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Decreased consumption of sweet fluids in mu opioid receptor knockout mice: a microstructural analysis of licking behavior

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Summary

Rationale—Evidence suggests that the palatability of food (i.e., the hedonic impact produced by its sensory features) can promote feeding and may underlie compulsive eating, leading to obesity. Pharmacological studies implicate opioid transmission in the hedonic control of feeding, though these studies often rely on agents lacking specificity for particular opioid receptors.

Objectives—Here, we investigated the role of mu opioid receptors (MORs) specifically in determining hedonic responses to palatable sweet stimuli.

Methods—In Experiment 1, licking microstructure when consuming sucrose solution (2 to 20 %) was compared in MOR knockout and wildtype mice as a function of sucrose concentration and level of food deprivation. In Experiment 2, a similar examination was conducted using the palatable but calorie-free stimulus sucralose (0.001 to 1%), allowing study of licking behavior independent of homeostatic variables.

Results—In Experiment 1, MOR knockout mice exhibited several alterations in sucrose licking. Although wildtype mice exhibited a two-fold increase in the burst length when food deprived, relative to the nondeprived test, this aspect of sucrose licking was generally insensitive to manipulations of food deprivation for MOR knockout mice. Furthermore, during concentration testing, their rate of sucrose licking was less than half that of wildtype mice. During sucralose testing (Experiment 2), MOR knockout mice licked at approximately half the wildtype rate, providing more direct evidence that MOR knockout mice were impaired in processing stimulus palatability.

Conclusions—These results suggest that transmission through MORs mediates hedonic responses to palatable stimuli, and therefore likely contributes to normal and pathological eating.

Keywords

opioid; feeding; reward; motivation; knockout; mice; appetite; licking; palatability; sugar

Introduction

The palatability of a food (i.e., the hedonic impact produced by tasting a food stimulus) is a major factor controlling feeding behavior (Yeomans et al. 2004), and excessive consumption of palatable food can lead to obesity and other serious health problems including metabolic syndrome, diabetes, cardiovascular disease and cancer (Guh et al. 2009). A vast psychopharmacological literature indicates that endogenous opioid systems are key

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mediators of palatability and its effects on feeding (Bodnar 2004; Nogueiras et al. 2012; Pecina and Smith 2010; Yeomans and Gray 2002). For instance, in rodents, opioid antagonists tend to suppress hedonic taste reactions to palatable taste stimuli such as sugar (see (Olszewski et al. 2011)), and reduce consumption and pursuit of such stimuli (Apfelbaum and Mandenoff 1981; Cleary et al. 1996; Kirkham 1990; Leventhal et al. 1995a; Levine et al. 1995; Marks-Kaufman and Kanarek 1981), whereas opioid agonists tend to produce opposite effects (Marks-Kaufman 1982; Stromberg et al. 1997). Studies using receptor-specific drugs have specifically identified the mu opioid receptor (MOR) as a critical player in mediating food palatability (Bodnar et al. 1995; Giraudo et al. 1999; Gosnell and Majchrzak 1989; Islam and Bodnar 1990; Leventhal et al. 1995a; Pecina and Berridge 2005; Zhang et al. 1998) although delta (Zhang and Kelley 1997) and kappa opioid receptors have also been implicated (Gosnell et al. 1986; Higgs and Cooper 1998).

A complementary strategy is to assess the effects of genetic deletion of MORs on processing food palatability. Given the pharmacological data one should expect MOR knockout (KO) mice to show reduced palatability responses; however, this hypothesis has not been tested. There is some evidence that MOR KO mice exhibit reduced or otherwise altered food-motivated behaviors (Kas et al. 2004; Laurent et al. 2012; Papaleo et al. 2007), but it is unclear if this reflects a reduction in the experienced palatability of foods or a deficit in processing other qualities of foods that contribute to the acquisition and/or expression of conditioned behaviors (i.e., its ability to reinforce or motivate reward-seeking). Arguing against the palatability hypothesis is the observation that MOR KO mice maintained on normal (Han et al. 2006) and high fat (Tabarin et al. 2005) diets show normal levels of food intake when measured over long periods. However, monitoring feeding over such long intervals may not reveal the specific influence of food palatability on ingestive behavior (Frisina and Sclafani 2002; Higgs and Cooper 1998).

In this study, we applied a microstructural lick analysis commonly used in pharmacological studies to evaluate and distinguish between various processes that control fluid intake (D'Aquila 2010; Davis and Smith 1988; Frisina and Sclafani 2002; Higgs and Cooper 1998). In Experiment 1, wildtype and MOR KO mice were allowed to freely consume a palatable 10% sucrose solution while in a state of mild (4-h) food deprivation. They were then administered a series of tests in which we manipulated the potential palatability of the sucrose solution by varying either their level of food deprivation (0- vs. 18-h food deprivation) or sucrose concentration (2% vs. 20% sucrose solution). We found that wildtype mice exhibited clear changes in palatability responses in the predicted manner, engaging in longer bursts of licking when in a higher food-deprivation state and when sucrose concentration was elevated. Although MOR KO mice exhibited a relatively normal pattern of licking during initial testing, they showed generally lower levels of licking behavior during sucrose concentration testing and displayed insensitivity to the change in food deprivation level, an effect that was preferentially expressed in the burst length measure. The consumption of nutritive stimuli, like sucrose, also engages post-ingestive processes that allow for homeostatic control over stimulus intake based on caloric need. Therefore, in Experiment 2, we conducted a similar analysis of licking behavior using sucralose solution, a calorie-free artificial sweetener, to assess consummatory behavior in wildtype and MOR KO mice in the absence of homeostatic regulation of feeding. Sucralose licking was profoundly reduced in MOR KO mice, relative to wildtypes, indicating a specific deficit in processing the palatability of sweet fluids. These results have implications for understanding the contributions of endogenous opioid systems to reward processing and may help explain how drugs and other hedonic stimuli take control of these systems to support compulsive reward-seeking behavior.

Methods

Animals

Female wildtype and MOR KO mice, maintained on a C57BL6 background, were derived from an in-house breeding colony produced by heterozygous-heterozygous matings (Matthes et al. 1996). We used female mice in this study to facilitate comparison with the results of previous studies using female MOR KO mice to study appetitive behavior (Kas et al. 2004; Papaleo et al. 2007). Mice were housed in cages of mixed genotypes of 3 to 4 mice per cage with food and water provided ad libitum in a temperature-controlled colony room. All measurements were taken approximately 6 to 7 h into the light period (06:00 – 18:00). Experimental protocols were approved by the UCLA Institutional Animal Care and Use Committee and were in accord with the National Research Council Guide for the Care and Use of Laboratory Animals.

Lickometer recording and analysis

Licking microstructure was recorded in a designated recording chamber outside of the home cage using a custom-made lickometer in 30-min sessions during which mice could freely consume sucrose solution through a standard, gravity-fed rodent drinking tube. For each session, mice were placed individually in a darkened cylindrical chamber (12.5 cm diameter, 22 cm height) located on a stainless steel floor warmed from below to approximately 28°C. The chamber had a single 1.5 cm hole in the wall 4.2 cm above the floor through which a stainless-steel drinking spout was inserted at an approximately 30° angle from the horizontal to provide access to sucrose solutions. The spout had a 5.5 mm internal diameter hole at the tip, and contained an approximately 7.5 mm diameter ball bearing. The outside of the spout was cuffed with a plastic sheath to provide electrical isolation thus avoiding electrical artifacts caused by mice touching areas of the spout other than the ball bearing with the tongue and paws. The spout was connected to a 13.8 V, 3 A DC power supply and in series to an analogue-digital converter (Model DI-148U, Dataq Instruments, Akron, OH) attached to a PC sampling at a rate of 30 Hz. Licking from the spout completed an electrical circuit, which could be detected as a change in potential at the tongue-spout interface. The current passing through the circuit at the time of tongue contact was estimated to be 0.9 μ A. Previous studies in rats suggest this to be below sensory threshold (Weijnen 1998). Digitized recordings were then analyzed using custom computer scripts written in Python that detected lick-related changes in current and computed the total number of licks, the total number of licking bursts and the average duration of licking bursts (see below). Licking bursts were defined as a series of two or more licks uninterrupted by a period of more than 1 s, based on recent studies showing this threshold to be ideal for determining palatability-related changes in licking microstructure in mice (Johnson et al. 2010). To further evaluate the validity of the 1-s cutoff parameter we analyzed inter-lick-intervals for data acquired during the last two sessions of task acquisition in Experiment 1 (i.e., days 9 and 10). Figure 1 plots these data as a cumulative frequency distribution illustrating the proportion of total inter-lick-intervals that are longer than time, t , for both wildtype and MOR KO mice. One mouse from the MOR KO group was excluded from this analysis because none of its inter-lick-intervals were less than 14 s. For both groups, inter-lick-intervals appeared to fall into one of two general distributions, a tightly clustered distribution of intervals shorter than 1 s and a second, much broader, distribution consisting of intervals over 1 s. Our burst analysis focused on licking related to the former (<1 s) distribution.

We computed both the average duration of licking bursts (i.e., time between the first and last lick in a burst, in seconds, averaged across all bursts in a session) and the number of licking bursts performed in each 30-min session, in addition to tracking the total number of individual licks, which included all burst-related licks as well as non-burst licks (i.e., those

licks that failed to meet the criteria for inclusion in the burst analysis). Although all of these measures may vary with stimulus palatability, it has been argued that they do so with varying degrees of fidelity and may be modulated by distinct processes, with licking burst duration serving as the most direct assay of stimulus palatability, particularly for caloric taste stimuli that likely to engage homeostatic regulatory processes (D'Aquila 2010; Davis 1989; Higgs and Cooper 1998).

Training and testing protocol

Experiment 1 – Sucrose testing—Mice (wildtype: $n = 8$, MOR KO: $n = 7$) underwent several phases of training and testing to identify genotype-dependent differences in the acquisition of licking behavior and sensitivity to manipulations of food deprivation and sucrose concentration. Mice were exposed to the lickometer in blocks of five contiguous days, interspersed with two-day periods during which animals remained in their home cages undisturbed. On all exposure days, mice were relocated temporarily to the testing room and allowed approximately 4-h to habituate in their home cages before placement in the lickometer apparatus for 30-min sessions during which continuous and free access to sucrose solution was provided. During the first phase of the study, mice were exposed to 10% sucrose for a total of 10 recording sessions each under 4-h food deprivation, providing them an opportunity to acquire stable licking behavior. For the second phase of the study, mice were given a single session of exposure to 10% sucrose (again while 4-h food-deprived) to re-establish baseline responding after the two-day break. On subsequent days, they were tested with 10% sucrose following either 0-h or 18-h of food deprivation. Testing under each condition was performed twice in a counterbalanced (ABAB or BABA) order and the results averaged together for analysis. In the third phase of the study, baseline responding to 10% sucrose was re-established (under 4-h food deprivation), and then mice were exposed to 2 or 20% sucrose, again following 4-h of food deprivation. Testing under each condition was performed twice and the results averaged.

Experiment 2 – Sucralose testing—Mice (wildtype: $n = 11$, MOR KO: $n = 16$) were initially given a series of 30-min exposure sessions to 0.01% sucralose for a total of 10 recording sessions under 4-h food deprivation, allowing them to acquire stable licking behavior, as in Experiment 1. The mice then underwent sucralose concentration testing, again under 4-h food deprivation. After a single session of exposure to 0.01% sucralose to re-establish baseline responding, the mice were given a series of four exposure sessions with varying concentrations of sucralose (0.001%, 0.01%, 0.1%, and 1%). Session order was varied across mice using a Latin square design. A second round of testing (1 re-acquisition session and 4 tests with varying sucralose concentrations) was conducted on the following week in an identical manner. Testing under each condition was performed twice and the results were averaged. Following lickometer testing, mice (wildtype: $n = 10$, MOR KO: $n = 14$) were given a test of sucralose consumption, which was conducted in distinct rectangular testing chambers (black opaque plastic walls and ceiling, 10 cm deep $\times$ 10 cm wide $\times$ 20 cm high, with hole in front wall for insertion of bottle spout). Mice were placed in separate chambers and allowed 30 min to habituate, at which point they were given access to a bottle containing 0.01% sucralose for 30 min. The bottles were weighed before and after testing to measure consumption.

Data presentation and statistical analyses

The effects of repeated testing, genotype and experimental manipulations (food deprivation or stimulus concentration) on licking microstructure were compared using mixed ANOVAs with within and between subject factors, followed by Bonferroni post-hoc testing (controlling for multiple comparisons) to identify between-genotype or between-manipulation differences wherever statistically significant interactions were detected in the

ANOVA. Statistical analyses were made using Statview software (Statview, Berkeley, CA). Probability values less than 0.05 were considered statistically significant. Data are expressed as group means $\pm$ standard error of the mean (S.E.M.) throughout.

Results

Experiment 1: Sucrose testing

Acquisition of sucrose licking behavior—Figure 2 shows changes in the three main measures of licking microstructure during daily training sessions to lick 10% sucrose following 4-h food deprivation. There was no main effect of genotype in any of the three measures when analyzed by repeated measures ANOVA over the 10 training sessions. There were, however, significant effects of session for each of the three measures (total licks, $F_{9,117}=2.77$, $p<0.01$; total bursts $F_{9,117}=3.23$, $p<0.01$; mean burst length, $F_{9,117}=2.30$, $p<0.05$) that generally reflected an increase in all three measures as training progressed. There were no significant interactions between session and genotype on responding for any of the three measures.

Effect of food deprivation on sucrose licking—There were no statistically significant differences between genotypes on any of the three measures on the re-exposure day prior to the 4-day block of testing for the effect of food deprivation: total licks: wildtype 337.1 ± 58.3 , KO 203.6 ± 82.3 ; number of bursts: wildtype 31.0 ± 5.6 , KO 26.7 ± 13.8 ; mean burst length: wildtype 1.80 ± 0.27 , KO 1.24 ± 0.35 . The results from the food deprivation test phase are presented in Figure 3. Over the 30-min recording sessions, all three measures of licking responses were significantly higher in the high food deprivation condition than in the low deprivation condition (total licks, $F_{1,13}=27.3$, $p<0.01$; total bursts, $F_{1,13}=20.1$, $p<0.01$; mean burst length, $F_{1,13}=12.3$, $p<0.01$). No significant main effects of genotype were observed for any of the three measures. However, a statistically significant interaction was observed between genotype and hunger state for mean burst length ($F_{1,13}=5.16$, $p<0.05$), indicating that the two groups differed in their sensitivity to this treatment. Wildtype mice showed an approximate doubling in mean burst length in the high deprivation state compared to the nondeprived state ($p < 0.05$, Bonferroni post-hoc test), whereas burst lengths in MOR KO mice were not significantly affected by this manipulation. No genotype effect was observed for the burst length measure in either deprivation condition.

Effect of sucrose concentration on licking—There were no statistically significant differences between the three measures on the re-exposure day prior to the 4-day block of test sessions for the effect of sucrose concentration: total licks: wildtype 343.8 ± 81.8 , KO 181.6 ± 68.0 ; number of bursts: wildtype 32.4 ± 6.1 , KO 24.3 ± 9.9 ; mean burst length: wildtype 1.34 ± 0.24 , KO 1.02 ± 0.19 . The results from sucrose concentration testing are shown in Figure 4. Increasing sucrose concentration from 2 to 20% produced statistically significant increases in the total number of licks per session and mean burst length (main effect of concentration: total licks, $F_{1,13}=10.6$, $p<0.01$; mean burst length, $F_{1,13}=11.0$, $p<0.01$). There were also significant main effects of genotype observed for these two measures (total licks, $F_{1,13}=11.9$, $p<0.01$; mean burst length, $F_{1,13}=5.89$, $p<0.05$) with MOR KO mice showing lower levels in both measures. No significant effect of concentration or genotype was detected for the total bursts measure. Although the attenuation in total licks and burst duration in MOR KO mice appeared to be greater in the 20% sucrose sessions, no statistically significant interactions between sucrose concentration and genotype on any of the three measures were found.

Experiment 2: Sucralose testing

Acquisition of sucralose licking behavior—Figure 5 shows changes in the three measures of licking microstructure during daily training sessions with 0.01% sucralose solution. MOR KO exhibited substantially lower levels of licking than wildtype mice, a profile that was apparent for each of the three measures of licking behavior (main effect of genotype: total licks, $F_{1,25} = 8.68$, $p < 0.01$; total bursts, $F_{1,25} = 9.62$, $p < 0.01$; mean burst length, $F_{1,25} = 20.1$, $p < 0.01$). Thus, unlike in Experiment 1, which used sucrose, MOR KO mice displayed a deficit early and throughout acquisition of the sucralose licking task. We also detected significant main effects of session for each of these measures (total licks, $F_{9,225} = 3.86$, $p < 0.01$; total bursts, $F_{9,225} = 3.27$, $p < 0.01$; mean burst length, $F_{9,225} = 1.98$, $p < 0.01$). Changes in licking behavior over sessions were also found to vary as a function of genotype for two of the licking measures; significant session by genotype interactions were detected for total licks ($F_{9, 225} = 2.20$; $p < 0.05$) and total bursts ($F_{9,225} = 2.37$; $p < 0.05$), but not for mean burst length.

Effect of sucralose concentration on licking—As during the initial training period, we found significant genotype differences for each of the three measures (total licks: $F_{1,25} = 13.2$, $p < 0.01$; number of bursts: $F_{1,25} = 15.0$; $p < 0.01$; mean burst length: $F_{1,25} = 5.68$, $p < 0.05$) for the data collected during re-exposure days following the two day breaks, which were administered before the first and second round of concentration testing (data averaged across 2 sessions): total licks: wildtype 263.8 ± 45.8 , KO 104.5 ± 18.6 ; number of bursts: wildtype 52.3 ± 9.11 , KO 18.2 ± 3.84 ; mean burst length: wildtype 0.87 ± 0.08 , KO 0.58 ± 0.08 . The results from sucralose concentration testing are shown in Figure 6. All three measures of licking were significantly affected by varying sucralose concentration (total licks, $F_{3,75} = 12.3$, $p < 0.01$; number of bursts, $F_{3,75} = 4.45$, $p < 0.01$; mean burst length, $F_{3,75} = 12.9$, $p < 0.01$), with the measures generally increasing as the concentration of sucralose increased. We also detected significant genotype effects in the total number of licks ($F_{1,25} = 9.58$, $p < 0.01$) and total number of bursts ($F_{1,25} = 15.8$; $p < 0.01$). No interactions between concentration and genotype were observed, indicating that although MOR KO mice tended to exhibit generally lower levels of sucralose licking, both groups were sensitive to changes in concentration. After lickometer testing, mice were given a 30-min consumption test to directly measure the quantity of sucralose solution consumed. During this session, wildtype mice drank significantly more 0.01% sucralose (mean: 162 mg, SEM: 35.0) than MOR KO mice (mean: 80 mg, SEM: 19.1) ($t_{(22)} = 2.21$, $p < 0.05$), indicating that effects observed during lickometer testing reflected differences in sucralose intake.

Discussion

Here, we show that signaling through MORs is a critical mediator of palatability. MOR KO mice displayed generally reduced levels of sucrose- and sucralose-licking behavior, relative to their wildtype littermates. For sucrose solution, the effects of the MOR KO were most consistently expressed in the licking burst length measure, but were also evidenced in our other measures of licking under certain conditions. A similar – if not more profound – attenuation of licking behavior was observed in MOR KO mice during the sucralose consumption study. Because sucralose is palatable but noncaloric, it is used to study consummatory behavior supported by hedonic, as opposed to homeostatic, processes. Thus, when considered together, the current findings indicate that MORs play an important role in processing the hedonic attributes of sweet stimuli.

These findings largely corroborate those obtained using pharmacological approaches (Kelley et al. 2002; Olszewski et al. 2011; Pecina et al. 2006). Although the genetic deletion approach used here has its own limitations (e.g., lifelong knockout of MORs may trigger

compensations in intact systems, including other opioid receptors), it ensures for complete and selective manipulation of MORs, and therefore provides an important complementary strategy for investigating the functions of this receptor system. The current findings are also noteworthy because a recent report found that MOR KO mice were relatively unimpaired in a sucrose consumption task. In this study, MOR KO and wildtype mice drank similar levels of 5% sucrose solution when given extended (3 days) access to this stimulus in their home cage (Kas et al. 2004). The current results, however, suggest that the palatability phenotype exhibited by MOR KOs may be specific to certain test parameters, like deprivation state or sucrose concentration, and may be clearest when mice are confronted with a change in these parameters (resulting in a change in sucrose palatability). It is also likely that our use of a relatively short-access (30-min) procedure and a microstructural lick analysis provided a more sensitive test of reward palatability.

It has been shown that pharmacological blockade of opioid receptors, generally, and MORs, specifically, leads to shorter bouts of palatable fluid/food consumption, in some cases without affecting the initiation of consumption (Beczowska et al. 1992; Higgs and Cooper 1998; Kirkham and Blundell 1984; 1987; Leventhal et al. 1995b; Wise and Raptis 1986). In the current study, we measured three major parameters of licking microstructure: total licks, number of bursts, and mean burst length, the latter of which has been argued to be a fairly selective index of stimulus palatability (D'Aquila 2010; Davis 1989; Higgs and Cooper 1998). Interestingly, MOR KO and wildtype mice exhibited distinct microstructural profiles of sucrose licking during tests in which food deprivation levels were manipulated. As expected, wildtype mice increased their overall licking behavior, the length of their licking bursts, and the frequency of these bursts when tested food deprived, relative to the nondeprived test. MOR KO mice, on other hand, appeared to be less sensitive to the change in food deprivation level. Most prominently, MOR KO mice did not significantly increase the length of their licking bursts when tested in the high deprivation state. Given the argument presented above that burst length may better reflect subjective assessments of palatability than total licks (D'Aquila 2010; Davis 1989; Higgs and Cooper 1998) it would appear MORs contribute to the influence of food deprivation on sucrose palatability. However, MOR KO mice did display elevations in total licks and total bursts when shifted from the nondeprived to the deprived state, indicating that their sucrose drinking behavior was not completely insensitive to the influence of food deprivation. A similar pattern of results was observed when we manipulated sucrose concentration. During this test, we found that increasing sucrose concentration from 2% to 20% resulted in significant increases in total licks performed and the duration of licking bursts. Interestingly, these two measures of licking were also generally attenuated in MOR KO mice. Although this genotype effect was not completely restricted to the burst duration measure, this aspect of sucrose licking was the one most consistently disrupted in MOR KO mice.

While the current findings provide strong support for the hypothesis that MORs play a critical role in processing stimulus palatability, it is important to consider alternative explanations for the above findings. Because the mouse must approach and interact with spout to ingest the palatable stimulus, it is possible that instrumental (response-stimulus) and/or Pavlovian (stimulus-stimulus) learning contributes to performance in the current task, and that these processes are the source of the group differences reported here. Similarly, it has been suggested that incentive motivational processes are likely influence licking behavior during voluntary drinking studies (D'Aquila 2010; Higgs and Cooper 1998). However, rather than affecting the length of licking bursts, it is argued that the incentive properties of the palatable stimulus, or cues that have become associated with that stimulus, have the ability to provoke new bouts of reward-seeking behavior, which is primarily reflected in the total burst measure. Although we did not find any significant genotype effects for this measure during sucrose testing, MOR KO mice did show significantly fewer

licking bursts during sucralose testing, which according to this account would reflect a deficit in incentive motivation. However, this framework for analyzing licking microstructure data requires further validation before such conclusions can be made. That said, other lines of research have more directly implicated MORs in incentive motivation (Barbano and Cador 2006; Barbano et al. 2009; Kas et al. 2004; Papaleo et al. 2007; Solinas and Goldberg 2005; Zhang et al. 2003). For instance, recent studies using pharmacological (Wassum et al. 2011; Wassum et al. 2009) and genetic knockout (Laurent et al. 2012) approaches show that MORs contribute to instrumental incentive learning, the process through which changes in reward palatability are encoded and come to influence reward-seeking behavior.

Our use of a voluntary licking task also leaves open the possibility that the generally lower levels of licking behavior observed in MOR KO mice reported here actually reflect an underlying deficit in exploration of the test chamber and drinking spout. Indeed, previous studies show that MOR KO mice display lower levels of exploratory behavior in novel environments (Hall et al. 2003). While this could explain group differences in the total licks and total bursts measures, such an account does not provide an adequate explanation for why MOR KO mice displayed shorter bursts of licking, since this measure actually reflects a subject's tendency to remain at the spout once it has come in contact with the palatable stimulus. In fact, according to this account, MOR KO mice might be expected to display *longer* licking bursts due to lack of response competition between licking and exploratory behavior.

Another issue to consider is that the lickometer device used here operates by passing a small electrical current through subject's tongue as it comes in contact with the drinking spout. It is possible that the genotype effects reported here reflect group differences in behavioral sensitivity to any oral sensation that may have been produced by that electrical current. Although it is difficult to provide a definitive test of this account, it should be noted that our estimates indicate that the current passing through mice during licking behavior (see Methods) is below that believed to be discriminable by rats (Weijnen 1998) and more than two orders of magnitude below that used in studies on the punishing effects of electrical tongue shock on licking in rodents (see Millan and Brocco 2003). Furthermore, we found that MOR KO mice drank less sucralose solution than wildtype mice during a 30-min consumption test, which was conducted without attaching bottles to the lickometer device. This strongly suggests that the attenuated licking exhibited by MOR KO mice in the current study reflects an underlying deficit in processing stimulus palatability, rather than differential sensitivity to electrical currents.

It is also worth noting that we used female mice in the current study to facilitate our comparison with previous studies that also used females to examine appetitive behavior in MOR KO mice (Kas et al. 2004; Papaleo et al. 2007). This approach leaves open questions about the sex-specificity of the current findings. Future studies should explore this possibility.

In summary, we show here that signaling through MORs determines palatability responses to the sweet tastants sucrose and sucralose, and would predict that this effect extends to other palatable stimuli. Given that palatability majorly influences overall food consumption, these findings are clearly relevant to understanding the etiology of eating disorders and obesity.

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Figure 1.

Cumulative frequency distribution plotting proportion of total inter-lick-intervals longer than time, t , for wildtype and MOR KO mice during sucrose exposure sessions 9 and 10 in Experiment 1. The gray vertical line marks the cut-off used for lick burst analysis.

Figure 2.

Licking responses during acquisition of 10% sucrose licking behavior in wildtype (closed circles) and MOR KO (open circles) mice. Total licks (top), total bursts (center), and burst lengths (bottom) are plotted separately. Data are plotted over 10 successive 30-min test sessions. Note that there was a 2-day gap between sessions 5 and 6. Horizontal bar with asterisk (*) indicates significant main effect of session.

Figure 3.

Effect of food deprivation state on licking responses in wildtype (closed circles) and MOR KO (open circles) mice. Tests were conducted after 0-h or 18-h of food deprivation. Horizontal bar with asterisk (*) indicates significant main effect of deprivation state. Pound sign (#) indicates significant genotype × state interaction. Double dagger (‡) above line indicates significant state effect in the wildtype condition.

Figure 4.

Effect of manipulating sucrose concentration on licking responses in wildtype (closed circles) and MOR KO (open circles) mice. Tests were conducted using 2% or 20% sucrose solutions. Horizontal and vertical bars with asterisks (*) indicating significant main effects of concentration and genotype respectively.

Figure 5.

Licking responses during acquisition of 0.01% sucralose licking behavior in wildtype (closed circles) and MOR KO (open circles) mice. Total licks (top), total bursts (center), and burst lengths (bottom) are plotted separately. Data are plotted over 10 successive 30-min test sessions. Note that there was a 2-day gap between sessions 5 and 6. Horizontal and vertical bars with asterisks (*) indicate significant main effects of session and genotype respectively. Pound sign (#) indicates significant session $\times$ genotype interaction.

Figure 6.

Effect of manipulating sucralose concentration on licking responses in wildtype (closed circles) and MOR KO (open circles) mice. Tests were conducted using 0.001%, 0.01%, 0.1% or 1% sucralose solutions. Horizontal and vertical bars with asterisks (*) indicate significant main effects of concentration and genotype respectively.