Linoleic and Oleic Acids Alter the Licking Responses to Sweet, Salt, Sour, and Bitter Tastants in Rats

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Abstract

The free fatty acids (FFAs), linoleic and oleic acids, commonly found in dietary fats can be detected by rats on the basis of gustatory cues following conditioned taste aversion pairings. FFAs depolarize the membrane potential of isolated rat taste receptor cells by inhibiting delayed rectifying potassium channels. This study examined the licking response of rats to sweet, salt, sour, and bitter taste solutions when 88 μM linoleic acid, 88 μM oleic acid, or an 88 μM linoleic–oleic acid mixture was added to the solutions. The presence of linoleic, oleic, and the linoleic–oleic acid mixture in sweet solutions produced increases in the licking responses, whereas adding linoleic, oleic, and the linoleic–oleic acid mixture to salt, sour, or bitter taste solutions produced decreases in licking responses when compared with the licking responses to the solutions in the absence of the FFAs. We conclude that FFAs may act in the oral cavity to depolarize taste receptor cells and therefore to increase the perceived intensity of concomitant tastants, thus contributing to the enhanced palatability associated with foods containing high dietary fat.

Key words: behavior, dietary fat, free fatty acids, gustatory, taste

Introduction

Obesity has become an increasing health concern throughout industrialized countries (World Health Organization 2000; Cameron et al. 2003; Contaldo and Pasanisi 2004), with obesity contributing to greater than 300,000 deaths annually in the United States alone (Mokdad et al. 2004). The morbidity of obesity is linked to several health conditions such as cardiovascular disease, hypertension, dyslipidemia, type II diabetes mellitus, osteoarthritis, stroke, asthma, and certain cancers (Must et al. 1999; Mokdad et al. 2003). Greater than 30% of adult Americans exceed the obese criterion of 30 on the body mass index (Ogden et al. 2006). This increased prevalence of obesity is strongly associated with high levels of daily dietary fat consumption (Miller et al. 1990, 1994; McCrory et al. 1999). Although postingestive signals providing feedback of food energy density and the glycemic index contribute to the reinforcement of dietary fat consumption (Elizalde and Sclafani 1990; Greenberg and Smith 1996; McCrory et al. 2000), increased taste palatability is most often cited as the primary influence in the overconsumption of dietary fat (Drewnowski and Greenwood 1983; Warwick and Schiffman 1990; McCrory et al. 1999, 2000).

A preference for dietary fat has been well-documented in rodent research models. Based on research that has controlled for textural (Elizalde and Sclafani 1990; Mindell et al. 1990; Ramirez 1992; Greenberg and Smith 1996; Takeda et al. 2001), olfactory (Takeda et al. 2001), and postingestive (Mindell et al. 1990; Greenberg and Smith 1996) cues, it appears that the rodent preference for dietary fat is based in part on another orosensory cue such as gustation. Corn oil, a prototypical dietary fat, consists of free fatty acid (FFA) triglycerides with linoleic acid (52%) and oleic acid (31%) representing a majority of the total FFAs (Gunstone 1996). Lipolysis through lingual lipase secreted by the rats' von Ebner's glands into the oral cavity can produce significant amounts of oleic acid (1.5% w/w or 53 mM) from the triacylglyceride triolein after only 1 s of exposure to the surface of the tongue (Kawai and Fushiki 2003). Furthermore, fatty acid transporter proteins localized to the rodent gustatory epithelium (Fukuwatari et al. 1997) may act to facilitate access of FFAs to the taste receptor cells. The ability of rats to detect the presence of FFAs by orosensory cues has been suggested through evidence of specific preferences for the FFAs, linoleic and oleic acids, in studies that have controlled for textural cues (Tsuruta et al. 1999; Takeda et al. 2001; Fukuwatari et al. 2003; Kawai and Fushiki 2003) and blocked olfactory cues (Takeda et al. 2001; Fukuwatari

et al. 2003). In addition, our laboratory recently demonstrated that rats can detect and avoid linoleic and oleic acids at concentrations equal to or greater than 66 μM following a single-conditioned taste aversion pairing (McCormack et al. 2006).

An FFA transduction mechanism for afferent gustatory signals has been identified through patch-clamp recordings of isolated rat taste receptor cells. The extracellular application of 10 μM linoleic and oleic acids to rat taste receptor cells inhibited a delayed rectifying potassium channel producing depolarization of the membrane potential (Gilbertson et al. 1997, 1998; Hansen et al. 2003). Calcium imaging of murine taste receptor cells supports the transduction of FFAs with increased levels of intracellular Ca^{2+} detected within 1 min of extracellular application of 50 μM linoleic or oleic acid (Nishizuka et al. 2004).

Based on the characterization of the gustatory transduction mechanism for FFAs, it is hypothesized that taste receptor cell depolarization as a result of linoleic or oleic acid application may act to increase the perceived intensity of any concomitant tastants. This study tests this hypothesis by examining the licking responses of rats when 88 μM linoleic acid, 88 μM oleic acid, or an 88 μM mixture of linoleic and oleic acids is added to sweet, sour, salt, or bitter taste solutions. An FFA concentration of 88 μM is above the behavioral detection threshold (66 μM) and well below the concentration of FFA (53 mM) that can be produced by lipolysis after a 1 s of exposure to the lingual epithelium. The inclusion of a mixture of linoleic and oleic acids intends to examine synergistic or competitive actions of the 2 FFAs. The ratio of linoleic to oleic acid used in the mixture is representative of the ratio of each FFA as found in corn oil. It is predicted that the addition of FFAs will act to depolarize taste receptor cells and therefore increase the perceived intensity of concomitant taste stimuli. This hypothetically would result in increased licking responses to innately appetitive taste stimuli, such as sweet, and decreased licking responses to innately aversive taste stimuli, such as moderate to high concentrations of salt, sour, and bitter stimuli.

Materials and methods

This study was conducted in 3 phases. Phase 1 (linoleic acid) examined the licking responses of rats to concentration series of sucrose, glucose, NaCl, citric acid, and quinine hydrochloride (quinine HCl) presented alone and with 88 μM linoleic acid present in the solutions. Phase 2 (oleic acid) examined the licking responses of rats to concentration series of the same taste stimuli presented alone and with 88 μM oleic acid present in the solutions. Phase 3 commenced immediately following phase 2 and utilized the same animal subjects. In phase 3 (linoleic–oleic mixture), the licking response of rats to concentration series of the same taste stimuli presented alone and with an 88 μM mixture of linoleic and oleic acids present in the solutions was examined.

Subjects

Subjects were 38 naive male Sprague–Dawley rats (phase 1 n = 22 and phases 2 and 3 n = 16) greater than 90 days old obtained from Charles River Laboratories, Wilmington, MA. All rats were individually housed in transparent plastic cages in a temperature-controlled colony room on a 12:12-h light:dark cycle with lights on at 0700. Animals had free access to Harlan Teklad 8604 rodent chow and deionized distilled water ad libitum unless otherwise noted. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Wofford College.

Apparatus

All testing was conducted in the MS-160 gustatory apparatus (DiLog Instruments, Tallahassee, FL). The MS-160 or "Davis Rig" allows the controlled presentation of 16 chemical stimuli while recording the licking behavior of the rat at 1-ms resolution. The combination of a sliding stimulus rack and motor-controlled lever shutter covering the access port permits programmed presentations of the taste stimuli for controlled durations, wait periods, and interstimulus intervals (Smith 2001). The Davis Rig is housed within an acoustic isolation chamber containing intake and exhaust fans located on opposing walls of the chamber in order to maintain a constant flow of air along the longitudinal axis of the stimulus delivery system, thus minimizing the olfactory cues for individual stimuli. An 8-watt light illuminates the acoustic chamber, and the behavior of the rats was monitored by the experimenters via a real-time internet camera.

Chemical stimuli

All taste solutions were mixed daily from reagent grade chemicals (Sigma-Aldrich [St Louis, MO] and VWR [West Chester, PA]) dissolved in deionized distilled water and presented at room temperature (22 °C). The FFAs were stored in a freezer at −20 °C prior to use. A minimum 99% pure, freely dissociated form of linoleic and oleic acids were used to make the FFA solutions. The 88 μM linoleic and oleic FFA mixture consisted of 55 μM linoleic acid and 33 μM oleic acid, which is the same proportion of each FFA found in corn oil, a quintessential dietary fat (Gunstone 1996). A small amount of ethanol (5 mM) was required to facilitate complete dissolution of the FFAs into the solutions; therefore, 5 mM ethanol was added to all solutions including the taste stimuli presented in absence of the FFAs. The following taste solutions were presented alone and with 88 μM linoleic acid, 88 μM oleic acid, or an 88 μM linoleic and oleic mixture added to the solutions: sucrose (15, 31, 62, 125, and 250 mM), glucose (15, 31, 62, 125, 250, and 500 mM), NaCl (31, 62, 125, 250, 500, and 1000 mM), citric acid (1.5, 3, 7, 15, 30, and 60 mM), and quinine HCl (0.003, 0.01, 0.03, 0.1, 0.3, and 1 mM). The addition of FFAs to the taste

solutions produced negligible differences in viscosity (linoleic acid = 0.908 cP, oleic acid = 0.901 cP, FFA mixture = 0.905 cP, water = 0.894 cP), acidity (linoleic acid = 6.2 pH, oleic acid = 6.5 pH, FFA mixture = 6.3 pH), and caloric density (calculated at 0.004 calories per 17 ml stimulus delivery bottle) compared with water.

Procedure

Training

All rats were placed on a 23-h water restriction schedule during the training period prior to testing and the day preceding the testing of each aversive taste stimulus. Phase 1 received a 2-week training period. During week 1 of training, rats were familiarized with licking in the Davis Rig by receiving 15 trials of a water stimulus using 90-s stimulus durations and 10-s interstimulus intervals. In the second week of training, the rats received 20 randomized trials consisting of 10 trials of water and 10 trials of 500 mM sucrose with 20-s durations and 40-s interstimulus intervals. Based on the performance of the rats during the training period of phase 1, in phase 2, the training period was effectively shortened to 2 days of water stimulus training (16 trials, 45-s duration, 10-s interstimulus interval) and 1 day of tastant training with 250 mM sucrose (16 randomized trials: 8 water and 8 sucrose, 30-s duration, 40-s interstimulus interval).

Testing

The stimulus delivery parameters during testing in all phases were 20-s stimulus durations with 40-s interstimulus intervals. In phase 1, each tastant was tested over 4 consecutive days with the taste stimulus presented alone on 2 days and the taste stimulus with 88 μM linoleic acid presented on the other 2 days. The order of tastants was randomly determined as sucrose, NaCl, citric acid, quinine, and glucose. Each daily test session consisted of 2 trials of each concentration of the taste stimulus. During testing of the salt, sour, and bitter taste stimuli, rats were placed on a 23-h water restriction schedule and 6 water trials were inserted in the stimulus order. The order of all stimuli was randomly selected. In phase 2, each tastant was tested over 2 consecutive days with the taste stimulus presented alone on one day and the taste stimulus presented with 88 μM oleic acid on the other day. The randomly selected order of tastants for phase 2 was NaCl, glucose, citric acid, sucrose, and quinine. Each daily test session consisted of 4 trials of each concentration of the taste stimulus with the order of the stimuli randomly determined. During testing of the salt, sour, and bitter taste stimuli, rats were motivated to sample each stimulus through 23-h water restriction and 8 water trials were inserted in the stimulus order. Immediately following phase 2, phase 3 commenced with each tastant tested over 2 consecutive days in an identical manner as phase 2, except the 88 μM linoleic and oleic acid mixture was added to the tastants instead of oleic acid. The randomly assigned order of tastants for phase 3 was glucose, citric acid, sucrose, quinine, and NaCl.

Data analysis

The latency to the first lick and the total number of licks per 20-s stimulus duration were recorded for each trial. Within each daily test session, each subject's mean lick response was calculated for all presentations of each stimulus. For the water-replete testing of appetitive taste stimuli, sucrose and glucose, the raw lick data were analyzed and presented. Since the animals were motivated to drink with water restriction during the testing of the aversive taste stimuli, NaCl, citric acid, and quinine, the raw lick data were standardized for individual differences in the rate of licking. A lick ratio of the number of licks per stimulus divided by the mean number of licks to water trials per daily test session for each animal was calculated such that lick ratios near zero indicate an avoidance to licking the taste stimulus and lick ratios of approximately one indicate maximal licking of the taste stimulus similar to the licking of the water-restricted animals during water stimulus trials. The raw lick data for the appetitive stimuli and the lick ratios for the aversive stimuli were analyzed for significant differences between the licking responses with and without the FFAs added to each tastant using a within-subject analysis of variance (ANOVA) statistical test. When significant main effects were found, post hoc analyses using paired t-tests were conducted to identify the source of the significance.

Results

Across the 3 experimental phases, the subjects maintained a stable, high rate of sampling the stimuli when presented in a trial. The average response rate was 73 ± 5.1% standard error. The minimum response rate of 66% occurred during testing of quinine mixed with linoleic acid, and the maximum response rate of 80% occurred during the testing of NaCl mixed with the FFA mixture. The average response rate for appetitive stimuli (74.6 ± 4.8%) was no different than the average response rate for aversive stimuli (72.0 ± 5.3%), and the average response rate for taste stimuli alone (72.7 ± 5.1%) was no different than the average response rate for taste stimuli presented with FFAs present (73.4 ± 5.2%).

The latency until the first lick can reflect the use of olfactory cues to discriminate stimuli during testing. In particular, large delays until the first lick may be indicative of the use of olfactory cues in order to avoid aversive stimuli. The latency until first lick values was consistently low across all stimuli with a mean latency until the first lick of 6.2 ± 2.0 s standard error. The mean latency until the first lick for water stimuli (5.5 ± 1.3 s) was not significantly different than the latency until first lick of either appetitive (4.4 ± 1.4 s) or aversive (7.5 ± 2.6 s) stimuli. There was no difference between the average latency until first lick of either taste stimuli presented alone (6.2 ± 2.1 s) or with the FFAs present (6.3 ± 2.1 s).
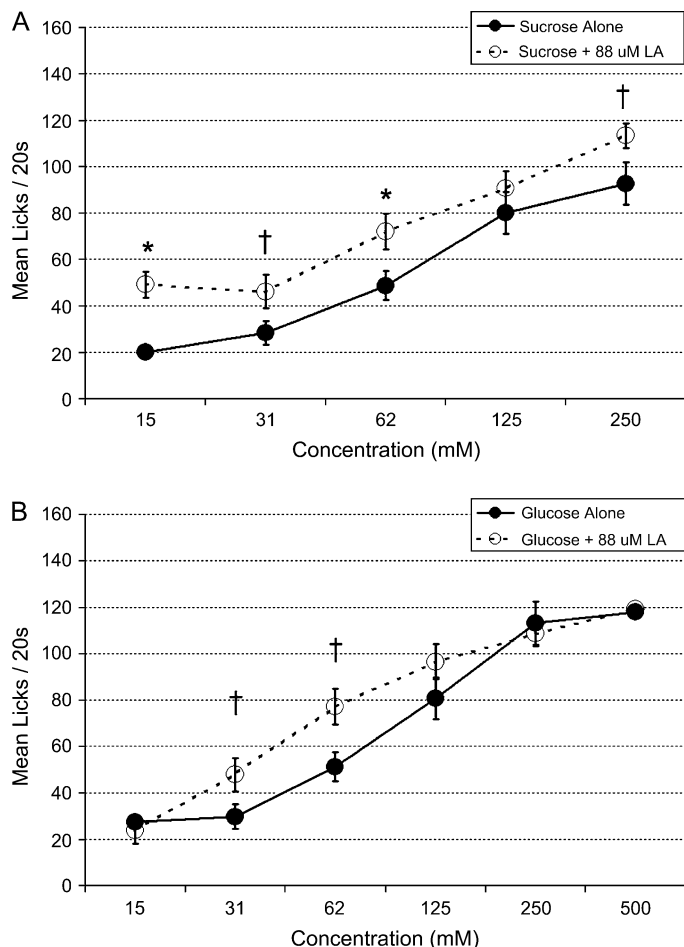
Linoleic acid

The addition of 88 μM linoleic acid to the appetitive taste stimuli, sucrose and glucose, increased the licking responses at several stimulus concentrations (Figure 1). In addition to a significant main effect for stimulus concentration [$F(4,84)$ = 72.341, $P < 0.05$], there was a significant main effect [$F(1,21)$ = 29.404, $P < 0.01$] of adding linoleic acid to the sucrose concentration series and no interaction between sucrose concentration and presence of linoleic acid. Post hoc tests revealed significant increases in licking at the 15 ($P < 0.01$), 31 ($P < 0.05$), 62 ($P < 0.01$), and 250 mM ($P < 0.05$) sucrose concentrations when 88 μM linoleic acid was added to the solutions.

There were significant main effects of the glucose concentration [$F(5,105)$ = 128.323, $P < 0.01$] and adding linoleic acid to glucose [$F(1,21)$ = 5.605, $P < 0.05$], as well as a significant interaction between the presence of linoleic acid and glucose concentration [$F(5,105)$ = 3.818, $P < 0.01$]. Post hoc

Figure 1   Mean (±standard error) licks per 20-s stimulus duration for sucrose **(A)** and glucose **(B)** concentrations presented without (closed symbols and solid line) and with (open symbols and dotted line) 88 μM linoleic acid present. Crosses indicate $P < 0.05$ and stars indicate $P < 0.01$ significant increases in the licking response when linoleic acid is present.

tests identified significant ($P < 0.05$) increases in licking to the 31 and 62 mM glucose concentrations when 88 μM linoleic acid was added to the sweet solutions.

Whereas adding 88 μM linoleic acid to the sweet stimuli produced increases in licking responses, the addition of 88 μM linoleic acid to the salt, sour, and bitter taste solutions resulted in decreased licking responses to several moderate to high concentrations of NaCl, citric acid, and quinine (Figure 2). For the sodium salt, there were significant main effects for stimulus concentration [$F(5,105)$ = 175.651, $P < 0.01$] and adding linoleic acid [$F(1,21)$ = 16.023, $P < 0.01$] with a significant interaction between NaCl concentration and the presence of linoleic acid [$F(5,105)$ = 2.765, $P < 0.05$]. Post hoc analyses identified significant decreases in the licking responses to 125 mM ($P < 0.01$) and 250 and 500 mM ($P < 0.05$) NaCl concentrations when 88 μM linoleic acid was present in the solutions. There were significant main effects for stimulus concentration [$F(5,105)$ = 135.309, $P < 0.01$] and adding linoleic acid to the citric acid solutions [$F(1,21)$ = 16.023, $P < 0.01$] with a significant interaction between the citric acid concentration and the presence of linoleic acid [$F(5,105)$ = 2.747, $P < 0.05$]. Post hoc tests indicated significant decreases in the licking responses at the 15 and 30 mM ($P < 0.01$) and 60 mM ($P < 0.05$) concentrations of citric acid when linoleic acid was added. For the bitter tastant, quinine, there was a significant main effect for stimulus concentration [$F(5,105)$ = 182.544, $P < 0.01$] and a significant interaction between the concentration of quinine and the presence of linoleic acid [$F(5,105)$ = 5.515, $P < 0.01$]. The lack of a significant main effect for adding linoleic acid in the presence of a significant interaction between stimulus concentration and adding linoleic acid indicates that linoleic acid did not produce an overall effect but rather was only effective at specific quinine concentrations. Post hoc tests revealed significant decreases in the licking response to the 0.03 mM ($P < 0.05$) and 0.1 mM ($P < 0.01$) concentrations of quinine.
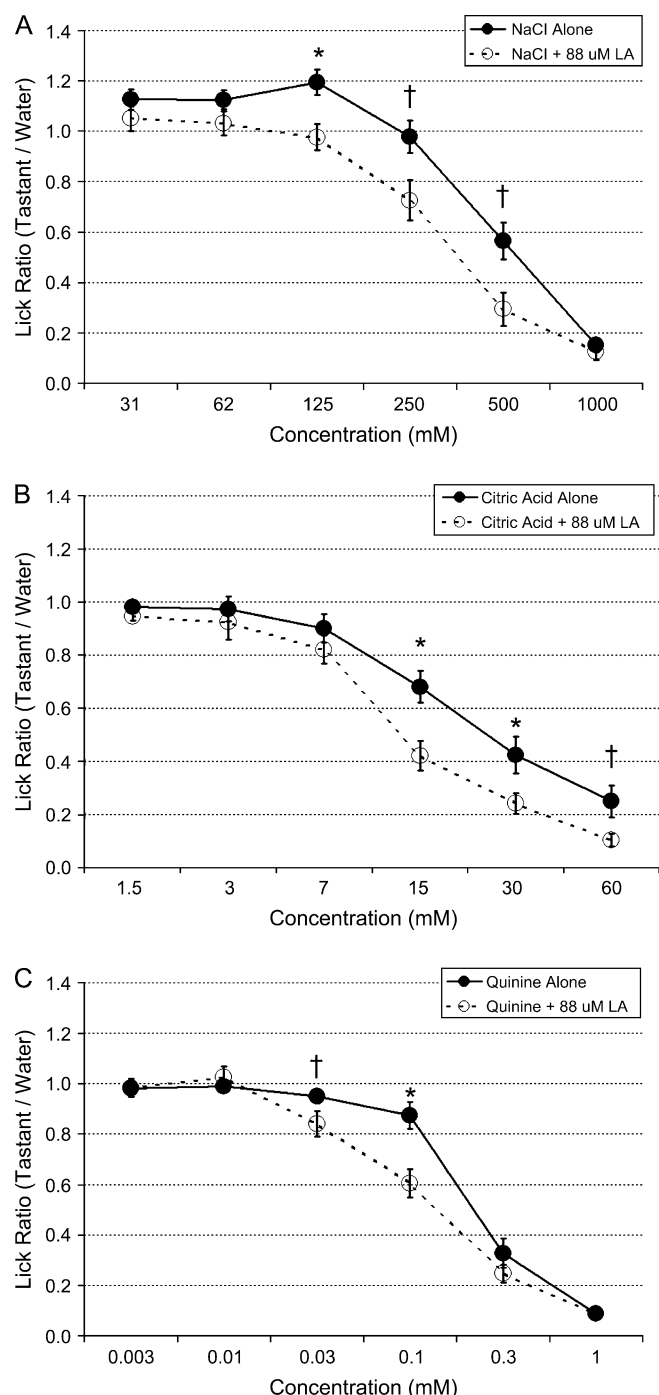
Oleic acid

When 88 μM oleic acid was added to the sweet taste stimuli, sucrose and glucose, the licking responses increased slightly (Figure 3). There were significant main effects on the licking responses to sucrose for both stimulus concentration [$F(4,60)$ = 233.385, $P < 0.01$] and adding oleic acid [$F(1,15)$ = 5.042, $P < 0.05$] as well as a significant interaction between the 2 conditions [$F(4,60)$ = 4.015, $P < 0.01$]. Post hoc tests indicated that adding 88 μM oleic acid produced significant ($P < 0.05$) increases in licking at the midrange concentrations of 31 and 62 mM sucrose.

There were significant main effects on the licking responses for the glucose concentrations [$F(5,75)$ = 101.625, $P < 0.01$] and adding oleic acid to the glucose stimuli [$F(1,15)$ = 33.451, $P < 0.01$] with no interaction between concentration and the presence of oleic acid. Post hoc analyses showed significant ($P < 0.05$) increases in the licking responses for the 62 and
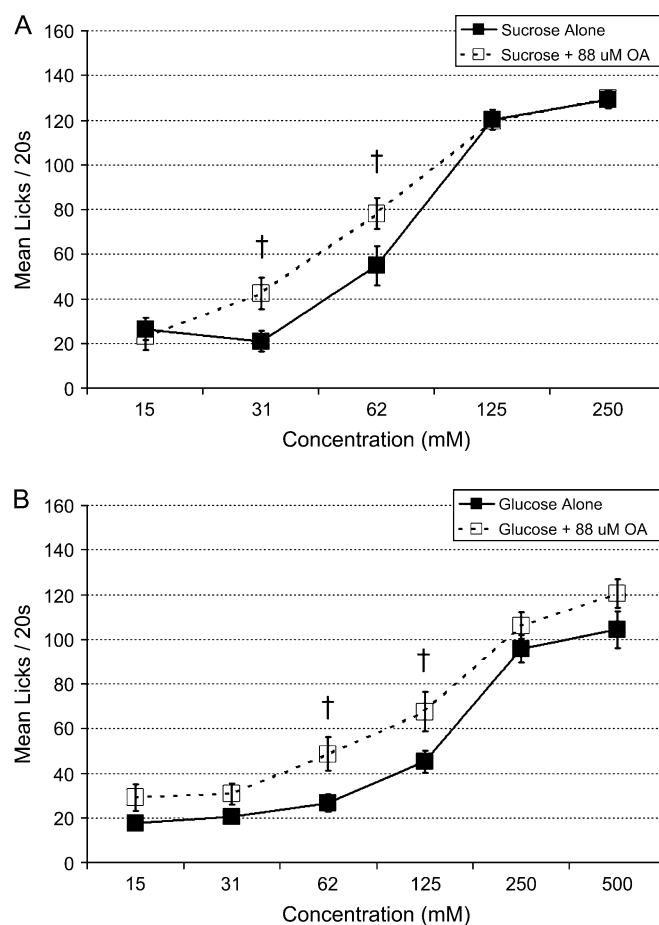
Figure 2   Mean (±standard error) lick ratio for NaCl (**A**), citric acid (**B**), and quinine (**C**) concentrations presented without (closed symbols and solid line) and with (open symbols and dotted line) 88 μM linoleic acid present. Crosses indicate $P < 0.05$ and stars indicate $P < 0.01$ significant reductions in the licking response when linoleic acid is present.

125 mM glucose concentrations when 88 μM oleic acid was added to the stimulus series.

As shown in Figure 4, the addition of oleic acid to the aversive taste stimuli produced decreases in the licking responses to moderate range concentrations of NaCl, citric acid, and
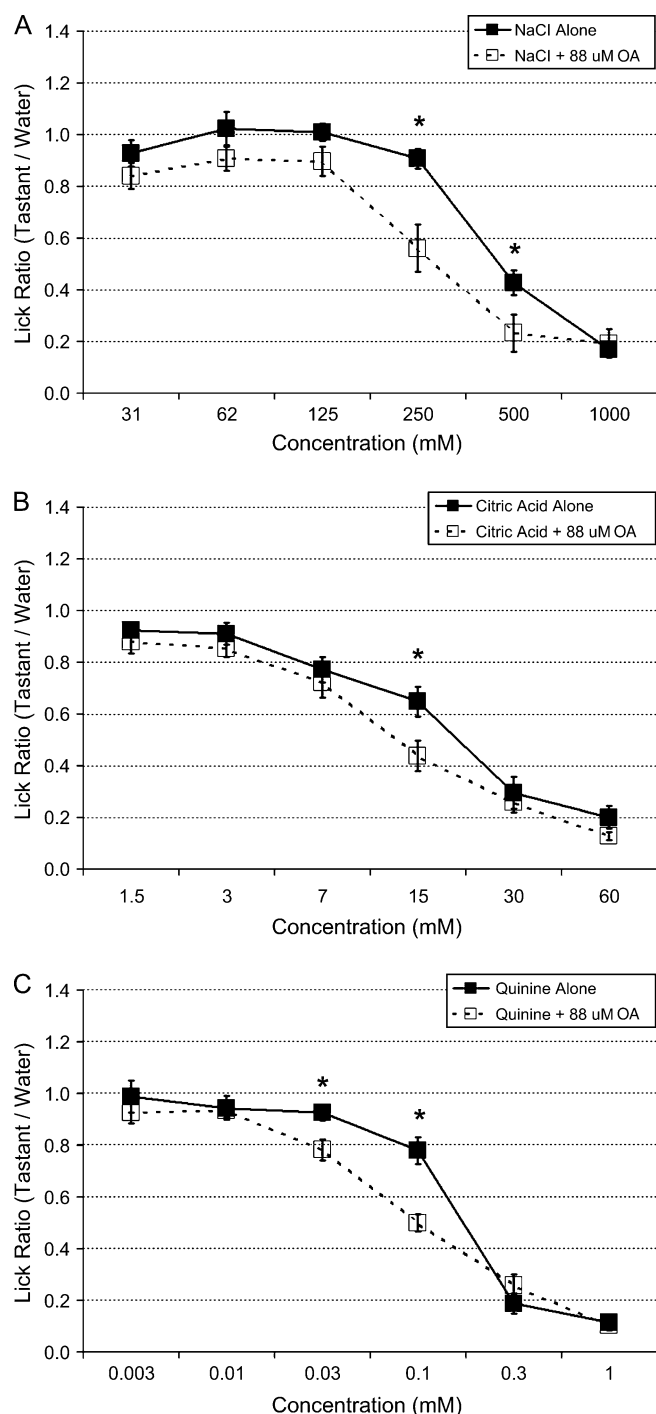

Figure 3   Mean (±standard error) licks per 20-s stimulus duration for sucrose (**A**) and glucose (**B**) concentrations presented without (closed symbols and solid line) and with (open symbols and dotted line) 88 μM oleic acid present. Crosses indicate $P < 0.05$ significant increases in the licking response when oleic acid is present.

quinine. There were significant main effects on the licking responses due to increasing concentrations of NaCl [$F_{(5,75)} = 78.028$, $P < 0.01$], citric acid [$F_{(5,75)} = 119.527$, $P < 0.01$], and quinine [$F_{(5,75)} = 243.360$, $P < 0.01$]. Also, the addition of 88 μM oleic acid to the taste solutions produced a significant main effect for NaCl [$F_{(1,15)} = 16.404$, $P < 0.01$], citric acid [$F_{(1,15)} = 8.850$, $P < 0.01$], and quinine [$F_{(1,15)} = 7.357$, $P < 0.05$]. There were significant interactions between the stimulus concentration and the presence of oleic acid for NaCl [$F_{(5,75)} = 3.608$, $P < 0.01$] and quinine [$F_{(5,75)} = 5.960$, $P < 0.01$]. Post hoc analysis showed significant ($P < 0.01$) reductions in licking to 250 and 500 mM NaCl, 15 mM citric acid, and both 0.03 and 0.1 mM quinine. Oleic acid was only effective in increasing the licking responses to the appetitive taste stimuli and reducing licking responses to the aversive taste stimuli at concentrations that were in the middle, transitional range between concentrations at the asymptotes that elicited either maximal or minimal licking responses.
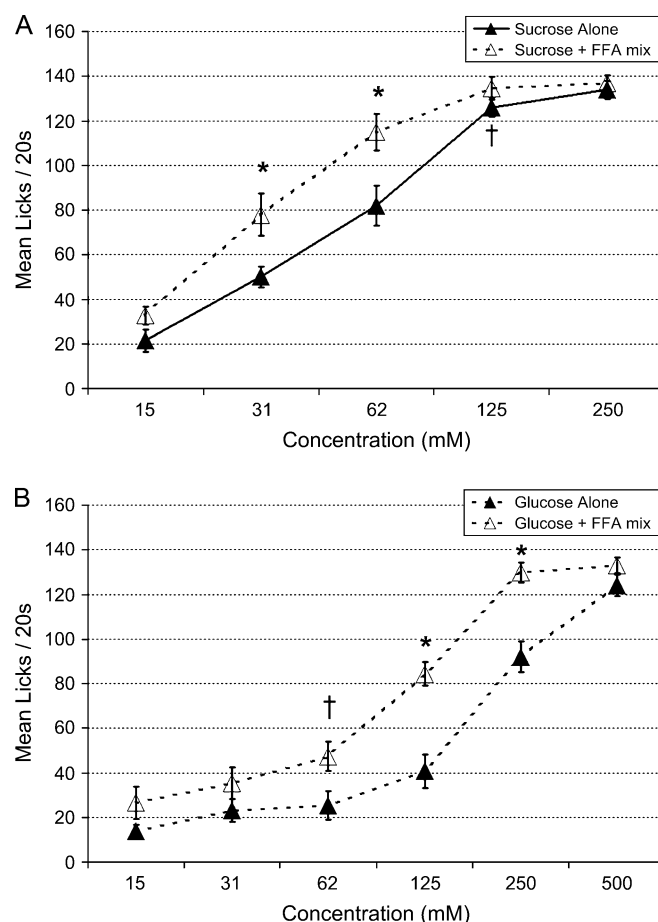
Linoleic–oleic mixture

Adding an 88 μM FFA mixture containing 55 μM linoleic and 33 μM oleic acids produced increases in the licking
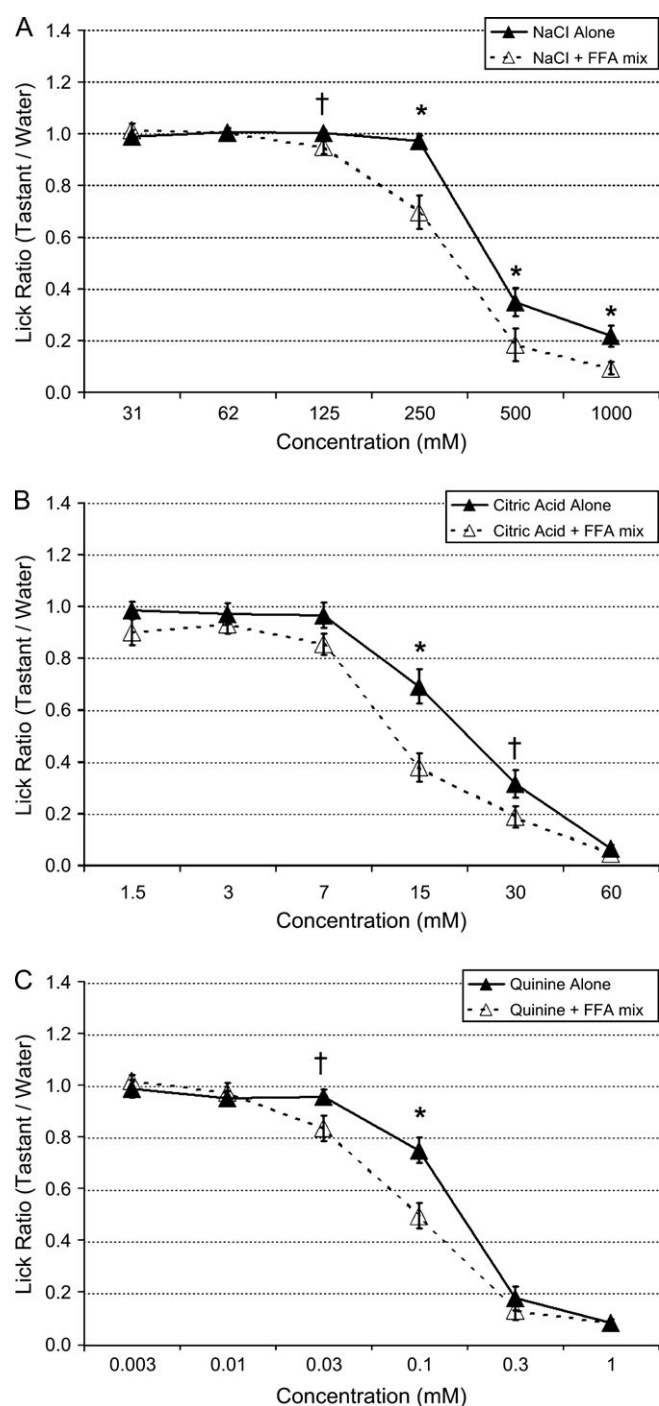

Figure 4   Mean (±standard error) lick ratio for NaCl **(A)**, citric acid **(B)**, and quinine **(C)** concentrations presented without (closed symbols and solid line) and with (open symbols and dotted line) 88 μM oleic acid present. Stars indicate $P < 0.01$ significant reductions in the licking response when oleic acid is present.

responses to several concentrations of the sweet stimuli, sucrose and glucose (Figure 5). There were significant main effects of the stimulus concentration for sucrose [$F(4,60)$ = 151.221, $P < 0.01$] and glucose [$F(5,75)$ = 164.864, $P < 0.01$]. There were significant main effects of adding the FFA mixture to both the sucrose stimuli [$F(1,15)$ = 58.894, $P < 0.01$] and glucose stimuli [$F(1,15)$ = 49.855, $P < 0.01$] as well as significant interactions between the presence of the FFA mixture and sucrose concentration [$F(4,60)$ = 4.742, $P < 0.01$] and glucose concentration [$F(5,75)$ = 5.045, $P < 0.01$]. Post hoc tests identified significant increases in licking to 3 moderate concentrations of sucrose, 31 and 62 mM ($P < 0.01$) and 125 mM ($P < 0.05$), and glucose, 62 mM ($P < 0.05$) and 125 and 250 mM ($P < 0.01$).

The presence of the FFA mixture in the aversive taste solutions predominately resulted in decreases in the licking responses to the midrange concentrations of NaCl, citric acid, and quinine as shown in Figure 6. There were significant

Figure 5   Mean (±standard error) licks per 20-s stimulus duration for sucrose **(A)** and glucose **(B)** concentrations presented without (closed symbols and solid line) and with (open symbols and dotted line) an 88 μM linoleic–oleic acid mixture present. Crosses indicate $P < 0.05$ and stars indicate $P < 0.01$ significant increases in the licking response when the FFA mixture is present.

Figure 6 Mean (±standard error) lick ratio for NaCl **(A)**, citric acid **(B)**, and quinine **(C)** concentrations presented without (closed symbols and solid line) and with (open symbols and dotted line) an 88 μM linoleic–oleic acid mixture present. Crosses indicate $P < 0.05$ and stars indicate $P < 0.01$ significant reductions in the licking response when the FFA mixture is present.

main effects of stimulus concentration for NaCl [$F(5,75)$ = 218.225, $P < 0.01$], citric acid [$F(5,75)$ = 159.590, $P < 0.01$], and quinine [$F(5,75)$ = 237.060, $P < 0.01$] as well as significant main effects for adding the FFA mixture to NaCl

[$F(1,15)$ = 36.155, $P < 0.01$], citric acid [$F(1,15)$ = 18.047, $P < 0.01$], and quinine [$F(1,15)$ = 10.249, $P < 0.01$]. In addition, there was a significant interaction between the presence of the FFA mixture and the concentration series for each aversive stimulus, NaCl [$F(5,75)$ = 8.598, $P < 0.01$], citric acid [$F(5,75)$ = 4.801, $P < 0.01$], and quinine [$F(5,75)$ = 7.050, $P < 0.01$].

Post hoc tests identified significant decreases in the licking response to 125 mM ($P < 0.05$) and 250, 500, and 1000 mM ($P < 0.01$) NaCl concentrations when the FFA mixture was added to the solutions. The presence of the FFA mixture significantly reduced licking to 15 mM ($P < 0.01$) and 30 mM ($P < 0.05$) concentrations of citric acid. Similarly, 2 midrange concentrations of quinine, 0.03 mM ($P < 0.05$) and 0.1 mM ($P < 0.01$), were also identified by post hoc analyses to have significant reductions in licking when the FFA mixture was present in the stimulus solutions.

Two analyses were performed to quantify and compare across the effects of adding linoleic acid, oleic acid, or the FFA mixture to the taste stimuli. Neither analysis revealed systematic differences between the ability of linoleic acid, oleic acid, or the FFA mixture to increase the licking responses to sweet tastants or decrease the licking to salt, sour, and bitter tastants. The first analysis compared the calculated percent increases or decreases for each of the respective concentrations of the stimuli at which significant effects were observed. An ANOVA statistic neither revealed any significant differences in the ability of the 3 fatty acid stimuli to increase the licking response to sucrose (LA = 70%, OA = 72%, FFA mixture = 34%) or glucose (LA = 56%, OA = 65%, FFA mixture = 78%) nor were there significant differences in the ability of each fatty acid stimulus to reduce the licking response to NaCl (LA = −31%, OA = −42%, FFA mixture = −34%), citric acid (LA = −47%, OA = −32%, FFA mixture = −43%), or quinine (LA = −21%, OA = −26%, FFA mixture = −23%). The second analysis involved calculating the area under the logarithmically plotted concentration curves for each stimulus with and without each fatty acid present in order to compare differences in the upward shifts in area for the sweet stimuli and the downward shifts in area for the salt, sour, and bitter stimuli between the 3 fatty acid stimuli. The only significant difference was for the glucose stimuli [$F(2,53)$ = 5.834, $P < 0.01$], in which post hoc least significance difference tests revealed that the FFA mixture produced a significantly greater area under the curve than linoleic acid ($P < 0.01$) and oleic acid ($P < 0.05$), which did not differ from one another. There were no significant differences in the upward shifts in area under the curve for the sucrose stimuli or the downward shifts in area under the curve for the NaCl, citric acid, and quinine stimuli. The lack of systematic significant differences in these 2 analyses indicate that linoleic acid, oleic acid, and a linoleic–oleic acid mixture were equally effective in modulating the licking responses to sweet, salt, sour, and bitter taste stimuli.

Discussion

This study demonstrated that the addition of suprathreshold concentrations of FFAs to taste solutions can alter the licking responses of rats in a consistent manner. These changes in licking response are similar to the results one would expect to observe in response to an increase in the perceived intensity of the taste stimuli. For innately appetitive taste stimuli, such as sucrose and glucose, adding FFAs produced increases in the licking response, whereas adding FFAs to innately aversive taste stimuli, such as NaCl, citric acid, and quinine, produced decreases in the licking responses. In general, larger changes in the licking responses were observed for the sweet stimuli mixed with FFAs than the mixtures of FFAs and salt, sour, or bitter stimuli. The effect of adding FFAs to the tastants was most prominent at moderate concentrations of the taste stimuli that were intermediately between stimulus concentrations that elicited minimal or maximal licking responses. That is, adding FFAs to the taste stimuli tended to not affect the licking responses at the lowest and highest concentrations of each tastant where floor and ceiling effects of the licking response are typically observed but rather acted to shift the transitional section of the licking response curve to the left and upward for appetitive taste stimuli and to the left and downward for aversive taste stimuli. These shifts in the licking response curves would be expected in response to a perceived increase in the intensity of the tastant such that concentrations previously deemed less appetitive or aversive appear to gain hedonic strength with the addition of the FFAs.

Consistent response rates and low latency values indicated good experimental control across all phases that, respectively, tested the effect of adding linoleic acid, oleic acid, and the linoleic–oleic acid mixture to the battery of taste stimuli. Linoleic acid was predicted to be a stronger stimulus than oleic acid based on previously published research using 5-min, 2-bottle preference tests between 1% (35.7 mM) linoleic and 1% (35.4 mM) oleic acids in which rats consumed 4-fold more linoleic acid than oleic acid (Tsuruta et al. 1999). Furthermore, although Gilbertson and colleagues have reported that both linoleic and oleic acids inhibit a delayed rectifying potassium channel in isolated rat taste receptors, there is a differential sensitivity for each FFA. Polyunsaturated fatty acids, such as 10 µM linoleic acid, are capable of depolarizing taste receptor cells harvested from either the fungiform papillae or the circumvallate papilla, but a subset of monounsaturated fatty acids, including oleic acid, only appears to depolarize taste receptor cells harvested from the circumvallate papilla with no effect on taste receptor cells collected from the fungiform papillae (Gilbertson et al. 1997, 1998; Hansen et al. 2003). This differential sensitivity of the taste receptor cell population, such that receptors in both the anterior and posterior taste buds respond to linoleic acid but only the posterior taste buds respond to oleic acid, would also predict that linoleic acid

could be a more effective modulator of licking behavior than either the oleic acid or the linoleic and oleic acid mixture. Using our current methodology, we were not able to demonstrate statistically significant differences between the effectiveness of linoleic acid, oleic acid, and the linoleic–oleic acid mixture in modulating the licking responses to a wide variety of taste stimuli; however, this does not rule out potential differences in the effectiveness of linoleic and oleic acids as modulatory stimuli. Our study was limited to the use of a single concentration of each FFA applied to a series of taste stimuli concentrations in which the FFAs tended to be most effective in altering the responses to moderate concentrations of each tastant. A future study examining the effect of multiple concentrations of the FFAs applied to moderate concentrations of taste stimuli may represent a better model with which to quantify the relative strength of each FFA.

An early series of experiments conducted by Smith et al. (2000) suggested that corn oil produced salient, nontactile orosensory cues through the demonstration that rats formed stimulus generalizations between corn oil alone and a sucrose and corn oil mixture in a manner that identified corn oil rather than the appetitive sucrose tastant as the salient feature of the mixture. Furthermore, short-term intake tests suggested a role for gustation in the selection of corn oil as the salient feature of the sucrose and corn oil mixture. In a series of taste aversion experiments examining stimulus generalizations, Smith and colleagues (2000) tested one FFA, 22 µM linoleic acid, reporting that although the rats could discriminate the presence of linoleic acid in a solution of linoleic acid and sucrose, sucrose rather than linoleic acid appeared to be the dominant stimulus. Our findings support the idea that the addition of FFAs to taste solutions acts to increase the perceived intensity of concomitant tastants rather than contributing an additional dominant taste sensation. In addition, Smith et al. (2000) found that corn oil and 22 µM linoleic acid formed reciprocal stimulus generalizations with one another. Because rats show a strong preference for corn oil and it appears that FFAs are a salient feature of corn oil, we examined the effectiveness of applying a mixture of linoleic and oleic acids that approximated the proportion of each of these 2 principle FFAs found in corn oil to our battery of taste stimuli. The linoleic–oleic acid mixture was found to be equally effective in shifting the licking responses to sweet stimuli upward and reducing the licking responses to salt, sour, and bitter stimuli as either linoleic acid or oleic acid alone. The lack of a synergistic effect of the FFA mixture suggests that linoleic and oleic acids likely act on the same transduction mechanism as predicted by isolated taste receptor cell electrophysiological recordings (Gilbertson et al. 1997, 1998; Hansen et al. 2003).

The current study contributes to a growing collection of data supporting the ability of FFAs to influence gustatory behavior. Postingestive influences were minimized through short-term (20 s) stimulus trials and low caloric–density solutions. Nongustatory orosensory cues were minimized

through negligible viscosity changes, and a constant airflow across the stimulus rack limited the use of olfactory cues as evidenced by consistently low latencies until the first lick of each trial. Across 3 testing phases utilizing 2 FFAs and a mixture of both fatty acids, rats consistently altered their licking to all 5 prototypical taste stimuli concentration series in a manner reflective of a perceived increase in stimulus intensity. Our results are in agreement with the previous isolated rat taste receptor cell recordings that showed increased depolarization when FFAs are concomitantly present with another taste stimulus. Our results also agree with human studies citing increased palatability as a primary influence in the overconsumption of dietary fat.

Several major questions remain in the investigation of the ability of FFAs to influence ingestive behavior through the gustatory system. These questions include whether increasing the concentration of the FFAs will produce a dose-dependent shift of the licking responses to appetitive and aversive taste stimuli. In addition, the ability of FFAs to influence afferent gustatory neural signals must also be characterized through in vivo neural recordings. Finally, it is important to replicate the influence of FFAs on taste as described in the rodent model using human research studies. A more complete understanding of the influence of FFAs on the gustatory system may result in the future manipulation of foods such that the palatability associated with dietary fats can be retained without the high caloric density of the fats.

Acknowledgements

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