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Orosensory Detection of Fatty Acids by Obesity-Prone and Obesity-Resistant Rats: Strain and Sex Differences

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Abstract

A series of brief-access (15s) behavioral assays following the formation of a conditioned taste aversion (CTA) to linoleic acid were performed in order to follow up on observations showing differences in the chemosensory responses to dietary fat in obesity-prone (Osborne-Mendel [O-M]) and obesity-resistant (S5B/Pl) rat strains. Strong aversions to linoleic acid (conditioned stimulus 100 μM) were generated in both O-M and S5B/Pl rats to concentrations as low as 2.5 μM. Observed strain differences were in contrast to expectations based upon electrophysiological studies previously showing greater fatty acid–induced inhibition of delayed rectifying K^+ channels in S5B/Pl rats. In the CTA assays, the O-M rats showed aversions at lower fatty acid concentrations with more resistance to extinction in brief-access orosensory tests, suggesting that the obesity-prone strain may be more sensitive in the detection and subsequent avoidance of linoleic acid than the obesity-resistant strain. The independent variable of sex produced even greater differences in the avoidance of linoleic acid following conditioning than the effects of strain. Female rats of both strains were significantly more sensitive to fatty acids, showed greater cross-generalization from linoleic to oleic acid, and showed greater avoidance of linoleic acid than male counterparts. These findings suggest genetic influences on yet to be identified mechanisms potentially within the gustatory system that affect the sensitivity to detect the fatty acid chemicals found in dietary fat during brief-access orosensory testing.

Key words: behavior, dietary fat, fatty acid, obesity, taste

Introduction

The incidence of obesity worldwide continues to escalate and with it there has been a corresponding increase in cardiovascular disease, diabetes, cancer, and other nutrition-related disorders. Given that an increase in dietary fat intake is regarded as one of the factors closely linked with the obesity epidemic (Bray and Popkin 1998, 1999; Bray et al. 2004), it has become increasingly important to understand the sensory cues responsible for the recognition of dietary fat. To this end, there has been an increase in research in recent years that has attempted to identify the ability of fats to activate the gustatory system, consistent with there being a taste of fat. Although the textural properties of fats have been suggested to represent its most salient sensory cue (Rolls et al. 1999; Verhagen et al. 2003), the ability to detect the presence of dietary fat is maintained in studies designed to minimize or mask the textural cues of fats (Elizalde and Sclafani 1990; Mindell et al. 1990; Greenberg and Smith 1996; Smith et al. 2000; Takeda et al. 2001; Chalé-Rush et al. 2007a, 2007b), suggesting a role for nontextural orosensory cues in dietary fat detection. Furthermore, there have been a number of mechanisms proposed to account for the ability of fats, specifically fatty acids, to activate taste cells. The putative ''receptors'' for fatty acids include fatty acid–sensitive delayed rectifying K^+ (DRK) channels (Gilbertson et al. 1997, 1998, 2005), the fatty acid–binding protein, CD36 (Baillie et al. 1996; Fukuwatari et al. 1997; Laugerette et al. 2005; Sclafani et al. 2007), and in a preliminary report, several members of the family of fatty acid–activated G protein–coupled receptors (GPCRs) (Hansen et al. 2006), a finding verified for one of the GPCRs in posterior tongue (Matsumara et al. 2007). Though several receptive mechanisms have been proposed, it is not clear if they function independently or, as in the case of insulin-secreting pancreatic β cells (Feng et al. 2006; Gromada 2006), as part of an integrated transduction pathway for fatty acids.

Of the fatty acid transduction pathways proposed in the gustatory system, the inhibition of DRK channels by fatty acids and subsequent activation of taste cells have been the most intensively investigated at the cellular level. The mechanism proposed involves a direct inhibition of DRK channels by unsaturated fatty acids, especially polyunsaturated fatty acids (PUFAs), in the low micromolar range ($EC_{50} \sim 1$ μM; Gilbertson et al. 2005), consistent with fatty acid concentrations found in the oral cavity during fat feeding (Kawai and Fushiki 2003). The importance of DRK channels in the repolarization of electrically excitable cells following activity has led to the suggestion that inhibition of DRK channels may play a role in enhancing stimulus-induced responses in taste cells (Gilbertson et al. 1997). Behavioral studies in rodents (Pittman et al. 2006) support the ability of PUFAs (e.g., linoleic acid) to act as a taste enhancer. Human psychophysical studies have been more ambiguous with some evidence supporting the role of linoleic acid as a taste enhancer (Kamphuis et al. 2003), whereas other evidence suggests that the linoleic acid may either not affect perceived intensity or increase the threshold for salty, sour, and bitter taste detection (Mattes 2007). However, there is emerging evidence from both rodent (Pittman et al. 2007) and human (Chalé-Rush et al. 2007a, 2007b) behavioral research to support the potential of fatty acids to generate taste sensations in absence of other taste stimuli.

Osborne-Mendel (O-M) and S5B/Pl (S5B) rats have long been used as models of obesity-prone and obesity-resistant rats. One of the defining phenotypes of these rats is that on chow (grain) diets, O-M rats are typically about 50% heavier than S5B rats; however, on a high-fat diet, O-M rats are over twice as heavy, eat more fat, and have significantly greater fat depositions as S5B rats (Schemmel et al. 1970, 1972). Recently, we followed up on a preliminary study (Gilbertson et al. 1998) that showed differences in fatty acid responsiveness in taste cells from obesity-prone and obesity-resistant rats using patch-clamp recording on taste cells from the anterior tongue (i.e., fungiform papillae). In an obesity-resistant rat model (S5B/Pl; Okada et al. 1992), outward K^+ current through DRK channels was inhibited to a significantly greater degree than that from O-M rats, an obesity-prone strain (Gilbertson et al. 2005), though inhibition constants were identical in the 2 strains ($EC_{50} \sim 1$ μM for effective fatty acids; see Table 2 in Gilbertson et al. [2005]). Having measured expression of all 9 DRK channel types in fungiform taste cells in S5B and O-M rats, we proposed a model to account for the expression data and the differences in cellular responses to fatty acids in S5B and O-M rats that was predicated on the hypothesis that different subfamilies of DRK channels (KCNA, KCNB, and KCNC) were differentially sensitive (or insensitive) to fatty acids. Thus, the greater responsiveness of taste receptor cells in S5B rats was due, we hypothesized, to expression of a greater ratio of fatty acid–sensitive:fatty acid–insensitive DRK channels than was found in O-M rats (cf., Figures 3 and 7 in Gilbertson et al. [2005]).

In the present study, we have begun to explore in vivo differences in the responsiveness to fatty acids in S5B and O-M rats in the context of our previous cellular and molecular data. By establishing a LiCl-induced conditioned taste aversion (CTA) for linoleic acid (100 μM), we have compared the subsequent strength of the aversion formed in both males and females in these 2 rat strains and the generalized avoidance to other fatty acids using brief-access taste testing procedures.

Materials and methods

Subjects

Four groups of adult (>60 days) rats were used in the present study. The 80 rats were equally divided into groups of 20 by strain (O-M or S5B) and sex. Each group of O-M and S5B males and females was further assigned to categories to receive either LiCl (experimental manipulation, CTA) or saline (control) injections during testing. Two rats were removed from the analysis due to their failure to lick to water or test stimuli during the behavioral testing resulting in the following sample sizes: O-M male LiCl, $n = 10$; O-M female LiCl, $n = 10$; O-M male NaCl, $n = 10$; O-M female NaCl, $n = 10$; S5B male LiCl, $n = 10$; S5B female LiCl, $n = 10$; S5B male NaCl, $n = 9$; and S5B female NaCl, $n = 9$. All rats were bred at the Laboratory Animal Research Center at Utah State University and reared on Harland Teklad 8604 rodent chow provided ad libitum prior to and after being shipped to Wofford College where the behavioral studies were carried out. Rats were individually housed in clear polycarbonate cages on a 12:12 h light:dark cycle with lights on at 7:00 AM at both institutions. Rats had ad libitum access to water until 6 days prior to conditioning and testing at which time the rats were placed on a 23-h water restriction schedule for the duration of the experiment. At the time of testing, there were significant differences between the mean body weight of the obesity-prone (males 298.4 ± 6.2 g, females 211.6 ± 3.9 g) and obesity-resistant strains (males 230.4 ± 6.2 g, females 144.4 ± 1.9 g); therefore, all unconditioned stimulus (US) injections were dose dependent on body weight. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the Institutional Animal Care and Use Committees of Wofford College and Utah State University.

Stimuli

All taste stimuli were mixed daily from reagent grade chemicals (Sigma-Aldrich, St Louis, MO) and presented at room temperature. Prior to use, fatty acids were kept stored in a freezer at −20 °C. The fatty acid stimuli consisted of lauric acid and the water-soluble forms of linoleic acid (sodium linoleate) and oleic acid (sodium oleate). Although the natural hydrolysis of triglycerides found in dietary fat produces

unesterified fatty acids in the oral cavity, previous research has shown that rats detect the sodium salt forms of fatty acids such as linoleate and oleate in manners similar to the unesterified linoleic and oleic acids (McCormack et al. 2006). Using the unesterified acids as test stimuli requires ethanol to facilitate dissolution, whereas using the aqueous linoleate and oleate as test stimuli results in the presence of sodium ions; however, the concentrations of linoleate and oleate used in this experiment would produce sodium ion concentrations less than 100 μM which should not present a taste stimulus confound as 100 μM is far below the threshold of sodium detection (≈5–10 mM). Fatty acid stimulus concentrations were selected to be similar to concentrations previously shown to activate O-M and S5B taste receptor cells (Gilbertson et al. 2005), and the conditioned stimulus (CS) of 100 μM linoleic acid was selected based on previous reports that this is a suprathreshold concentration for the Sprague–Dawley strain of rat (McCormack et al. 2006; Pittman et al. 2007). In addition to water, there were 9 test stimuli consisting of 2.5, 5, 20, 50, 75, and 100 μM linoleic acid, 50 and 100 μM oleic acid, and 100 μM lauric acid. The viscosity and pH of micromolar concentrations linoleic and oleic acids have been shown to be similar to distilled, deionized water (McCormack et al. 2006).

CTA paradigm

Taste aversions were conditioned through 3 consecutive daily pairings of the CS and the US. At 9:00 AM on each conditioning day, rats were given 10-min access to a single bottle containing 100 μM linoleic acid as the CS. Consumption of the CS was measured by the difference in bottle weight (0.01 g resolution) before and after the 10-min access period. Rats that consumed less than 2 g of CS received a 5-ml intraoral application of the CS solution. Approximately 30 min following CS consumption, the US was administered through intraperitoneal injections (20 ml/kg body weight dosage) of 150 mM LiCl to induce gastric distress or 150 mM NaCl (saline) as a control condition. All rats receiving a LiCl injection showed behavioral signs of gastric malaise, the unconditioned response, within 45 min of the injection. Signs of gastric malaise include lying in an extended prone position, immobility, and a lack of rearing or cage exploration compared with the saline-injected controls. At 4:15 PM on each of the water restriction days, all rats were given 45-min access to water.

Behavioral training and testing procedures

All testing was conducted in the MS-160 Davis Rig gustatory behavioral apparatus (DiLog Instruments, Tallahassee, FL). The Davis Rig measures rat licking behavior at a resolution of 1 ms during the controlled presentation of up to 16 taste stimuli as previously described (Smith 2001; Pittman et al. 2006). The Davis Rig is housed inside an acoustic isolation chamber utilizing a white noise generator. Intake and exhaust fans are located on opposing walls of the chamber in order to direct constant airflow along the longitudinal axis of the stimulus delivery tray serving to reduce olfactory cues for any given stimulus. Rats were trained to lick during water stimulus trials in the Davis Rig for 3 consecutive days prior to the initial conditioning day. Following the third conditioning day, 3 consecutive days of testing in the Davis Rig assessed the formation of conditioned and generalized taste aversions. Each daily test session consisted of 4 blocks of 12 trials with stimulus durations of 15 s, wait times for the first lick of 45 s, and interstimulus intervals of 15 s. Each block included 1 trial of each test stimulus and 3 trials of water stimuli. The stimulus order within each block was randomly assigned.

Data analysis

The latency until first lick and total number of licks per stimulus were averaged across the 4 trials per test session. All rats included in the data analysis sampled each stimulus at least once during each daily test session. Trials in which the rat did not lick were excluded from analysis. The mean number of licks per trial was normalized using a lick ratio (licks per test stimulus/licks to water) in order to account for individual variances in the water-restricted motivation across the rats. A repeated measures mixed factorial analysis of variance analyzed the main effects and interactions of the between-subject (injection, sex, and strain) and within-subject (test day and concentration) independent variables. Post hoc paired *t*-test analyses were used to identify the source of significant effects and interactions. A criterion of $P < 0.05$ was used to report significant results.

Results

CTA to linoleic acid

In both strains and sexes of rats used in the present study, 3 consecutive daily pairings of linoleic acid (100 μM) with the LiCl injection resulted in the formation of a robust CTA. As shown in Table 1, there were significant decreases in CS

Table 1 Mean consumption (±SEM) of the CS

	Day 1	Day 2	Day 3
O-M male LiCl	12.5 ± 1.3	4.6 ± 0.4	3.0 ± 0.6
O-M male NaCl	12.1 ± 0.5	11.8 ± 0.8	12.7 ± 1.1
O-M female LiCl	9.5 ± 0.6	5.5 ± 0.4	2.5 ± 0.5
O-M female NaCl	10.7 ± 0.5	11.9 ± 0.4	11.9 ± 0.3
S5B/P1 male LiCl	11.2 ± 0.6	4.5 ± 0.4	2.1 ± 0.5
S5B/P1 male NaCl	11.2 ± 0.7	12.3 ± 0.9	12.5 ± 0.6
S5B/P1 female LiCl	8.9 ± 0.4	2.5 ± 0.4	1.9 ± 0.5
S5B/P1 female NaCl	9.0 ± 0.5	8.2 ± 0.5	9.4 ± 0.5

consumption by the LiCl-injected groups on both conditioning days 2 and 3 (main effect injection: $F_{1,70} = 366.058$, $P < 0.01$; interaction injection/days: $F_{2,140} = 137.425$, $P < 0.01$). As might be expected, due to increased body weight, males of both strains consumed more CS solution on the initial conditioning day than female rats; however, there was no significant difference between male and female consumption of the CS after CTA formation on day 2 or 3. Furthermore, there was no difference in the CS consumption between the strains on any of the conditioning days. Therefore, differences in the strength of the CTA observed during testing days cannot be explained by differential exposure to the CS during the conditioning period.

Following conditioning, for 3 consecutive testing days, the avoidance of linoleic acid was further assessed across decreasing concentrations in discrete trials of 15 s. Examining the lick ratios for linoleic acid across the 3 days of testing revealed a significant main effect of injection ($F_{1,70} = 295.287$, $P < 0.01$) on the lick ratios of linoleic acid meaning that overall there were reduced responses to linoleic acid across the concentrations for LiCl-injected groups (0.57 ± 0.03) compared with the NaCl-injected groups (0.98 ± 0.03). In addition, there were multiple significant interactions, indicating that the avoidance of linoleic acid during the testing was affected not only by the injection condition but also by the conditions of sex, day of testing, and concentration of linoleic acid tested. The significant interaction between injection and sex ($F_{1,70} = 5.937$, $P < 0.01$) reflected the overall greater avoidance of linoleic acid across concentrations by females (LiCl, 0.53 ± 0.03; NaCl, 1.00 ± 0.04) than by males (LiCl, 0.60 ± 0.03; NaCl, 0.96 ± 0.02) across the 3 testing days. There was also a significant 4-way interaction between injection, strain, concentration, and testing day ($F_{10,700} = 2.123$, $P < 0.05$) indicating that the O-M and S5B rats showed different patterns of avoidance across the 3 test days warranting an examination of the cohort group lick ratios for each concentration of linoleic acid on each test day. As shown in Figure 1, the avoidance of linoleic acid was strongest on test day 1 with extinction of the CTA lessening the avoidance of the lower linoleic acid concentrations on testing days 2 and 3 (injection/day/concentration interaction $F_{10,700} = 3.224$, $P < 0.01$). Post hoc paired *t*-tests revealed significant ($P < 0.05$, as indicated on Figure 1) avoidance of almost all linoleic acid concentrations, with the exception of 2 μM for the female O-M group, by all LiCl-injected groups on test day 1. The strength of the conditioned aversion on the first day of testing hindered the ability to examine differences in the sensitivity to linoleic acid due to a floor effect as both strains and sexes of rats were maximally avoiding all but the lowest linoleic acid concentrations; however, as the conditioned aversion weakened on testing day 2, strain and sex differences in the sensitivity to linoleic acid became evident. The emergence of group differences in the avoidance of linoleic acid following the first day of testing was not unexpected, as on testing day 1, the data show a floor effect with all groups robustly avoiding linoleic acid to the maximum extent. In contrast, by third testing day, the extinction of the conditioned aversion had progressed such that the most groups were no longer avoiding the linoleic acid compared with the cohort control groups. Therefore, on testing day 2 when the animals are strongly influenced by neither the conditioned aversion state nor the water-restricted motivation to lick solutions, strain and sex differences are most likely to be observed.

Analysis of the lick ratios for linoleic acid on testing day 2 revealed significant interactions between injection and sex ($F_{1,70} = 9.556$, $P < 0.01$) and injection and strain ($F_{1,70} = 3.690$, $P < 0.05$), respectively, indicating a greater avoidance of linoleic acid for females (0.51 ± 0.07) compared with males (0.69 ± 0.09) and O-M rats (0.55 ± 0.08) compared with S5B rats (0.65 ± 0.09) across all concentrations. Figure 2 redraws the testing day 2 lick ratio data for LiCl-injected groups in order to compare the effects of sex within the strains (O-M, Figures 2A and S5B, Figure 2B) and the effect of strain within each sex (females, Figure 2C and males, Figure 2D). Post hoc paired *t*-tests revealed significantly greater avoidance of linoleic acid by female than male LiCl-injected O-M rats (50 μM, $t_9 = 2.281$, $P < 0.05$; 75 μM, $t_9 = 2.570$, $P < 0.05$) and S5B rats (2.5 μM, $t_9 = 2.430$, $P < 0.05$; 75 μM, $t_9 = 2.338$, $P < 0.05$; 100 μM, $t_9 = 2.781$, $P < 0.05$) for concentrations at which the animals were licking neither maximally (no avoidance) nor minimally (complete avoidance). Likewise, within each sex, the O-M rats significantly avoided linoleic acid more than S5B rats, with the greater effects seen in the more sensitive female rats (2.5 μM, $t_9 = 2.214$, $P = 0.05$; 20 μM, $t_9 = -2.308$, $P < 0.05$; 50 μM, $t_9 = -2.330$, $P < 0.05$) than male rats (50 μM, $t_9 = -2.239$, $P = 0.05$).

Changes in the patterns of avoidance of linoleic acid on testing day 2 are unlikely due to differential exposure to linoleic acid on the first day of testing as there was no significant difference between the cumulative number of licks to linoleic acid (O-M male 28.3 ± 10.5, O-M female 28.5 ± 9.9, S5B male 20.5 ± 8.7, S5B female 23.9 ± 11.0) within the LiCl injection conditions on test day 1. Furthermore, the licking responses to water trials across the 3 testing days were consistent across injection conditions and within each sex and strain condition. There were no significant main effects of injection or interactions between injection and sex or injection and strain for the cumulative number of licks to water on any of the testing days indicating that regardless of CTA condition, O-M rats (day 2: LiCl males 88.9 ± 4.3, NaCl males 85.5 ± 3.9; LiCl females 80.2 ± 3.3, NaCl females 81.2 ± 4.7) and S5B rats (day 2: LiCl males 91.2 ± 4.4, NaCl males 92.7 ± 3.0; LiCl females 94.2 ± 5.1, NaCl females 88.6 ± 7.1) licked equivalently during the water stimulus trials.

Generalization of the CTA to other fatty acids

Our previous electrophysiological assays characterizing the fatty acid specificity of inhibition of DRK channels in male

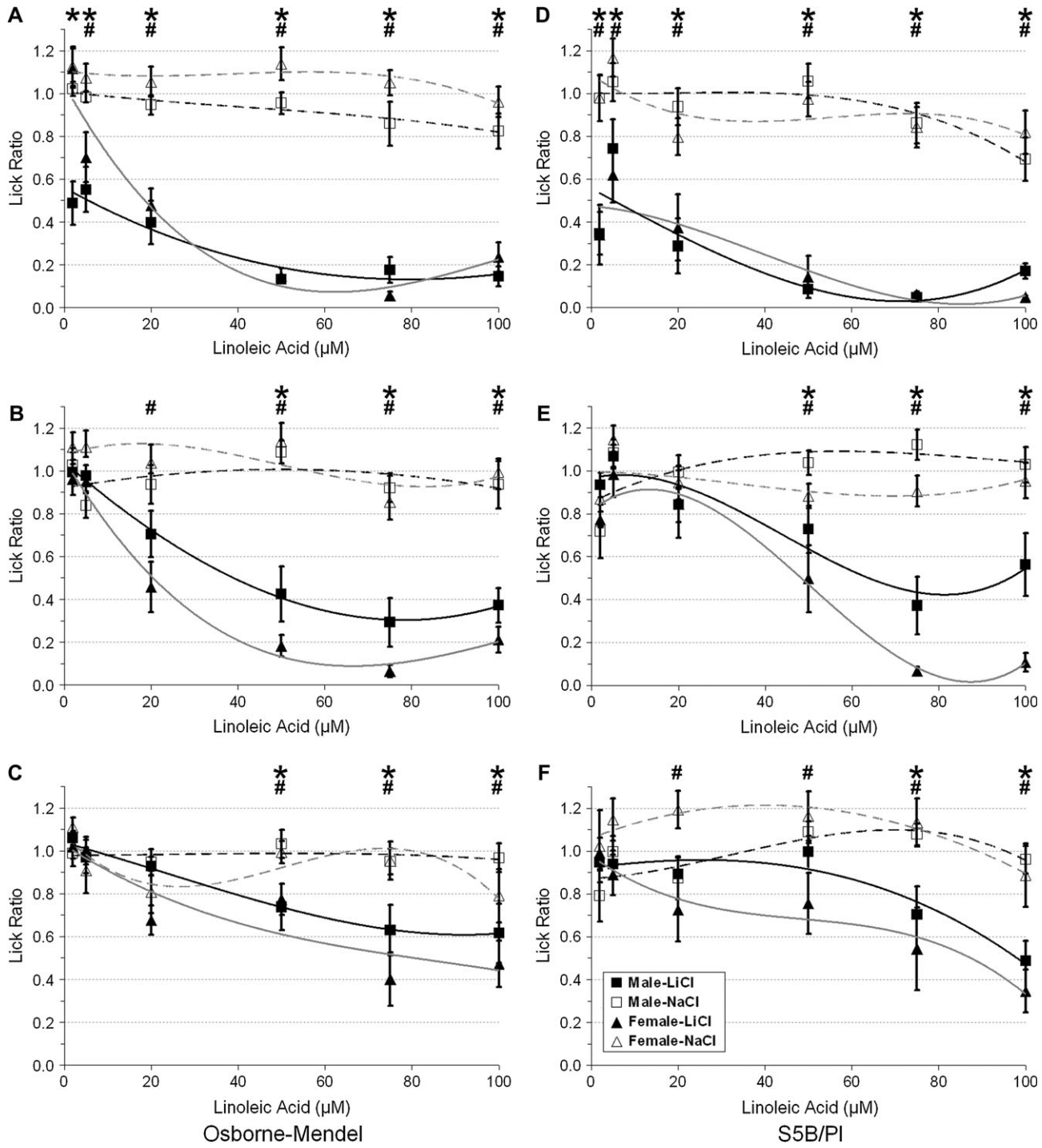

Figure 1 Mean lick ratios (±standard error of the mean) for linoleic acid organized by rat strain on day 1 (O-M, **A**; S5B, **D**), day 2, (O-M, **B**; S5B, **E**), and day 3 (O-M, **C**; S5B, **F**). Asterisks indicate significant differences between male LiCl-injected (closed square, solid black line) and NaCl-injected (open square, dashed black line) groups ($P < 0.05$), and numeric symbols indicate significant differences between female LiCl-injected (closed triangle, solid gray line) and female NaCl-injected (open triangle, dashed gray line) groups ($P < 0.05$).

rats have shown that anterior (fungiform) taste buds respond to PUFAs, whereas those in the posterior tongue (foliate/vallate taste buds) respond to both monounsaturated fatty acids and PUFAs (Hansen et al. 2003). Taste cells from neither area were responsive to saturated fatty acids. To test the generalization of the linoleic acid CTA to other fatty acid types, we included 2 concentrations of a monounsaturated fatty acid, oleic acid, and a single concentration of a saturated fatty acid, lauric acid, among our test stimuli. Across the 3 test days, there was an overall main effect of injection

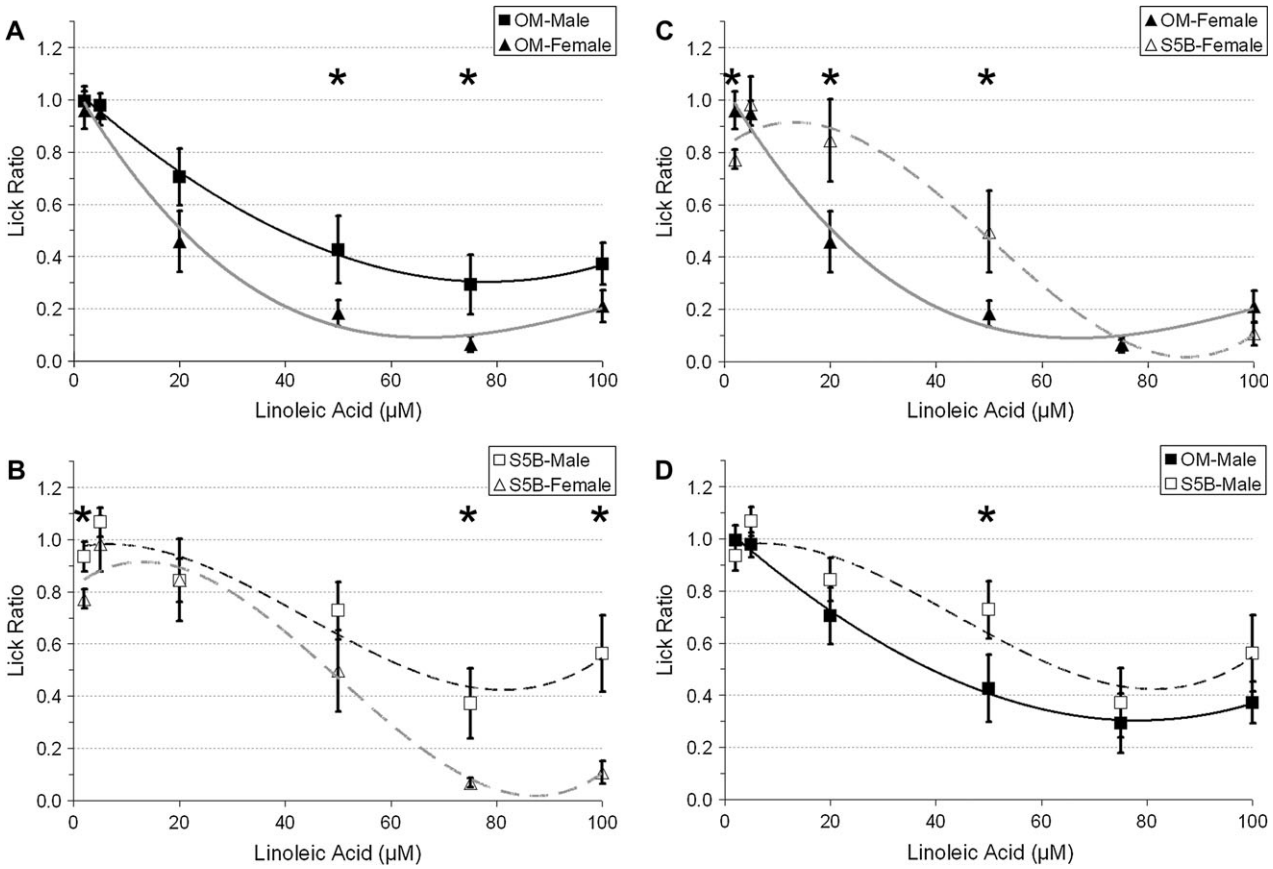

Figure 2 Mean lick ratios (±standard error of the mean) for linoleic acid from LiCl-injected groups on test day 2 comparing sex differences within the O-M **(A)** and S5B/Pl **(B)** strains and strain differences within the female **(C)** and male **(D)** rats. Asterisks indicate significant differences between comparison groups ($P < 0.05$).

($F_{1,70} = 25.998$, $P < 0.01$) on the lick ratios for oleic acid as well as multiple significant interactions including injection by strain ($F_{1,70} = 7.028$, $P < 0.01$) and injection by sex ($F_{1,70} = 7.379$, $P < 0.01$) indicating, respectively, that across sex the LiCl-injected O-M rats (0.77 ± 0.03) showed greater generalized avoidance of oleic acid than the LiCl-injected S5B rats (0.97 ± 0.03) and within each strain female rats (0.83 ± 0.04) showed greater avoidance than male rats (0.91 ± 0.03) across all 3 testing days. Furthermore, there was a significant 3-way interaction between injection, concentration, and testing day ($F_{2,140} = 4.629$, $P < 0.01$) as well as a 4-way interaction between injection, strain, sex, and testing day ($F_{2,140} = 6.239$, $P < 0.01$). As can be seen in Figure 3, these interactions indicate that the generalized avoidance of oleic acid varied according to both test day and concentration of oleic acid with the greatest avoidance demonstrated on day 1. Furthermore, the interactions between injection and both sex and strain are clearly shown in Figure 3 with the order of the strength of generalized avoidance of 100 μM oleic acid being O-M male = S5B female < O-M female (Figure 3A). Compared with the saline-injected controls, the LiCl-injected, male O-M rats and female S5B rats only avoided 100 μM oleic acid (O-M, $t_9 = -4.708$, $P < 0.01$; S5B, $t_8 = -2.321$, $P < 0.05$) on the first testing day. Whereas, the LiCl-injected, female O-M rats avoided both 50 μM ($t_9 = -3.411$, $P < 0.01$) and 100 μM ($t_9 = -8.336$, $P < 0.01$) oleic acid on the first testing day. On the second testing day, the aversion of oleic acid for LiCl-injected, female O-M rats (Figure 3C) was lessened but still significantly present (50 μM, $t_9 = -2.636$, $P < 0.05$; 100 μM, $t_9 = -3.934$, $P < 0.01$) compared with the licking responses of saline-injected, female O-M rats (Figure 3D). Thus, strain and sex differences in sensitivity to oleic acid were evident such that the obesity-prone rats showed greater generalized avoidances than the obesity-resistant rats and female rats showed greater generalized avoidances of oleic acid than male rats. Overall, the strength of the generalized avoidance to oleic acid was less than the conditioned avoidance of linoleic acid that is consistent with previous research that proposed the sensory salience of oleic acid to be less than linoleic acid (Hansen et al. 2003; Pittman et al. 2007).

This same sex difference in sensitivity to fatty acids was also observed in a generalized avoidance of lauric acid with an overall significant main effect of injection ($F_{1,70} = 41.605$, $P < 0.01$) and a significant interaction between injection and sex ($F_{1,70} = 24.418$, $P < 0.01$). As shown in Figure 3A,B, the LiCl-injected male rats do not avoid lauric acid, whereas

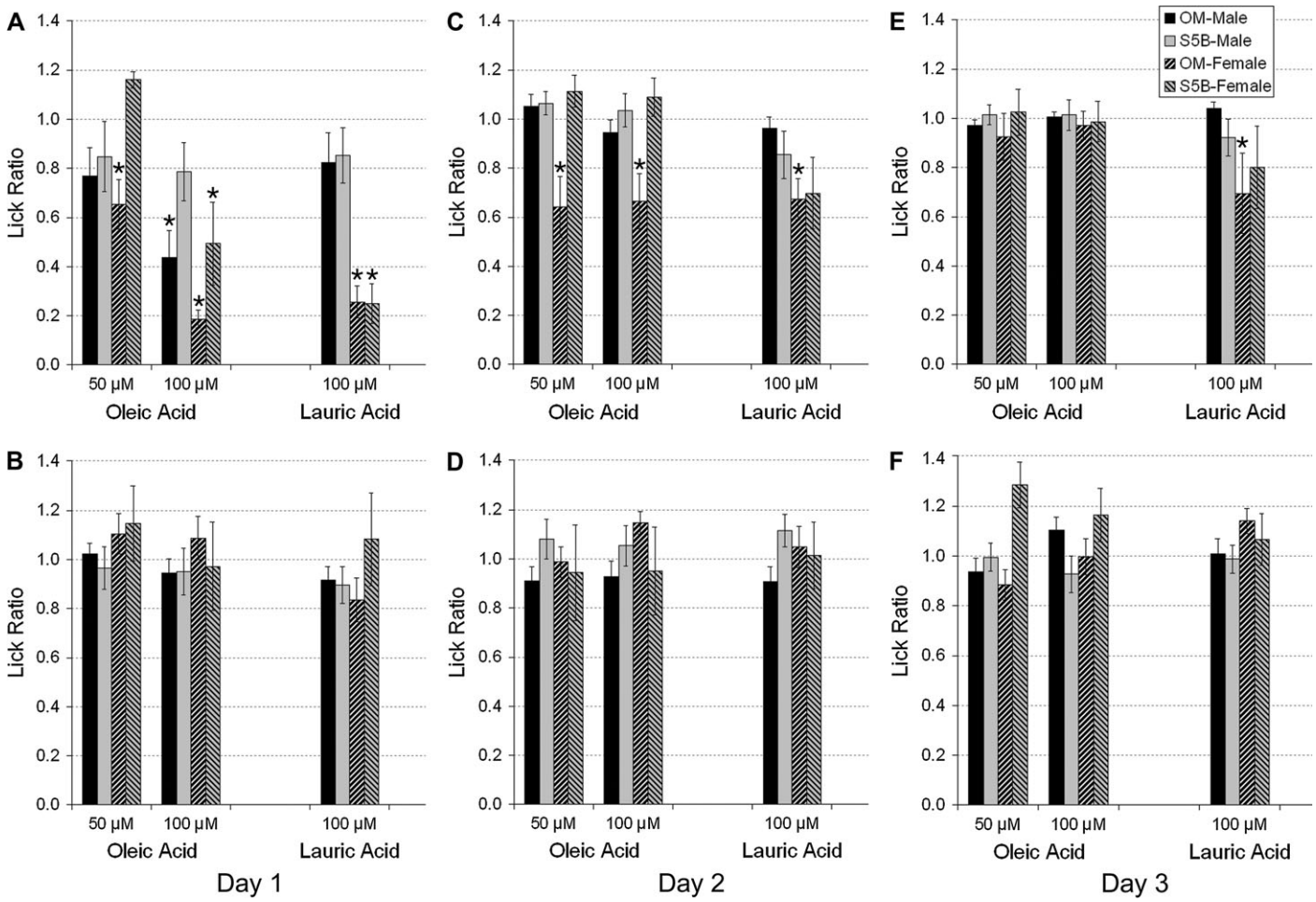

Figure 3 Mean lick ratios (±standard error of the mean) for oleic and lauric acids from the 4 cohort groups across the 3 days of testing with LiCl-injected groups shown in the top panels **(A, C, and E)** and NaCl-injected groups shown in the bottom panels **(B, D, and F)**. Asterisks indicate significant differences ($P < 0.05$) between LiCl-injected and NaCl-injected groups within the same cohort group on a given test day.

both LiCl-injected O-M and S5B female groups significantly avoid lauric acid when compared with the female NaCl-injected O-M ($t_9 = -5.591$, $P < 0.01$) and S5B ($t_8 = -3.805$, $P < 0.01$) groups on test day 1. As shown in Figure 3C, there is rapid extinction of the conditioned generalized avoidance of oleic and lauric acid on test day 2 with only the LiCl-injected, female O-M rats weakly avoiding lauric acid on test day 2 ($t_9 = -3.134$, $P < 0.01$) and test day 3 ($t_9 = -2.739$, $P < 0.05$).

Latency to lick during test trials

Brief-access tests of licking responses to tastants are designed to minimize extraneous influences such as postingestive feedback and hunger/satiety states, thereby maximizing the ability to associate licking behavior with the influences of immediate sensory signals such as olfaction, somatosensation, and gustation. With that said, within our gustatory behavioral testing parameters, it is not feasible to control for all orosensory cues and, therefore, we cannot rule out roles for somatosensation or olfaction in favor of gustatory detection of fatty acids. We have previously measured the viscosity and pH of 88 µM concentrations of fatty acids and found no differences between fatty acid solutions and water (McCormack et al. 2006), suggesting that viscosity is unlikely a salient somatosensory cue for detecting fatty acids. We have also made efforts to control olfactory cues by directing constant airflow along the axis of the stimulus tray to mix olfactory cues across the stimuli, and we assessed the latency until the first lick of each trial as measure of olfactory influences on our data. Particularly with regard to taste avoidance testing, latency times that are longer for stimulus trials than for water trials are indicative of rats using olfactory cues to detect and avoid consuming aversive stimuli. As shown in Figure 4, there were no significant differences in the latency until the first lick across the stimuli, linoleic acid, oleic acid, lauric acid, or water, for the strain, sex, or injection conditions. Although rats were given a 45-s period to initiate the first lick before a trial was terminated, the average latencies until the first lick were very brief, ranging from

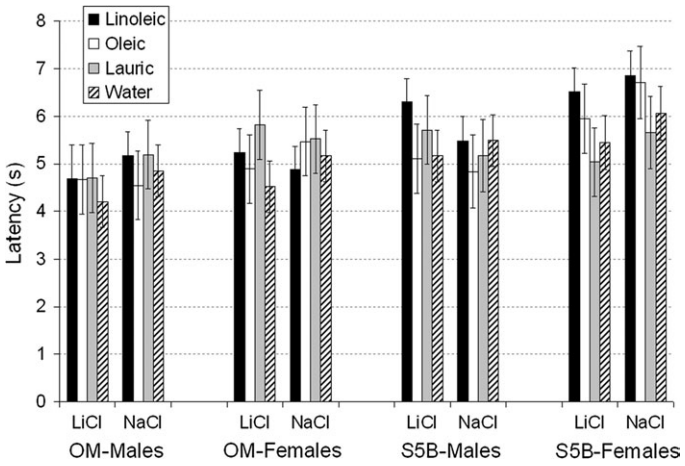

Figure 4 Mean latency (±standard error of the mean) in seconds until the first lick during testing trials for linoleic acid, oleic acid, lauric acid, and water from the 4 cohort groups organized by injection condition.

4.2 to 6.5 s with standard errors ranging from 0.5 to 0.8 s, indicating little variability across trials and stimuli that is indicative of good stimulus control and minimal olfactory influences on licking behavior.

Discussion

An obesity-prone strain, O-M, and an obesity-resistant one, S5B/Pl (S5B), have been shown to exhibit marked differences in the preferences for dietary fat in 3-choice macronutrient selection tests (Okada et al. 1992). Gilbertson et al. (1998, 2005) have shown that these 2 strains also differ markedly in their taste cell responses to fatty acids, specifically PUFAs like linoleic acid. That is, in vitro patch-clamp recordings show that fatty acids inhibited DRK channels to a greater degree in taste cells from S5B rats than in the obesity-prone O-M rats, a difference that was linked to differential DRK channel expression between the 2 strains (Gilbertson et al. 2005). Interestingly, the same report showed that although the magnitude of inhibition differed, the concentration required to produce a half-maximal block of DRK channels in the 2 strains was identical (inhibition constants [IC_{50}] $\sim$ 1 μM for PUFAs). Based upon these data, it has been theorized that S5B rats would be more responsive to the sensory cues contained in dietary fat than O-M rats. Due to their ability to act as open-channel blockers of DRK channels (Gilbertson et al. 1997; Liu et al. 2005), fatty acids, including linoleic acid, have been suggested to alter the gustatory response to other tastants, a phenomenon verified in behavioral assays for both appetitive and aversive tastants (Pittman et al. 2006). Consistent with this notion and the effects of fatty acids on taste cells, a recent report showed that linoleic acid was more effective at enhancing preference for subthreshold concentrations of saccharin in S5B rats than in O-M rats in preference tests (Gilbertson et al. 2005). In the present study, we have attempted to use a CTA paradigm as a more direct measure to explore the innate behavioral sensitivity to fatty acids in these 2 rat strains that differ in their dietary preference for fat.

Strain differences

Based upon the discussion above, one might speculate that the S5B rats may form a stronger, more salient aversion to linoleic acid than the O-M rats. Alternatively, because the IC_{50} of DRK channels by fatty acids were identical in both strains, an equally plausible expectation would be that there would be no difference in the sensitivity to linoleic acid following the formation of the CTA to 100 μM linoleic acid. However, in the present study, in which rats were maintained on normal chow diets, we found evidence that both male and female obesity-prone O-M rats showed greater sensitivity for linoleic acid than the obesity-resistant S5B rats as supported by increased avoidance of middling concentrations of linoleic acid on day 2 (cf., Figure 2C,D). In general, the avoidance of lower concentrations of the CS in CTA paradigms is reflective of a greater sensitivity to the CS. Previous research by Pittman et al. (2007) using similar methodology conducted on the Sprague–Dawley strain of rat provided the basis for determining the linoleic acid concentrations for this specific study. In that study, male Sprague–Dawley rats did not avoid linoleic acid concentrations ≤20 μM and female Sprague–Dawley rats did not avoid linoleic acid concentrations ≤5 μM. Therefore, in the present study, we had sought to include concentrations spanning the subthreshold to the CS range, 2.5–100 μM, respectively. Unexpectedly, all LiCl-injected groups avoided 20, 5, and 2.5 μM concentrations of linoleic acid on the first testing day with the exception of female O-M rats that did not show avoidance to the 2.5 μM concentration. As we were unable to determine a threshold for the detection of linoleic acid, we were therefore unable to use threshold measurements as indicators of differential sensitivity to the fatty acids. Based on our findings from this initial examination of the behavioral responses to linoleic acid in O-M and S5B strains, future studies may provide additional evidence of differential sensitivity between strains and sexes by testing linoleic acid concentrations ranging from 0.2 to 20 μM concentrations. Given the strong avoidance to all concentrations of linoleic acid on the first testing day, our analyses of strain and sex differences were confined to data collected on the second testing day when the aversion to linoleic acid had weakened. In theory, differences in the expression of DRK channels or other fatty acid transduction mechanisms would be masked by maximal avoidance of a stimulus under the condition of a strong aversion as on conditioning days 2 and 3 and testing day 1, whereas differential sensitivities in the responsiveness to fatty acids could affect the licking responses to linoleic acid under the conditions of a weaker aversion such as testing day 2. On testing day 3, the patterns of cohort group avoidance of linoleic acid were similar in nature to day 2, although

the further extinction of the aversion had increased licking to a point that there were no discernable significant differences in the responses across concentrations. In addition to examining the responsiveness to linoleic acid concentrations spanning subthreshold levels, lessening the strength of the conditioned aversion through either weaker CS concentrations or fewer pairings of the CS and US may also represent effective means of further examining the differences in strain and sex sensitivity to fatty acids.

Our observations of differences in strain sensitivity to fatty acids run counter to the behavior previously predicted from electrophysiological assays showing that fatty acids, like linoleic acid, were more effective at inhibiting DRK channels in S5B rats than in O-M rats (Gilbertson et al. 1998, 2005). It has been hypothesized that a greater inhibition of fatty acid–sensitive DRK channels would result in greater activation of the taste cells and that the molecular mechanism underlying this was due to expression of a higher ratio of fatty acid–sensitive:fatty acid–insensitive DRK channels (Gilbertson et al. 2005). Moreover, in this same study, linoleic acid was more effective in enhancing preference for a subthreshold concentration of saccharin in S5B than in O-M rats consistent with the greater effect of linoleic acid on DRK channels in obesity-resistant rats (Gilbertson et al. 2005). A possible resolution to these apparent contradictory findings could center on the identification of additional putative fatty acid receptive proteins expressed in taste cells. The fatty acid–binding protein CD36, which had initially been identified by Fukuwatari et al. (1997), has been shown to be critical for fatty acid preference in preference tests using CD36-deficient mice (Laugerette et al. 2005; Sclafani et al. 2007). In addition, several types of long-chain PUFA-activated GPCRs have been shown to be expressed in rat taste buds including GPR120 (Gilbertson et al. 2007; Matsumara et al. 2007) and GPR40 (Hansen et al. 2006), both receptors for linoleic acid. Thus, it is possible that the taste recognition of fatty acids in isolation (i.e., as a taste primer) may reflect activity at CD36 and/or fatty acid–activated GPCRs, as yet unexamined in the O-M and S5B strains, whereas the ability of fatty acids to enhance taste perception (Pittman et al. 2006) may be mediated through the action of fatty acids on DRK channels (Gilbertson et al. 1997). Further experiments aimed to separate the role of fatty acids as taste primers from those where fatty acids may act on the perception of other sapid molecules will be required to distinguish among the contributions of these potentially differential fatty acid transduction mechanisms. Finally, although much research has focused on the recognition of fatty acids in the oral cavity of rodents, little is known about either the afferent neural coding for fatty acids or the processing of those afferent neural signals in the central nervous system. The differential sensitivity of the S5B and O-M rat strains evidenced in this CTA experiment may reflect differential higher order processing of the fatty acid afferent signals as opposed to differences at the level of fatty acid transduction mechanisms.

Alternatively, the differential sensitivity to fatty acids demonstrated between the S5B and O-M strains of rats may be related to differences in nongustatory fatty acid detection mechanisms. We attempted to minimize olfactory cues by directing constant airflow down the axis of the stimulus delivery tray in order to mix olfactory stimuli across stimulus tube presentations. This method has revealed little evidence of olfactory influences in previous gustatory experiments (Pittman et al. 2006, 2007). Furthermore, analysis of the latency to first lick durations indicates that rats consistently sample the test stimulus within the first 6 s of the 45-s presentation period regardless of whether the stimulus is a fatty acid or water.

Although care was taken to minimize olfactory cues in the fatty acid–containing stimuli during our brief-access, behavioral experiments, there was no concerted effort to mask the textural properties of the fatty acid stimuli. This is due in part to the fact that the viscosity of micromolar fatty acid–containing solutions is only negligibly higher than water (Pittman et al. 2006); however, other textural cues such as lubricity may be present at these micromolar fatty acid concentrations. A preliminary study by Gilbertson et al. (Yu et al. 2007) has shown that fatty acids at these concentrations (3–100 μM) are capable of causing significant changes in intracellular Ca^{2+} in subsets of lingual trigeminal neurons, the cells responsible for the somatosensory perception of texture. Although Sprague–Dawley rat trigeminal neurons have been shown to respond to fatty acids via an inhibition of DRK channels (Gilbertson et al. 2004), this has not been compared in S5B and O-M rats. Thus, the data contained in the present study not only could be attributable to a taste component but also may reflect the activity of fatty acids on the somatosensory neurons in the oral cavity.

Sex differences in the response to linoleic acid

Interestingly, differences between males and females in the present study were much more pronounced than were the strain differences. Previous comparisons of sex differences in the acquisition and extinction of CTAs have shown that male rats form stronger CTAs than female rats, and this increased conditioned aversion is specifically related to the presence of testosterone (Foy MR and Foy JG 2003). Therefore, it is unlikely that our observed sex differences are related to the effects of conditioning but rather likely reflect differential responses to the sensory properties of the CS. The majority of existing studies that have explored fatty acid taste transduction in rodents have investigated only males (Gilbertson et al. 1997, 1998, 2005; Tsuruta et al. 1999; Smith et al. 2000; McCormack et al. 2006; Pittman et al. 2006), did not report data segregated by sex (Laugerette et al. 2005), or found equivocal sex differences (Stratford et al. 2006). Recently, Pittman et al. (2007) demonstrated a stronger, more salient taste aversion to both linoleic and oleic acids in female Sprague–Dawley rats when compared with male

rat counterparts. The data in the present study are consistent with those findings and lend additional evidence to the interpretation that females exhibit a greater responsiveness to the sensory cues elicited by fatty acids than their male counterparts. Thus, a comparison of the cellular responses to fatty acids and the molecular expression of putative fatty acid receptors in the gustatory and somatosensory systems of male and female rats would seem a critical next step toward providing insights into the molecular underpinnings of these sex differences in the sensitivity to detect fatty acids. Our findings also warrant inclusion of female rats in longer term behavioral studies in order to characterize fatty acid preferences and ingestive consumption patterns for fatty acids and dietary fat as related to body weight, regulating caloric intake, and incidence of obesity in the O-M and S5B strains of rat.

Cross-generalization to other fatty acids

On the first testing day, with the exception of S5B males, all other groups of rats receiving LiCl injections showed a moderate or strong aversion to 100 μM oleic acid (Figure 3A), which may indicate that this fatty acid cross-generalizes to the conditioned fatty acid, linoleic acid (100 μM), as has been previously demonstrated for linoleic and oleic acids in the Sprague–Dawley rat strain (McCormack et al. 2006; Pittman et al. 2007). There was a rapid extinction of this aversion by the second testing day with all groups except O-M females no longer significantly avoiding licking the oleic acid (Figure 3C) despite a continued, robust avoidance of linoleic acid on the second testing day by all cohort groups. The hypothesis that oleic acid is less salient than linoleic acid was proposed in previous research demonstrating that multiple CS–US pairings were necessary to effectively condition an avoidance of oleic acid as compared with a single CS–US pairing being effective to condition a taste aversion to linoleic acid (Pittman et al. 2007). The weaker generalized avoidance of oleic acid in this experiment further supports the hypothesis that oleic acid may have less sensory salience than linoleic acid. Individual taste cells from the posterior tongue (foliate and circumvallate papillae) have been shown to respond to both monounsaturated fatty acids and PUFAs (Hansen et al. 2003), whereas the anterior taste buds from the fungiform papillae respond significantly only to PUFAs (Gilbertson et al. 1997). Therefore, based on this molecular evidence supporting a greater distribution of linoleic acid–sensitive taste receptor cells in the oral cavity, it may be predicted that linoleic acid would elicit stronger behavioral responses than oleic acid.

Cross-generalization between linoleic and oleic acid provided further evidence of the differences in both sex and strain sensitivity to fatty acids. Within each sex, the O-M rats showed the strongest avoidance of oleic acid. Of the male rats, only the O-M strain avoided 100 μM oleic acid with no avoidance of oleic acid by the S5B male rats. Within the female LiCl-injected groups, O-M rats showed stronger avoidance for both 50 μM (0.65 ± 0.10) and 100 μM (0.19 ± 0.04) oleic acid compared with the S5B rats (50 μM, 1.16 ± 0.03; 100 μM, 0.49 ± 0.17). The greater sensitivity of females to fatty acids than males was also clear within each strain. Within the less sensitive S5B strain, only females showed generalized avoidance of oleic acid. Whereas within the more sensitive O-M strain, male rats only avoided 100 μM oleic acid on day 1 in contrast to the avoidance of both 50 and 100 μM oleic acid by female rats on both testing days 1 and 2.

Based on previous in vitro electrophysiological studies, which reported saturated acids as ineffective to depolarize taste receptor cells harvested from male rats, we hypothesized that the saturated fatty acid, lauric acid, would be an ineffective fatty acid stimulus, and therefore, rats would show no evidence of a generalized avoidance of lauric acid. Our hypothesis was partially supported by the male rats of both strains that showed no avoidance of lauric acid on any test day; however, female rats in both strains did robustly avoid lauric acid on test day 1. The rapid extinction of the avoidance on day 2 suggests that similar to oleic acid, lauric acid, while sharing some sensory similarities with linoleic acid, also can be effectively discriminated from linoleic acid that was robustly avoided on testing day 2 and weakly avoided on testing day 3. Although our study of rodent behavior is the first to report similar stimulus qualities shared by the saturated lauric acid, and the polyunsaturated linoleic acid, there is evidence that humans can detect the saturated acid, stearic acid, at similar threshold levels as both linoleic and oleic acids (Chalé-Rush et al. 2007a, 2007b). The sex difference in sensitivity to lauric acid supports the theory of an increased sensitivity for female rats to fatty acid stimuli in general. This intriguing sex difference in sensitivity to saturated fatty acids further emphasizes the need to include female subjects in further electrophysiological and behavioral studies seeking to understand the sensorineural properties of fatty acids.

Many questions remain to be answered regarding the ability of the gustatory system to detect fatty acids. This study introduces the potential for genetic differences to influence the behavioral responses to the ingestion of micromolar quantities of fatty acids. Furthermore, additional evidence has been provided to support an increased female sensitivity to fatty acid ingestion. Based on previous molecular evidence that the obesity-prone rats had a decreased DRK sensitivity to fatty acids compared with an obesity-resistant strain, it had been speculated that a reduction in the behavioral responsiveness to fatty acids could underlie previously observed increases in consumption of high-fat food by the obesity-prone strain compared with the obesity-resistant strain. Our behavioral evidence appears to support an alternative hypothesis that an increased sensitivity to fatty acids in the obesity-prone rats might actually drive the consumption of high-fat food as a more palatable stimulus compared with the obesity-resistant rats. Although the previously reported decrease in the expression of fatty acid–sensitive DRK

channels in obesity-prone rats as compared with obesity-resistant rats appears incongruent with our behavioral results, it is important to recognize that multiple other physiological mechanisms, such as additional fatty acid transduction mechanisms, differences in afferent neural coding, or central processing of the afferent taste signals, could underlie the strain differences in fatty acid avoidance. Little is known about the general gustatory responsiveness of these obesity-prone and obesity-resistant strains of rat; therefore, it is important to characterize the behavioral responses of these rat strains to other prototypical tastants as well as examine the responsiveness of these strains of rat to fatty acids using other behavioral assays that do not utilize avoidance of a CS as the primary motivation for responding to the taste stimuli. Our data suggest subtle differences between the responsiveness of each strain to fatty acids. It is worth noting that both strains exhibited similar fatty acid detection capabilities on the first day of conditioning testing, and it was not until the second day that strain differences became apparent. Employing a positive reinforcement model of ingestion as the motivation to respond to the fatty acids or examining stimulus discriminations between fatty acids represents 2 additional behavioral techniques that may further elucidate these subtle strain differences. The differences in fatty acid responsiveness between male and female rats were robustly demonstrated in both strains of rat. As research continues to make progress toward understanding the neurophysiological basis of fatty acid sensitivity in the gustatory system, our findings highlight the necessity to explore potential sex differences in the future examination of transduction, signaling, and behavioral responsiveness related to the ingestion of fatty acids.

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