licking for aversive concentrations of QHCl eliminates some possible psychophysical effects of the hormone. If MCH enhanced the perceived gustatory intensity of weak taste stimuli, we would expect MCH to have left-shifted the sucrose and QHCl concentration-licking curves. Instead, the sucrose curve (including water) was vertically shifted whereas responses to the most aversive concentrations of QHCl were unchanged (Fig. 6). Conversely, if MCH caused taste stimuli to be reduced in perceived intensity, one would expect the curves to right-shift. However, after MCH the stronger concentrations of QHCl continued to suppress licking to a level comparable to aCSF control conditions (Fig. 6, top), whereas sucrose responses at all concentrations were increased rather than decreased. Finally, because MCH did not change the shape of the concentration function for sucrose, we conclude that MCH enhances the gain of responses to normally accepted stimuli at a phase of processing that occurs after initial gustatory processing and after the decision to accept the taste stimulus.

It was recently reported that intracerebroventricular MCH injection increased intake of saccharin-ethanol (0.1%/10%)
and sucrose-QHCl (0.5 M/0.35 mM) mixtures in 2-h tests (22). It is unclear whether these intake increases were related to gustatory, caloric, or other rewarding aspects of the taste solutions offered; however, it is worth noting that the sucrose-QHCl mixture was more preferred than water, even under baseline conditions (22). The current data suggest that MCH more likely enhanced avidity for sucrose in the mixture rather than producing a decrease in aversion to the QHCl. Overall, combined with the observed inconsistency regarding MCH-induced increases in saccharin intake across availability conditions (discussed above), the effects of this hormone appear to vary as a function of tantast availability, acceptability, and preference.

**MCH and lateral hypothalamic stimulation.** It has been suggested that the MCH system may mediate the behavioral effects of electrical stimulation of the lateral hypothalamus (ESLH), because MCH increases consumption of both food and water, and that its synthesis is limited to the LH (7, 8). The current results qualify this hypothesis: MCH enhanced responses to sucrose, but it failed to enhance brief responses to normally avoided concentrations of QHCl. By contrast, ESLH failed to increase ingestive taste reactivity for sucrose solutions (0.3 and 1 M), but it did increase such responses for 0.3 mM QHCl (6). Numerous investigators have also shown that ESLH increased consumption of a broad range of QHCl concentrations that were normally avoided under baseline conditions (e.g., 36, 48, 54, 57, 58). Furthermore, ESLH was shown to strongly disrupt lick frequency, an effect not observed in this study (27). Overall, prevailing data suggest that the orexigenic effects of MCH are more selective than for ESLH, in that they appear to be limited to normally accepted or preferred tastants.

**Comparison of NPY and MCH effects on licking microstructure.** The neuropeptides MCH, NPY, and orexin A are well-known orexigenics that are implicated in hypothalamic signal cascades that influence ingestive behavior and metabolism (42). It is believed that NPY and POMC-containing neurons in the arcuate nucleus may stimulate and inhibit the orexin- and MCH-containing neurons, respectively, in the juxtacapsular and perifornical regions of the LH. Recently, we evaluated the effects of 3V NPY (5 µg/1.17 nM) infusions on intake and licking microstructure for saccharin, water, and a range of sucrose concentrations (0.03 to 1.0 M), using design and analysis parameters identical to the current study (5). If MCH at least partially mediates the influences of NPY, one might predict that the pattern of licking responses to these two orexigenic compounds shared some similarities, but they did not.

Although NPY and MCH both increased sucrose intake, they did so via dramatically different behavioral means. Table 2 shows a side-by-side comparison of the effects of intracerebroventricular NPY and MCH on various measures of licking microstructure for water and sucrose solutions. First, under NPY treatment, meals were vastly prolonged, containing more than four times more bursts compared with control conditions; MCH produced no such effects. Second, MCH increased the initial ingestion rates and the mean length of bursts, whereas NPY had a much smaller effect on initial lick rate and produced either no effect or suppressed mean burst size, depending on tantast concentration. Third, MCH reduced the mean pause time within meals, whereas NPY significantly slowed the average rate of ingestion in sucrose meals (Table 2). In addition, NPY increases both meal size and meal frequency for a range of tantasts (2, 5, 21, 46, 47), whereas the effects of MCH are limited to meal size (this study and 34). Finally, although MCH increased licking for water, we observed no such effect after NPY injection (Table 2; Ref. 5).

**Limitations.** Intracerebroventricular administration of any compound can never correlate absolutely with its natural stimulation patterns, and the anticipation that intracerebroventricular MCH would necessarily reproduce those of another centrally applied orexigen (NPY) must be considered in this light. Despite this constraint, the divergent effects of these two neuropeptides on licking microstructure suggest that forebrain ventricular MCH and NPY injections have separate sites of action. This is consistent with the recent report that 4V MCH injections failed to increase feeding (63), whereas 4V NPY injections do so with as much efficacy as hypothalamic or forebrain ventricle NPY infusions (10). Importantly, coinjection of low intracerebroventricular doses of NPY and MCH produced no synergistic effect on chow intake (39).

Overall, intracerebroventricular NPY effects on licking microstructure and electrophysiological gastric distension responses in the NST suggest that NPY treatment suppresses inhibitory gut feedback with little effect on taste evaluation (5, 32, 43, 46). Meanwhile, although MCH immunoreactivity in caudal NST areas associated with visceral processing is con-

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**Table 2. Comparison of MCH and NPY effects on licking microstructure**

<table>
<thead>
<tr>
<th>Effect of MCH</th>
<th>Effect of NPY</th>
<th>Effect of (2)</th>
<th>Effect of (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.1 M sucrose</td>
<td>1.0 M sucrose</td>
<td>Water</td>
</tr>
<tr>
<td>Meal size</td>
<td>↑ 60%</td>
<td>↑ 61%</td>
<td>↑ 29%</td>
</tr>
<tr>
<td>Lick volume</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Meal duration</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Latency</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Initial lick rate</td>
<td>↑ 141%</td>
<td>↑ 59%</td>
<td>↑ 22%</td>
</tr>
<tr>
<td>Mean lick rate</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Burst count</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Burst size</td>
<td>↑ 36%</td>
<td>↑ 29%</td>
<td>↑ 51%</td>
</tr>
<tr>
<td>Pause time</td>
<td>↓ 19%</td>
<td>↓ 6%</td>
<td>↓ 14%</td>
</tr>
<tr>
<td>Within-burst lick rate</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
</tbody>
</table>

MCH and NPY treatments differentially affected most measures of licking. Rats received injections of melanin-concentrating hormone (MCH) (5 µg/5 µl) or neuropeptide Y (NPY) (5 µg/5 µl) into the third ventricle 15 min before 90-min intake tests. Comparisons are relative to responses for the same tantast after 5-µl injection of artificial cerebrospinal fluid (vehicle). Bold indicates that the drug effect was statistically significant \((P < 0.05)\). “No effect” indicates that the difference between vehicle and drug conditions was not statistically significant. † From Ref. 5.
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