

Introduction

Free fatty acids have been shown to depolarize the membrane potential of isolated rat taste receptor cells through the inhibition of delay-rectifying potassium channels. Previously our laboratory has shown that the free fatty acids **linoleic acid (LA)** and **oleic acid (OA)** commonly found in dietary fats, such as corn oil, can be detected and avoided by rats during 2-bottle preference tests following a **conditioned taste aversion (CTA)**. Furthermore, we have also demonstrated that the detection of linoleic acid in this CTA paradigm requires an intact chorda tympani nerve.

This study extends our previous investigations of the ability of rats to detect free fatty acids on the basis of gustatory cues through three experimental objectives:

1. Assess the detection threshold for linoleic & oleic acid using short-duration stimulus presentations following a CTA;
2. Examine the effect of bilateral chorda tympani transections on the ability to detect linoleic & oleic acid following a CTA;
3. Characterize the effect of stimulus presentation duration on the ability to demonstrate an avoidance of linoleic & oleic acid following a CTA.

Methods

Chemical Stimuli

Linoleic Acid (LA)

EXP A: 44, 88, 176 μ M EXP B: 45, 55, 65, 75, 85 μ M

Oleic Acid (OA)

EXP A: 44, 88, 176 μ M EXP B: 40, 55, 70, 85, 100 μ M

Conditioning and Behavioral Testing

EXP A: Male Sprague-Dawley rats at least 90 days old were placed on a 23hr water restriction schedule and trained to reliably drink from bottle spouts in the MS-160 Davis Rig. After being trained to lick in the Davis Rig, 32 rats received bilateral chorda tympani transections and 20 rats received sham surgeries. Following 6 days of recovery from CTX or SHAM surgery with ad lib access to water, the rats were placed on a 23hr water restriction schedule, divided into four groups based on the surgery, CS free fatty acid, and UCS injection conditions (CTX/LA/LiCl n=8; CTX/LA/NaCl n=8; SHAM/LA/LiCl n=5; SHAM/LA/NaCl n=5; CTX/OA/LiCl n=8; CTX/OA/NaCl n=8; SHAM/OA/LiCl n=5; SHAM/OA/NaCl n=5), and given 15 min access to the CS (either 88 μ M LA or OA) followed by an i.p. injection of the UCS (either 150 mM LiCl or NaCl at a dosage of 13.33ml/kg). Following one day of rest after the injection day, all rats were tested in the MS-160 Davis Rig receiving 48 randomized trials consisting of 24 water trials and 4 trials each of 44, 88, and 176 μ M linoleic and oleic acid. All stimulus presentations had 8s durations and 8s inter-trial intervals. Following testing, rats were euthanized by pentobarbital overdose and the surgery conditions were verified through histological examination of the presence of open and closed taste pores on the tongue epithelium.

EXP B: Male Sprague-Dawley rats at least 90 days old were placed on a 23hr water restriction schedule and trained to reliably drink from bottle spouts in the MS-160 Davis Rig. After being trained to lick in the Davis Rig, the rats were divided into four groups based on the CS free fatty acid and UCS injection conditions (A/LiCl n=10; LA/NaCl n=10; OA/LiCl n=30; OA/NaCl n=10), given 15 min access to the CS (either 100 μ M LA or OA) followed by an i.p. injection of the UCS (either 150 mM LiCl or NaCl at a dosage of 13.33ml/kg). The following day, CTAs were tested for 50 rats in the MS-160 Davis Rig using both 8s and 30s stimulus presentation durations across a range of CS concentrations. Additionally, CTAs were assessed in 10 rats from the OA/LiCl condition with a 15 min 2-bottle preference test between water and 100 μ M oleic acid.

DATA ANALYSIS: As the rats were motivated to drink due to the water restriction schedule, the licking responses recorded in the MS-160 Davis Rig were standardized for each rat using a **lick ratio** (number of licks per tastant stimulus / the mean number of licks to water trials within each daily test session). The consumption of control and CS solutions during the 2-bottle preference tests was measured by weight (0.01 g) and reported as a **preference score** (intake of CS / total intake of control solution & CS).

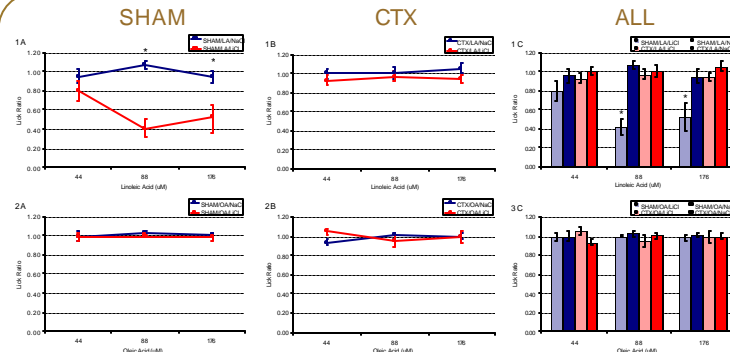


Figure 1. Lick ratios for linoleic acid concentrations following pairing of 88 μ M linoleic acid and either a LiCl or NaCl injection for the sham surgery condition (A), bilateral chorda tympani transection condition (B), and all experimental conditions (C). Significant differences are represented by stars ($p < 0.01$).

Figure 2. Lick ratios for oleic acid concentrations following pairing of 88 μ M oleic acid and either a LiCl or NaCl injection for the sham surgery condition (A), bilateral chorda tympani transection condition (B), and all experimental conditions (C).

Table 1. Results of histological verification of bilateral chorda tympani transections.

	Left Pores	Left FP	Right Pores	Right FP	Total Pores	Total FP	% FP with Pores	SE %:
CTX	3.2	55.8	2.7	55.7	5.9	111.4	5.3%	0.8%
Sham	66.8	74.5	69.8	77.4	136.6	151.9	89.5%	1.8%

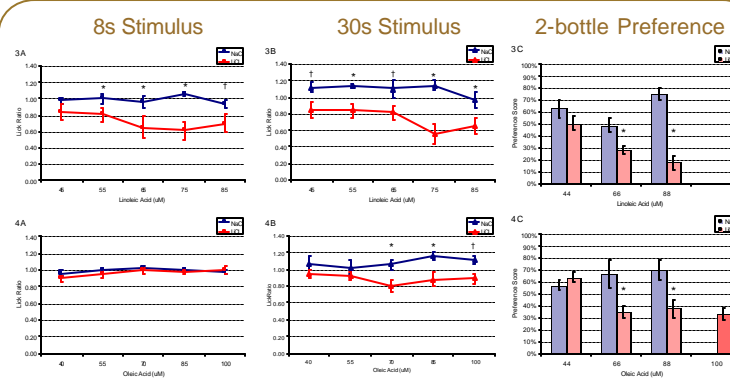


Figure 3. Lick ratios for linoleic acid concentrations following pairing of 100 μ M linoleic acid and either a LiCl or NaCl injection for stimulus presentations of 8s durations (A) or 30s durations (B). Preference score data from a previous study is shown in Fig. 3C as an example of a 15 min 2-bottle preference test assessment of CTA formation. Significant differences are represented by stars ($p < 0.01$) and crosses ($p > 0.05$).

Figure 4. Lick ratios for oleic acid concentrations following pairing of 100 μ M oleic acid and either a LiCl or NaCl injection for stimulus presentations of 8s durations (A) or 30s durations (B) in the MS-160 Davis Rig and preference scores for 15 min 2-bottle preference tests of CTA formation (C). Significant differences are represented by stars ($p < 0.01$) and crosses ($p < 0.05$).

Results

EXPA: An analysis of variance identified significant differences for the main effects of surgery and injection conditions. In addition, there was a significant interaction between the surgery, injection, and concentration conditions. Least significant difference post-hoc analyses identified significant avoidance of linoleic acid at 88 and 176 μ M concentrations for the sham surgery animals that received LiCl injections. There was no significant avoidance of linoleic acid by animals with bilateral chorda tympani transections. There was no avoidance of oleic acid by any of the experimental groups.

EXPB: An analysis of variance identified significant differences for the main effect of injection condition for both 8s and 30s linoleic acid stimulus durations. Least significant difference post-hoc analyses identified significant avoidance of linoleic acid at concentrations = 55 μ M for the 8s stimulus duration condition and all concentrations (44-88 μ M) for the 30s stimulus duration condition. An analysis of variance identified significant differences for the main effect of injection condition for the 30s oleic acid stimulus duration. Least significant difference post-hoc analyses identified significant avoidance of oleic acid at concentrations = 70 μ M for the 30s stimulus duration condition. In addition, a 15 min 2-bottle preference test between water and 100 μ M oleic acid showed a robust avoidance (37% \pm 7%) for animals receiving a LiCl injection.

Conclusions

- Following a CTA, rats avoid future consumption of linoleic acid at concentrations > 44 μ M regardless of assessment technique or stimulus duration.
- Rats lacking the chorda tympani gustatory pathway are unable to avoid linoleic acid following a CTA.
- Stimulus duration appears to affect the ability of rats to demonstrate avoidance of oleic acid following a CTA, such that 8s stimulus durations are insufficient to demonstrate the formation of a CTA, whereas 30s stimulus durations demonstrate significant decreases in licking responses to oleic acid at concentrations = 70 μ M.
- Given a single choice decision, thirst motivation may mask the formation of CTA compared to a two-choice decision assessment of conditioned taste aversions.

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