

# Chorda tympani nerve transection alters linoleic acid taste discrimination by male and female rats

Jennifer M. Stratford, Kathleen S. Curtis, Robert J. Contreras\*

*Department of Psychology and Program in Neuroscience, The Florida State University, Tallahassee, FL 32306-1270, USA*

Received 3 March 2006; received in revised form 17 May 2006; accepted 8 June 2006

## Abstract

Taste is intimately associated with food choice, yet little is known about the role of taste in preferences for dietary fat, a major component of many foods. We measured the taste threshold for linoleic acid (LA), an essential free fatty acid found in dietary fat, before and after bilateral transections of the chorda tympani nerve (CTX) in adult male and female rats. We conditioned a taste aversion to 88  $\mu\text{M}$  LA and assessed the generalization of the aversion to lower LA concentrations to determine LA discrimination thresholds. We discovered that female rats had a lower LA discrimination threshold ( $\sim 2.75$   $\mu\text{M}$  LA) than did male rats ( $\sim 11$   $\mu\text{M}$  LA). In another set of animals, we performed CTX and found that CTX elevated LA threshold to the same level ( $\sim 22$   $\mu\text{M}$  LA) in male and female rats. Finally, we evaluated licking responses to 11, 22, 44 and 88  $\mu\text{M}$  LA mixed in sucrose by male rats and ovariectomized (OVX) female rats treated with estradiol benzoate or oil vehicle. All rats increased licking to increasing LA concentrations, but OVX rats responded to a lower LA concentration (22  $\mu\text{M}$ ) than did males (44  $\mu\text{M}$ ) in 10-s trials. However, estradiol did not affect this outcome. Collectively, these experiments show that male and female rats use taste to discriminate LA and that the chorda tympani nerve, which innervates taste buds on the anterior tongue, plays a role in this discrimination. Furthermore, sex differences in fat preferences may depend on differences in fatty acid taste thresholds as well as on the taste stimuli with which fat is combined.

© 2006 Elsevier Inc. All rights reserved.

*Keywords:* Gustatory processing; Free fatty acids; Taste preferences; Fat taste; Conditioned taste aversion

## 1. Introduction

Taste is the gateway for ingestion as food choices are intimately associated with taste. In turn, food consumption ultimately influences body weight. Given the implications for the effect of taste on ingestion and body weight, it is surprising that little research has investigated the taste of fat—a substance directly related to body weight and, in extreme cases, to obesity. Traditionally, behavioral responses to fat have been attributed to smell, texture, or post-ingestive effects rather than to taste. However, emerging evidence suggests that fat has a taste in addition to its other sensory attributes. Rats prefer fat solutions even when olfaction and texture are minimized [1] and can make behavioral discriminations between different kinds of oils [2]. Ingested fats are rapidly broken down in the oral cavity by lingual lipase (within 1–5s; [3]), thereby providing free fatty

acids that may act on taste receptors on the tongue. In fact, the addition of a lipase inhibitor greatly reduces rats' preference for fat solutions [3]. Finally, essential free fatty acids, such as linoleic acid (LA), that are major components of many dietary fats, inhibit delayed rectifying potassium channels in isolated taste cells [4,5] and a fatty acid transporter has been found in the taste buds of the gustatory epithelium [6]. Together, these results suggest that fat has a taste and, more specifically, that the taste of fatty acids underlies the preference for fats.

Despite the evidence for the detection of fat and fatty acids in the oral cavity, peripheral neural mechanisms that mediate fat taste responses remain unknown. Most studies of peripheral gustatory nerves have focused on electrophysiological responses to classic taste stimuli (sweet, salty, bitter and sour), with very few exceptions (e.g. [7]). For example, it is well known that the chorda tympani (CT) branch of the facial nerve is highly responsive to salt and, to a lesser degree, to sour stimuli in rats and is also involved in appetitive behaviors [8]; however, CT sensitivity to fat or fatty acids has not been examined.

\* Corresponding author. Tel.: +1 850 644 1751; fax: +1 850 644 7739.

E-mail address: [contreras@psy.fsu.edu](mailto:contreras@psy.fsu.edu) (R.J. Contreras).

Accordingly, the following experiments were designed to examine fat taste in greater detail by evaluating the approximate taste threshold for LA and the role of peripheral neural signals in fat taste discrimination. We evaluated the generalization of a taste aversion conditioned to LA to determine approximate LA taste thresholds in male and female rats. In this protocol, we conducted 2-bottle (LA+water) tests and operationally defined the discrimination threshold for LA to be the concentration at which rats did not discriminate between LA and water. We also examined the effect of bilateral transections of the CT (CTX) on discrimination thresholds using the same conditioned taste aversion generalization protocol. Finally, given empirical as well as anecdotal evidence that women have a greater preference for ‘sweet-fat’ substances [9,10] than do men, we examined whether male and female rats respond differently to LA mixed with sucrose and the role of estrogen in such differences. We used short-term (10-s) intake tests, designed to minimize post-ingestive effects, to evaluate behavioral taste responses to LA mixed with sucrose and whether estrogen affected these taste responses.

## 2. Methods

### 2.1. Subjects

Age-matched adult male and female Sprague–Dawley rats (Charles River Laboratory) weighing 200–375 g at the beginning of testing were individually housed in a temperature-controlled (72 °F) room and maintained on a 12:12-h light–dark cycle with lights on at 07:00 h. Rats were given *ad libitum* access to Purina rodent chow (#5001) and water, except where noted. The Institutional Animal Care and Use Committee at Florida State University approved all procedures.

### 2.2. Chemicals

Due to its lipophilic nature, linoleic acid (LA; Sigma; 99% pure) was dissolved in 5 mM EtOH. All other reagent grade chemicals were mixed in deionized water.

### 2.3. Experiment 1: LA discrimination thresholds

Male and female rats were placed on a water restriction schedule during which they had access to deionized water for 10 min daily. Once they reliably drank volumes of water  $\geq 7$  ml, all rats were given 88  $\mu$ M LA in a graduated drinking tube (conditioning day, D1). After 10 min, fluid intake was recorded and rats were injected i.p. with 3 mEq/kg BW of 0.15 M LiCl (male  $n=10$ ; female  $n=9$ ) or 0.15 M NaCl (male  $n=9$ ; female  $n=10$ ).

Rats were given water for 10 min on D2 and the LiCl-induced conditioned taste aversion (CTA) to 88  $\mu$ M LA was verified on D3 in 10 min, 2-bottle (LA and water) tests. Rats were tested for the generalization of the CTA to less concentrated LA solutions (44, 22, 11, 5.5, 2.75, 1.375  $\mu$ M) in additional 2-bottle (LA and water) tests conducted on D4–9. During these generalization tests, one LA concentration was

given each day with presentation in descending order of concentration. To ensure that the results obtained did not reflect an extinction of the conditioned aversion, the CTA to 88  $\mu$ M LA again was assessed after the final day of generalization testing (D10).

### 2.4. Experiment 2: effect of CTX on LA discrimination thresholds

Following adaptation to water restriction as described previously, a different group of male (NaCl  $n=9$ , LiCl  $n=9$ ) and female (NaCl  $n=10$ , LiCl  $n=10$ ) rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Abbot Laboratories). The chorda tympani nerve was bilaterally transected (CTX) as described by O’Keefe et al. [11]. Briefly, a topical analgesic (bupivacaine) was applied to the external auditory meatus which was then retracted using blunted hypodermic needles to visualize the tympanic membrane and the malleus through an operating microscope. The tympanic membrane was removed using a hypodermic needle, a small caudal portion of the malleus was removed to expose the CT and the CT then was sectioned. Following transection, rats were allowed to recover for 7 days during which they had *ad libitum* access to food and water. Rats then were given 10 min daily access to water until stable intakes of  $\geq 7$  ml were re-established. Immediately afterward, rats were given a conditioned aversion to 88  $\mu$ M LA and tested for the generalization of the aversion to 44, 22, 11 and 5.5  $\mu$ M LA as described previously.

After the last day of testing (~2 weeks after CTX), animals were deeply anesthetized with urethane and the tongues were removed for histological verification of the efficacy of the CT transections as described by Geran et al. [12]. The anterior portion of the tongue was isolated and placed in distilled water and then dipped in 0.5% methylene blue for approximately 1 min. The epithelium then was separated from the underlying connective tissue and muscle and flattened between two glass slides. The number of visible fungiform papillae and taste pores were counted under a light microscope. Because CTX results in a large decrease in the number of taste pores on the anterior 2/3 of the tongue [13], rats that had  $\geq 20\%$  of papillae with taste pores after CTX ( $n=4$ ) were excluded from the analysis.

### 2.5. Experiment 3: lickometer training and testing

Rats were trained to consume fluids during 10-s presentations using the Davis MS80 Rig (Dilog Instruments and Systems, Tallahassee, FL), a programmable lickometer with a shutter that is opened or closed electronically to give the rat access to one of eight drinking tubes that are mounted on a sliding platform. A microcomputer controls the shutter and the position of the platform, thus determining the order and duration of the tube presentation, as well as the interval between presentations. Contact with the spout of a drinking tube completes a weak electrical circuit (<60 nA) that is recorded by the microcomputer as a lick.

Training and testing followed procedures described previously [14,15]. Briefly, rats were acclimated to the Davis

MS80 Rig by being placed in the test chamber with access to one tube of 0.2 M sucrose for 15 min/day on two consecutive days. For the next 7 days, rats were given water for only 30 min/day in their home cages following 15 min access to 0.2 M sucrose in the Davis MS80 Rig. On the last 4 days, rats were given three 200-s presentations of 0.2 M sucrose to acclimate them to the sound and motion of the sliding platform. Rats were then given *ad libitum* access to water for the remainder of the training and testing, which was conducted at 2–5 day intervals. Presentations of 0.2 M sucrose were decreased to 10 s and training continued for 3–5 sessions until the rats reliably licked during each 10-s presentation. In subsequent sessions, rats were given 10-s presentations of test solutions described below. In these sessions, if a rat did not lick within 180 s, the shutter closed and the platform moved to present a different tube.

#### 2.5.1. 0.0375 and 0.2 M sucrose

Our previous studies found that rats licked at maximal rates to most sucrose-test solution mixtures when only one sucrose concentration was used [14,16]. Rats reduced licking only to mixtures containing high concentrations of the test solution, which prevented the detection of increased licking. Therefore, we mixed LA in both 0.2 M and 0.0375 M sucrose to elicit different rates of licking (i.e., a contrast effect), thereby allowing the detection of both increases and decreases in licking.

#### 2.5.2. Sucrose vs. sucrose + EtOH

To control for the effect of the addition of EtOH, during initial training, rats were given trials with both sucrose concentrations with and without EtOH. A two-way (Sucrose concentration  $\times$  EtOH) repeated measures Analysis of Variance (ANOVA; Statistica, StatSoft, Tulsa, OK) revealed no significant effect of the addition of EtOH ( $p=0.13$ ). Therefore, for the rest of the training and during testing, all sucrose solutions (SUC) were mixed in 5 mM EtOH.

#### 2.5.3. Sucrose + LA training

Male and female rats (prior to ovariectomy) were given access to each LA concentration (11, 22, 44 and 88  $\mu$ M) mixed in both sucrose concentrations during training. Thus, both groups had previous experience with sucrose as well as with LA mixed in sucrose before the start of testing to minimize the possibility that the results obtained during LA testing were attributable to novelty.

#### 2.5.4. OVX rats: ovariectomy and estrogen replacement

After training as described, female rats ( $n=11$ ) were bilaterally ovariectomized (OVX) under sodium pentobarbital anesthesia (Nembutal Sodium; Abbott Laboratories, North Chicago, IL; 50 mg/kg BW, ip). A midline abdominal incision was made and the ovaries were removed. The abdominal muscles then were sutured and wound clips applied to the skin. Rats were permitted to recover for 7 days, during which time one additional training session was run.

We used an estrogen replacement schedule designed to mimic the pattern of estrogen fluctuations in intact, cycling

female rats [17]. OVX rats were randomly assigned to receive injections of estradiol benzoate (EB; 10  $\mu$ g/0.1 ml oil, sc; Sigma, St. Louis, MO) or the oil vehicle (OIL; 0.1 ml, sc) on two consecutive days. Plasma estrogen levels increase to the supraphysiological range immediately following EB injections, but are metabolized to physiologic levels over the subsequent 24 h using this protocol [17]. The treatment schedule was repeated at weekly intervals for a total of 4 weeks, during which EB/OIL injections for each rat were alternated. Thus, each OVX rat was tested in both EB and OIL conditions. Body weights were recorded daily during testing.

#### 2.5.5. Male rats

Male rats ( $n=9$ ) did not undergo surgery, but were injected with OIL or received no injection (CONTROL). As with OVX rats, the treatment schedule was repeated for a total of 4 weeks and the treatments were alternated. Thus, each male rat was tested in both OIL and CONTROL conditions.

#### 2.5.6. Lickometer testing

Licking responses to 11, 22, 44 and 88  $\mu$ M LA mixed in 0.0375 M or 0.2 M sucrose (LA+SUC) were evaluated 24 h after the first treatment and 48 h after the second treatment. Rats were given 10-s presentations of water, EtOH, SUC (0.0375 M and 0.2 M) and LA+SUC (0.2 M and 0.0375 M). Since the Davis MS80 Rig holds a maximum of eight drinking tubes, 11  $\mu$ M and 44  $\mu$ M LA were presented in testing sessions during weeks 1 and 2, and 22  $\mu$ M and 88  $\mu$ M LA were presented in testing sessions during weeks 3 and 4. Thus, all rats were tested in both treatment conditions (OVX: EB/OIL; male: OIL/CONTROL) for all LA concentrations (11, 44, 22 and 88  $\mu$ M).

Within a session, the order of presentation was randomized and each solution was presented twice with a 180-s limit to begin licking. Once licking began, rats had 10-s access to the test solution during each trial. Trials were separated by 30 s. The number of licks during each presentation was recorded. In trials when a rat failed to lick (e.g. during presentations of water), a zero was recorded. The average number of licks for each solution was calculated for each rat. Thus, within each treatment condition, the licking rate for each LA concentration was the average of four trials (two trials per day for two test days). Preliminary comparisons of the number of licks to 0.2 M and to 0.0375 M SUC using a two-way (Sucrose concentration  $\times$  Day) repeated measures ANOVA revealed no significant changes over the 4 weeks of testing ( $p=0.15$ ). Thus, the number of licks for each SUC solution was averaged over eight test days (two trials per day for four test days) for each rat in each treatment condition.

#### 2.6. Statistical analysis

All data are presented as group means  $\pm$  S.E.M. Statistical comparisons were made using repeated measures ANOVAs as described below. Pairwise comparisons of statistically significant ( $p<0.05$ ) main effects or interactions were evaluated using Student–Newman–Keuls tests and specific planned comparisons were made using Bonferroni corrections.

### 2.6.1. Experiment 1: LA discrimination thresholds

The results from the CTA tests were expressed as preference scores, calculated as [LA intake (ml)/total intake (ml)]. By convention, we considered scores  $\geq 0.55$  to indicate a preference for LA, scores  $\leq 0.45$  to indicate an aversion to LA and scores between 0.45 and 0.55 to indicate indifference to LA. The CTA to 88  $\mu\text{M}$  LA at the beginning and at the end of testing was compared using a three-factor (Sex  $\times$  Drug  $\times$  time) repeated measures ANOVA. Initial testing showed that all LiCl-treated rats generalized the CTA to both 44 and 22  $\mu\text{M}$  LA. Therefore, the main testing sequence consisted of daily generalization tests of the CTA to 11, 5.5, 2.75 and 1.375  $\mu\text{M}$  LA. This change did not alter the subsequent results, as revealed by statistical comparisons of the two protocols using a four-way (Sex  $\times$  Drug  $\times$  Protocol  $\times$  LA concentration) repeated measures ANOVA. Thus, data obtained from the two protocols were combined in subsequent analyses. The generalization of the CTA to 11, 5.5, 2.75 and 1.375  $\mu\text{M}$  LA solutions was compared using a three-factor (Sex  $\times$  Drug  $\times$  Concentration) repeated measures ANOVA.

### 2.6.2. Experiment 2: effect of CTX on LA discrimination

Statistical comparisons of the taste aversion conditioned to 88  $\mu\text{M}$  LA in CTX rats at the beginning and at the end of testing were made using a three-factor (Sex  $\times$  Drug  $\times$  Time) repeated measures ANOVA. Comparisons of the generalization of the conditioned aversion to LA were made using a three-factor (Sex  $\times$  Drug  $\times$  Concentration) repeated measures ANOVA.

### 2.6.3. Experiment 3: lickometer tests

Licking responses in 10-s tests were analyzed using a four-way (Sex  $\times$  Treatment  $\times$  SUC  $\times$  LA) repeated measures analysis of variance (ANOVA; Statistica, StatSoft, Tulsa, OK). Statistical comparisons of the change in body weight from D1 to D4 in OVX rats (EB vs. OIL) were made using paired *t*-tests.

## 3. Results

### 3.1. Experiment 1: LA discrimination thresholds

Fig. 1 shows the preference scores for 88  $\mu\text{M}$  LA at the beginning and end of testing for all CT-intact groups. As

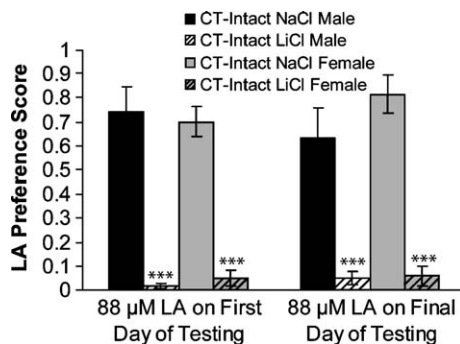


Fig. 1. CT-intact conditioned taste aversion testing: preference scores for 88  $\mu\text{M}$  LA on the first day of testing (D3) and the final day of testing (D8) by male (white bars) and female (gray bars) NaCl-treated (solid bars) and LiCl-treated (hatched bars) CT-intact rats. \*\*\* $p < 0.001$ .

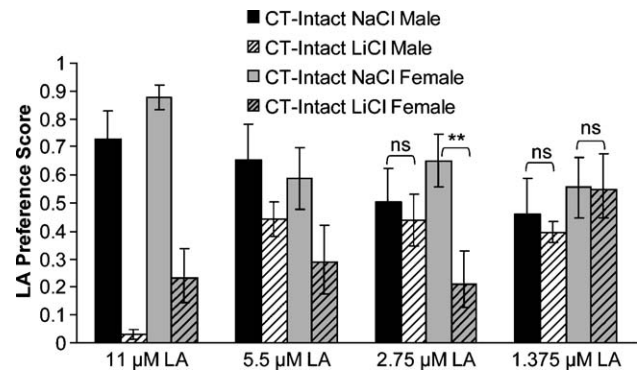


Fig. 2. CT-intact generalization testing: preference scores for LA (11, 5.5, 2.75, 1.375  $\mu\text{M}$ ) by male (white bars) and female (gray bars) NaCl-treated (solid bars) and LiCl-treated (hatched bars) CT-intact rats. \*\* $p < 0.01$ ; ns = not significant.

expected, LiCl treatment resulted in a robust aversion to 88  $\mu\text{M}$  LA ( $F(1,34) = 252.57$ ,  $p < 0.001$ ) that remained unchanged from the first to final day of testing ( $F(1,34) = 0.05$ ,  $p = 0.81$ ) in both male and female rats.

Fig. 2 shows LA preference scores for 11, 5.5, 2.75 and 1.375  $\mu\text{M}$  LA. Neither the main effect of Sex nor the main effect of Concentration was statistically significant for the generalization of the CTA; however, there was a significant effect of Drug ( $F(1,34) = 32.75$ ,  $p < 0.001$ ) as well as a significant interaction between Drug and Concentration ( $F(3,102) = 9.01$ ,  $p < 0.001$ ). Post hoc analyses of the Drug  $\times$  Concentration interaction revealed that, independent of Sex, preference scores for 11, 5.5 and 2.75  $\mu\text{M}$  LA by LiCl-treated rats were less than those by NaCl-treated rats (all  $p$ 's  $< 0.05$ ). However, the preference for 1.375  $\mu\text{M}$  LA did not differ between drug groups ( $p = 0.69$ ).

Unpublished observations by Smith et al. [18] found that male rats can distinguish between LA and water at low (10  $\mu\text{M}$ ) concentrations and our preliminary studies [19] suggested that female rats are able to distinguish lower LA concentrations than do male rats. Accordingly, we made specific planned comparisons of the preference for 2.75 and 1.375  $\mu\text{M}$  LA by NaCl- and LiCl-treated male and NaCl- and LiCl-treated female rats (Fig. 2), adjusted using Bonferroni corrections. These comparisons revealed that the preference for 2.75  $\mu\text{M}$  LA by LiCl-treated

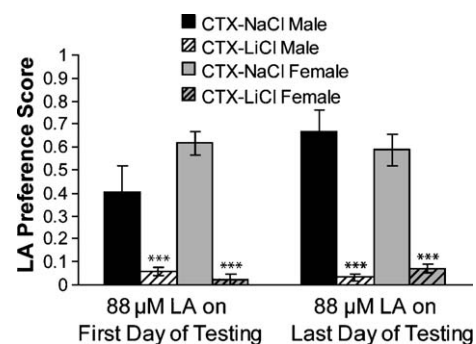


Fig. 3. CTX conditioned taste aversion testing: preference scores for 88  $\mu\text{M}$  LA on the first day of testing (D3) and the final day of testing (D8) by male (white bars) and female (gray bars) NaCl-treated (solid bars) and LiCl-treated (hatched bars) CTX rats. \*\*\* $p < 0.001$ .

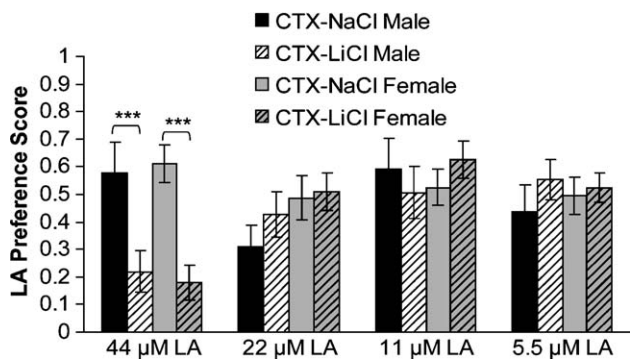


Fig. 4. CTX generalization testing: preference scores for LA (44, 22, 11, 5.5 μM) by male (white bars) and female (gray bars) NaCl-treated (solid bars) and LiCl-treated (hatched bars) CTX rats. \*\*\* $p < 0.001$ .

female rats was significantly less than the preference by NaCl-treated female rats ( $p < 0.01$ ). In contrast, the preference for 2.75 μM LA by LiCl-treated male rats was not different from the preference by NaCl-treated male rats ( $p = 2.41$ ). However, at 1.375 μM LA, the preference for LA was not different between LiCl-treated and NaCl-treated rats for either sex ( $p = 2.44$  and  $p = 3.81$ , respectively; Bonferroni corrections).

3.2. Experiment 2: effect of CTX on LA discrimination

Fig. 3 shows preference scores for 88 μM LA at the beginning and end of testing for all CTX groups. There was a significant effect of Drug ( $F(1,35) = 136.1, p < 0.001$ ), but no effects of Sex or Time. Moreover, there were significant interactions between Sex and Time ( $F(1,35) = 4.80, p < 0.05$ ) as well as between Drug and Time ( $F(1,35) = 4.15, p < 0.05$ ). Post hoc analysis of the Sex × Time interaction revealed that, independent of drug, male rats, but not female rats, had a significantly greater LA preference at the end of testing vs. the beginning of testing ( $p < 0.05$ ). Analysis of the Drug × Time interaction showed that, independent of sex, LA preference scores by NaCl-treated rats were greater at the end of testing compared to those at the beginning of testing ( $p < 0.05$ ). In contrast, LA preference scores by LiCl-treated rats remained low from the beginning to the end of testing ( $p = 0.62$ ).

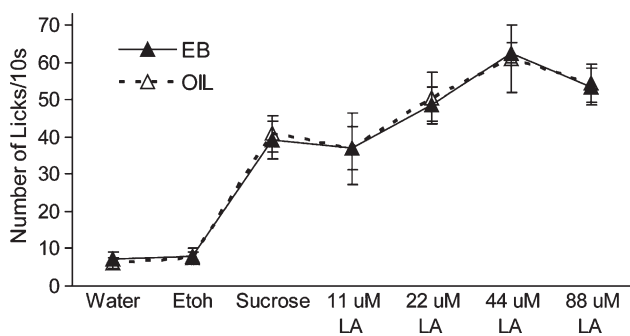


Fig. 5. OVX lickometer testing: mean licking responses in 10-s trials by OVX rats treated with EB (filled symbols) or OIL (open symbols) to water, 5 mM EtOH, 0.0375 M sucrose, and LA (11, 22 44 and 88 μM) mixed in 0.0375 M sucrose.

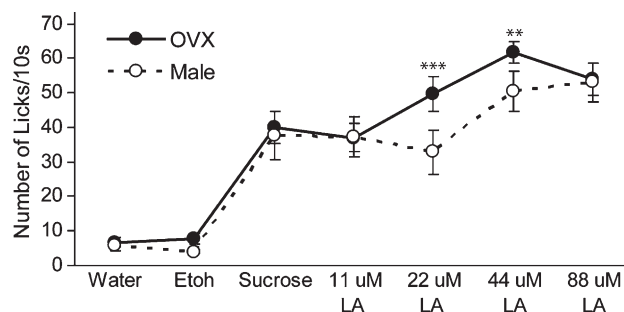


Fig. 6. OVX and male lickometer testing: mean licking responses in 10-s trials by OVX (filled symbols) and male (open symbols) rats, to water, 5 mM EtOH, 0.0375 M sucrose, and LA (11, 22 44 and 88 μM) mixed in 0.0375 M sucrose. \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Fig. 4 shows preference scores for 44, 22, 11 and 5.5 μM LA by male and female NaCl- and LiCl-treated CTX rats. Statistical analyses showed no effect of Sex or Drug, but a significant main effect of Concentration ( $F(3,105) = 3.60, p < 0.05$ ) and a significant interaction between Drug and Concentration ( $F(3,105) = 9.17, p < 0.001$ ). Post hoc analyses of the Drug × Concentration interaction revealed that, independent of Sex, LA preference scores were less by LiCl-treated rats compared to those of NaCl-treated rats at 44 μM LA ( $p < 0.001$ ), but not at 22, 11 or 5.5 μM LA ( $p = 0.64, 0.93$  and  $0.34$ , respectively).

3.3. Experiment 3: lickometer testing

Fig. 5 shows the mean number of licks during 10-s access to test solutions by OVX rats injected with EB or OIL. OVX rats increased their licking to increasing LA concentrations, regardless of treatment. Similar to OVX rats, licking responses by male rats (data not shown) were not affected by treatment (i.e. EB/OIL; OIL/CONTROL). A four-way ANOVA revealed no effect of treatment and no interaction between treatment and any other factor. Accordingly, Fig. 6 shows the data from male and OVX rats partitioned by sex. There was a significant interaction between Sex, SUC, and LA ( $F(4,72) = 2.95, p < 0.05$ ). Post hoc analyses showed that all rats licked at comparably high rates to 0.2 M SUC and to all LA solutions mixed in 0.2 M SUC, regardless of sex or LA concentration (see Table 1; all  $p > 0.42$ ). Moreover, both OVX and male rats licked at lower rates to 0.0375 M SUC ( $40 ± 5$  and  $35 ± 7$  licks/10 s, respectively). However, compared to licking to 0.0375 M SUC alone, OVX rats licked at significantly greater rates with increasing LA concentration beginning with 22 μM LA and at each LA concentration thereafter (all  $p < 0.001$ ). In contrast, although male rats also increased their licking to LA + 0.0375 M SUC, the increase was significant only at 44 and 88 μM

Table 1  
Licking responses in 10-s trials by OVX and male rats to test solutions mixed in 0.2 M sucrose

	0.2 M sucrose	11 μM LA	22 μM LA	44 μM LA	88 μM LA
OVX	68.67 ± 0.96	70.82 ± 1.24	69.47 ± 1.28	69.75 ± 1.29	68.40 ± 1.30
Male	62.71 ± 1.70	66.47 ± 1.79	65.09 ± 1.73	65.17 ± 1.84	64.81 ± 2.34

Data are means ± standard error, expressed as number of licks/10 s.

( $p < 0.001$ ). In fact, OVX rats licked significantly more to both 22  $\mu\text{M}$  ( $p < 0.001$ ) and 44  $\mu\text{M}$  ( $p < 0.01$ ) LA+0.0375 M SUC when compared to licking by male rats.

### 3.3.1. Body weight of OVX rats

During testing, the body weight of OVX rats decreased an average of  $15.4 \pm 2.3$  g after EB treatment but increased an average of  $7.8 \pm 1.4$  g after OIL treatment ( $p < 0.001$ ).

## 4. Discussion

Most investigations of fat preferences have focused on the consumption of fats, such as oils, and the related post-ingestive consequences [20,21], rather than on the taste properties of fat. This oversight is especially surprising because taste has a potent influence on food intake and preference. In this regard, results from several *in vitro* and behavioral studies are highly suggestive that fats and free fatty acids have taste properties [4,5,22]. The present study sought to further examine the taste of free fatty acids in a series of related experiments designed to examine the taste threshold for LA, an essential free fatty acid, and to assess the role of a peripheral gustatory nerve in LA taste discrimination by male and female rats.

Our results provide further support for a taste component of free fatty acids by demonstrating that the taste threshold for LA requires input from the gustatory chorda tympani nerve (Fig. 2 vs. Fig. 4). Furthermore, there are subtle sex differences in the LA taste thresholds (Fig. 2) that may be related to differences in licking responses (Fig. 6). It is unlikely that the effects seen are attributable solely to either smell or texture for several reasons. First, Smith [23] showed that rats can distinguish between LA and water at low (10  $\mu\text{M}$ ) concentrations even with ablation of the olfactory bulb, suggesting that the olfactory component of LA is small at best. Second, although fat has an oily texture, our experiments used the free fatty acid LA, which has a low viscosity (only about 1.5% greater than that of water; [22]). Lastly, and most importantly, the observation that transection of the CT, a gustatory nerve, impairs LA detection suggests that fatty acids have a taste component.

### 4.1. Experiment 1: LA discrimination thresholds

We first assessed the discrimination threshold for LA by male and female rats in 10 min 2-bottle tests, designed to minimize the post-ingestive consequence of LA intake. Both male and female rats developed a CTA to 88  $\mu\text{M}$  LA (Fig. 1) and generalized this aversion to lower (44  $\mu\text{M}$ –5.5  $\mu\text{M}$ ) concentrations of LA (Fig. 2; 44, 22  $\mu\text{M}$  data not shown). However, LiCl-treated female rats generalized the CTA to a lower concentration of LA than did LiCl-treated male rats (2.75  $\mu\text{M}$  LA vs. 11  $\mu\text{M}$  LA), suggesting that female rats can discriminate a lower concentration of LA from water than can male rats.

Due to the lipophilic nature of LA, all LA test solutions were mixed in a low (5 mM) EtOH concentration, which allows LA to go more readily into solution. However, it is doubtful that the addition of EtOH could explain our results, as an aversion

conditioned to LA mixed with 5 mM EtOH does not generalize to EtOH alone [22]. Moreover, in our conditioned taste aversion studies, the concentration of EtOH was held constant while LA concentration was varied. Our results show conclusively that rats failed to generalize the CTA to LA at lower concentrations, suggesting that the EtOH used in our test solutions does not provide an olfactory cue that affects detection thresholds.

These results support recent findings by McCormack et al. [22] who reported that male rats form a CTA to LA solutions. Differences in methodology may account for the differences in discrimination thresholds; nevertheless, these results together provide strong evidence for taste-mediated detection of LA. Although it is apparent that male and female rats have different discrimination thresholds, it should be noted that the protocol used (2-bottle tests) indicates an approximate threshold for LA discrimination rather than the absolute threshold. It is possible that employing methods such as the conditioned shock avoidance procedure used by St. John and Spector [24] may provide a more accurate determination of LA detection thresholds.

### 4.2. Experiment 2: effect of CTX on LA discrimination

Despite strong behavioral and electrophysiological evidence suggesting that fatty acids have a taste, little is known about the role of specific gustatory nerves. Therefore, we examined the role of the CT in LA discrimination as an initial examination of the peripheral neural mechanisms. NaCl-treated male and female CTX rats had a moderate preference for 88 and 44  $\mu\text{M}$  LA (Figs. 3 and 4, respectively) that was more variable, especially in the NaCl-treated male CTX rats, as compared to the CT-intact rats (Figs. 1 and 2). LiCl-treated male and female CTX rats showed a CTA to 88  $\mu\text{M}$  LA and generalized this aversion to 44  $\mu\text{M}$  LA. However, CTX impaired the ability of both male and female rats to detect and subsequently avoid LA concentrations  $\leq 22$   $\mu\text{M}$  in generalization tests. Interestingly, CTX shifted the discrimination threshold for LA to the same concentration (22  $\mu\text{M}$ ) in male and female rats, an observation that is in contrast to the lower discrimination threshold for LA in CT-intact female rats than in CT-intact male rats (Fig. 2). Thus, the CT is involved in LA discrimination in both male and female rats, but may play a greater role for female rats which may account for the lower LA discrimination threshold by female rats.

The effects seen after transection of the gustatory CT provide further support that fatty acids have a taste. Although CTX decreases saliva production through denervation of the submaxillary and sublingual glands which could result in decreased lipolysis, lingual lipase released from the von Ebner's gland is unaffected by CTX [24]. More importantly, our experiments used a free fatty acid, LA, thereby circumventing the need for lipase activity. Thus, the effects seen after CTX are unlikely to be attributable to elimination of nongustatory fibers within the CT. In addition, it is unlikely that CTX has non-specific effects on ingestion that influence the results, as we found that rats with CTX have stable body weights within a few days after surgery and other researchers have reported that CTX

has no effect on food or water consumption [18]. Moreover, our unpublished data show that sham CTX rats have similar licking responses to LA as do CT-intact animals, suggesting that the effects observed in our current studies are not attributable to the effects of surgery (i.e. anesthesia and removal of the tympani membrane). Finally, CTX rats develop a CTA to sucrose and quinine [24,25], providing further evidence that the effects of CTX on LA detection are attributable to the impairment of CT gustatory sensory information.

CTX did not affect the CTA to 88  $\mu$ M LA or the generalization of the CTA to 44  $\mu$ M LA by either sex, suggesting that other gustatory nerves also are involved. Alternatively, CTX rats may use other sensory cues, such as olfaction or texture, to detect more concentrated LA solutions. In any event, these results show that CTX impairs the ability of both male and female rats to discriminate LA from water. Moreover, CTX shifts the discrimination threshold to the same concentration in male and female rats, suggesting that CT input is more important for LA discrimination in female than male rats.

#### 4.3. Experiment 3: lickometer testing

Because previous research has shown that women have a greater preference for 'sweet-fat' foods than do men [9,10], we examined licking responses to LA mixed in sucrose by male and OVX rats and the role of estrogen in these responses. In 10-s tests, both OVX and male rats licked maximally to all LA concentrations mixed in 0.2 M SUC (Table 1) and licked at a comparatively lower rate to 0.0375 M SUC. OVX and male rats increased licking to increasing concentrations of LA mixed in 0.0375 M SUC; however, regardless of treatment, OVX rats significantly increased licking at a lower concentration of LA (22  $\mu$ M) than did male rats (44  $\mu$ M). At 22  $\mu$ M LA, OVX rats licked  $\sim$ 50 licks/10 s—a 42% increase over the licking rate by male rats at that concentration and a 24% increase from the SUC baseline licking by OVX rats. At 44  $\mu$ M LA, OVX rats licked  $\sim$ 62 licks/10 s, which is 28% greater than the licking rate by male rats and a 54% increase from SUC baseline. Thus, though the differences observed are subtle, they are noteworthy, especially given the maximum rate at which rats can lick ( $\sim$ 70 licks/10 s).

Sex differences have been reported in sucrose preferences [26,27] and our recent studies [14,28] showed that EB reduces 0.025 M sucrose detection. However, it is unlikely that the sex differences observed in this study are specific to the sucrose component of the LA–sucrose mixture. We used 0.0375 M sucrose in the present study to ensure that the concentration of sucrose used would be detectable by all groups. In fact, throughout the testing and regardless of treatment, OVX rats maintained constant rates of licking to 0.0375 M sucrose that were comparable to those by male rats. Finally, it is clear from the concentration-dependent increase in licking to LA mixed in 0.0375 M sucrose that both male and OVX rats were able to distinguish between the LA and sucrose components in the taste mixture, as has been reported for other mixed taste stimuli [16,29], including oil–sucrose mixtures [23]. Thus, there are

subtle sex differences in behavioral taste responses to sucrose–LA mixtures.

Interestingly, the acute effects of estrogen appear to play no role in taste responses to LA. This observation is surprising because acute estrogen treatment affects taste responses to sucrose [14]. What, then, could account for the sex differences observed? The body weight loss in OVX rats after EB treatment is typical of cyclic EB treatment [26] and confirms the effectiveness of treatment. It is possible that exposure to EB early in development may contribute to fundamental differences in the taste system that are responsible for the subtle sex differences in fat taste responses. Alternatively, other gonadal hormones may play a role in these sex differences. At present, there is no evidence that progesterone, either alone or in combination with EB, affects taste responses, but testosterone has been implicated in sex differences in salt preferences [30]. Additional studies will be necessary to address these issues.

## 5. Conclusions

These experiments were designed to provide further evidence for and insight into fat taste. By using both male and female rats, our experiments provided a more comprehensive examination of fat taste and revealed subtle differences in how male and female rats respond to the taste of LA. These results show that LA has a taste component and that the CT is involved in LA discrimination. Moreover, female rats have a lower taste threshold for LA than do male rats and the CT may be more important in LA discrimination in female rats, as CTX shifted the discrimination threshold to approximately the same level in male and female rats. In fact, the greater role of CT input for LA discrimination in female rats may account for their ability to discriminate a lower LA concentration from water. Finally, OVX rats increase their licking to a lower concentration of LA mixed in a dilute sucrose concentration than do male rats, but acute estrogen treatment plays no role in these differences. Collectively, these experiments show that free fatty acids have a taste component and that the CT is involved in fatty acid discrimination. Furthermore, sex differences in fat preferences may depend on differences in fatty acid discrimination thresholds as well as on the taste stimuli with which fat is combined.

## 6. Perspectives

Many evolutionary adaptations promote reproductive success, in part, by optimizing survival of the offspring. For example, enhanced sensitivity to environmental stimuli during pregnancy may improve the ability to detect resources necessary to support a viable pregnancy. In this regard, energy dense foods rich in fat are essential to good maternal health during pregnancy [31–34]. Thus, the detection of fat may have intrinsic survival value and, in fact, dietary fat preferences increase during pregnancy. However, it is disadvantageous from an evolutionary point-of-view for males and females to have markedly different capabilities to detect a resource so essential for survival. The subtle differences we found suggest

that both males and females can detect fats, but that females may be slightly more sensitive to the taste of fat. This enhanced sensitivity may, in turn, help promote offspring viability.

Reproductive hormones exert a powerful influence on food preferences. The preference for the taste of sucrose is greatest during ovulation in humans [35,36], when levels of reproductive hormones, including estrogen are elevated. Importantly, the ability to detect sucrose is also greatest during ovulation [36], suggesting that estrogen influences taste preferences by modulating taste detection thresholds. Fat preferences by women also fluctuate across the menstrual cycle [37,38] in tandem with changes in circulating levels of estrogen and other reproductive hormones. Thus, it is surprising that the acute effects of estrogen do not affect behavioral licking responses to ‘sweet-fat’ mixtures by OVX rats. Clearly, there are sex differences in free fatty acid preferences, but the mechanism underlying these differences remains unknown.

Finally, the implications of these findings are far-reaching as both the type and amount of foods consumed are influenced by taste. Traditionally, fat has been thought of as an “enhancer” of other tastes. Thus, sex differences in fatty acid taste perception may influence the specific kinds of fat ingested. There are sex differences in fat preferences in humans, with women having a greater preference for ‘sweet fats’ (e.g. chocolate) than do men [9,10]. Thus, the sex differences observed in our lickometer test solutions, which used sweet-fat mixtures, may mirror results seen in humans. Ultimately, sex differences in taste-driven preferences for fat may result in increased fat intake, which may lead to increased risk of obesity.

## Acknowledgements

This research was supported by NIH grants from the National Institute on Deafness and Communication Disorders (DC04785: R.J.C.; DC06360: K.S.C.; T-32 DC00044: J.M.S.). Portions of these data were presented in preliminary form at the 26th and 27th annual meeting of the Association for Chemoreception Sciences in Sarasota, FL as well as the 34th annual meeting of the Society for Neuroscience.

## References

- [1] Fukuwatari T, Shibata K, Iguchi K, Saeki T, Iwata A, Tani K, et al. Role of gustation in the recognition of oleate and triolein in anosmic rats. *Physiol Behav* 2003;78(4-5):579–83.
- [2] Larue C. Oral cues involved in the rat’s selective intake of fats. *Chem Senses Flavor* 1978;3:1–6.
- [3] Kawai T, Fushiki T. Importance of lipolysis in oral cavity for orosensory detection of fat. *Am J Physiol Regul Integr Comp Physiol* 2003;285(2):R447–54.
- [4] Gilbertson TA. Gustatory mechanisms for the detection of fat. *Curr Opin Neurobiol* 1998;8(4):447–52.
- [5] Gilbertson TA, Liu L, York DA, Bray GA. Dietary fat preferences are inversely correlated with peripheral gustatory fatty acid sensitivity. *Ann N Y Acad Sci* 1998;855:165–8.
- [6] Fukuwatari T, Kawada T, Tsuruta M, Hiraoka T, Iwanaga T, Sugimoto E, et al. Expression of the putative membrane fatty acid transporter (FAT) in taste buds of the circumvallate papillae in rats. *FEBS Lett* 1997;414(2):461–4.
- [7] Lundy Jr RF, Contreras RJ. Taste prestimulation increases the chorda tympani nerve response to menthol. *Physiol Behav* 1993;54(1):65–70.
- [8] Spector AC. Linking gustatory neurobiology to behavior in vertebrates. *Neurosci Biobehav Rev* 2000;24(4):391–416.
- [9] Dube L, LeBel JL, Lu J. Affect asymmetry and comfort food consumption. *Physiol Behav* 2005;86(4):559–67.
- [10] Yanovski S. Sugar and fat: cravings and aversions. *J Nutr* 2003;133(3):835S–7S.
- [11] O’Keefe GB, Schumm J, Smith JC. Loss of sensitivity to low concentrations of NaCl following bilateral chorda tympani nerve sections in rats. *Chem Senses* 1994;19(2):169–84.
- [12] Geran LC, Garcea M, Spector AC. Nerve regeneration-induced recovery of quinine avoidance after complete gustatory deafferentation of the tongue. *Am J Physiol Regul Integr Comp Physiol* 2004;287(5):R1235–43.
- [13] Geran LC, Garcea M, Spector AC. Transecting the gustatory branches of the facial nerve impairs NH(4)Cl vs. KCl discrimination in rats. *Am J Physiol Regul Integr Comp Physiol* 2002;283(3):R739–47.
- [14] Curtis KS, Davis LM, Johnson AL, Therrien KL, Contreras RJ. Sex differences in behavioral taste responses to and ingestion of sucrose and NaCl solutions by rats. *Physiol Behav* 2004;80(5):657–64.
- [15] Curtis KS, Krause EG, Contreras RJ. Altered NaCl taste responses precede increased NaCl ingestion during Na(+) deprivation. *Physiol Behav* 2001;72(5):743–9.
- [16] Contreras RJ, Carson CA, Pierce CE. A novel psychophysical procedure for bitter taste assessment in rats. *Chem Senses* 1995;20(3):305–12.
- [17] Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J Comp Neurol* 1993;336(2):293–306.
- [18] Smith JC, Fisher EM, Maleszewski V, McClain B. Orosensory factors in the ingestion of corn oil/sucrose mixtures by the rat. *Physiol Behav* 2000;69(1-2):135–46.
- [19] Stratford JM, Curtis KS, Contreras RJ. Bilateral chorda tympani transections affect behavioral taste responses to linoleic acid in rats. *Abstr-Soc Neurosci* in press.
- [20] Scalfani A, Glendinning JI. Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: oral and postoral interactions. *Am J Physiol Regul Integr Comp Physiol* 2005;289(3):R712–20.
- [21] Takeda M, Imaizumi M, Sawano S, Manabe Y, Fushiki T. Long-term optional ingestion of corn oil induces excessive caloric intake and obesity in mice. *Nutrition* 2001;17(2):117–20.
- [22] McCormack DN, Clyburn VL, Pittman DW. Detection of free fatty acids following a conditioned taste aversion in rats. *Physiol Behav* 2006.
- [23] Smith JC. Gustation as a factor in the ingestion of sweet and fat emulsions by the rat. *Physiol Behav* 2004;82(1):181–5.
- [24] St. John SJ, Spector AC. Combined glossopharyngeal and chorda tympani nerve transection elevates quinine detection thresholds in rats (*Rattus norvegicus*). *Behav Neurosci* 1996;110(6):1456–68.
- [25] Grill HJ, Schwartz GJ. The contribution of gustatory nerve input to oral motor behavior and intake-based preference. II. Effects of combined chorda tympani and glossopharyngeal nerve section in the rat. *Brain Res* 1992;573(1):105–13.
- [26] Geary N. Estradiol, CCK and satiation. *Peptides* 2001;22(8):1251–63.
- [27] Scalfani A, Hertwig H, Vigorito M, Feigin MB. Sex differences in polysaccharide and sugar preferences in rats. *Neurosci Biobehav Rev* 1987;11(2):241–51.
- [28] Curtis KS, Stratford JM, Contreras RJ. Estrogen increases the taste threshold for sucrose in rats. *Physiol Behav* 2005;86(3):281–6.
- [29] Hsiao S, Fan RJ. Additivity of taste-specific effects of sucrose and quinine: microstructural analysis of ingestive behavior in rats. *Behav Neurosci* 1993;107(2):317–26.
- [30] Kreczek J. Sex differences in salt taste: the effect of testosterone. *Physiol Behav* 1973;10(4):683–8.
- [31] Decsi T, Campoy C, Koletzko B. Effect of N-3 polyunsaturated fatty acid supplementation in pregnancy: the Nuheal trial. *Adv Exp Med Biol* 2005;569:109–13.
- [32] Decsi T, Koletzko B. N-3 fatty acids and pregnancy outcomes. *Curr Opin Clin Nutr Metab Care* 2005;8(2):161–6.

- [33] Facchinetti F, Fazio M, Venturini P. Polyunsaturated fatty acids and risk of preterm delivery. *Eur Rev Med Pharmacol Sci* 2005;9(1):41–8.
- [34] Loosemore ED, Judge MP, Lammi-Keefe CJ. Dietary intake of essential and long-chain polyunsaturated fatty acids in pregnancy. *Lipids* 2004;39(5):421–4.
- [35] Parlee MB. Menstrual rhythms in sensory processes: a review of fluctuations in vision, olfaction, audition, taste, and touch. *Psychol Bull* 1983;93(3):539–48.
- [36] Than TT, Delay ER, Maier ME. Sucrose threshold variation during the menstrual cycle. *Physiol Behav* 1994;56(2):237–9.
- [37] Cross GB, Marley J, Miles H, Willson K. Changes in nutrient intake during the menstrual cycle of overweight women with premenstrual syndrome. *Br J Nutr* 2001;85(4):475–82.
- [38] Reimer RA, Debert CT, House JL, Poulin MJ. Dietary and metabolic differences in pre- versus postmenopausal women taking or not taking hormone replacement therapy. *Physiol Behav* 2005;84(2):303–12.