GUSTATORY DETECTION OF OLEIC ACID AND STIMULUS GENERALIZATION TO LINOLEIC ACID IN RATS.

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Introduction

On the basis of gustatory cues, rats can selectively consume dietary oils (corn oil) as opposed to non-nutritive oils (mineral oil)[4]. We believe that free fatty acid components of dietary oils are responsible for the gustatory cues. There is evidence that lingual lipase in the rat oral cavity can produce up to 5% oleic acid from complex triglyceride fats within 10 seconds of exposure[2]. Additionally, a potential transduction mechanism for free fatty acids has been identified in isolated rat taste receptor cells[1]. Previously, we demonstrated that rats can detect and avoid 66µM linoleic acid, the principle free fatty acid component of corn oil (52%), on the basis of gustatory cues[3]. Oleic acid is the second principle free fatty acid constituent of corn oil (31%).

This experiment examined the ability of rats to behaviorally detect and avoid low concentrations of oleic acid. Furthermore, we examined the effect of using free fatty acids dissolved in 5mM ethanol versus the sodium salt form of fatty acids on the behavioral detection ability of rats. Finally, given that rats can detect and avoid both linoleic acid and oleic acid, we assessed the ability of a conditioned taste aversion to either linoleic or oleic acid to generalize to the other free fatty acid.

Methods

- 32 male Sprague-Dawley rats were placed on a 23.5hr water restriction 4 days prior to the conditioning day.
- On the conditioning day all rats received 1hr access to 2-bottles of the conditioned tastant. 30 min following the 1hr access rats received i.p. injections (20ml/kg dosage) of either 150mM LiCl or 150mM NaCl.
- On the test day all rats received 1hr access to 1 bottle containing a tastant solution and 1 bottle containing a control solution (5mM ETOH or 88µM NaCl) – the bottle positions (left/right) were randomized daily.
- The following taste stimuli were tested:
  88µM linoleic acid dissolved in 5mM ethanol (LA-ETOH)
  88µM aqueous sodium linoleate (LA-SALT)
  44, 66, & 88µM oleic acid dissolved in 5mM ethanol (OA-ETOH)
  88µM aqueous sodium oleate (OA-SALT)

PHASE 1	Conditioning Day	Test Day 1	Test Day 2	Test Day 3
Group 1: LiCl-injection (n=8)	88µM LA-ETOH	88µM LA-ETOH	88µM LA-SALT	88µM OA-ETOH
Group 2: LiCl-injection (n=8)	88µM LA-SALT	88µM LA-SALT	88µM LA-ETOH	88µM OA-SALT
Group 3: NaCl-injection (n=8)	88µM LA-ETOH	88µM LA-ETOH	88µM LA-SALT	88µM OA-ETOH
Group 4: NaCl-injection (n=8)	88µM LA-SALT	88µM LA-SALT	88µM LA-ETOH	88µM OA-SALT

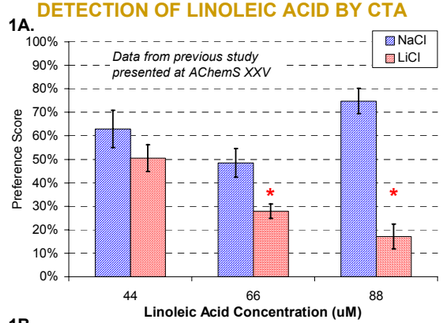
PHASE 2	Conditioning Day	Test Day 1	Test Day 2	Test Day 3
Group 1: LiCl-injection (n=8)	88µM OA-ETOH	88µM OA-ETOH	88µM OA-SALT	88µM LA-ETOH
Group 2: LiCl-injection (n=8)	88µM OA-SALT	88µM OA-SALT	88µM OA-ETOH	88µM LA-SALT
Group 3: NaCl-injection (n=8)	88µM OA-ETOH	88µM OA-ETOH	88µM OA-SALT	88µM LA-ETOH
Group 4: NaCl-injection (n=8)	88µM OA-SALT	88µM OA-SALT	88µM OA-ETOH	88µM LA-SALT

PHASE 3	Conditioning Day	Test Day 1
Group 1: NaCl-injection (n=8)	44µM OA-ETOH	44µM OA-ETOH
Group 2: NaCl-injection(n=8)	66µM OA- ETOH	66µM OA-SALT
Group 3: LiCl-injection (n=8)	44µM OA-ETOH	44µM OA-ETOH
Group 4: LiCl-injection (n=8)	66µM OA-ETOH	66µM OA-ETOH

Data Analysis

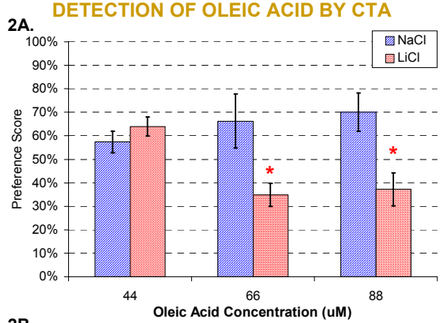
- Tastant intake was determined by difference in bottle weight measured before and after the 1hr access period.
- Preference Score = ratio of conditioned or tested tastant to total solution intake
  For example: PS = Intake of linoleic acid / Total Solution Intake (Intake of linoleic acid & control solution)
  Thus preferences scores that are **50% indicate indifference of the two solutions (tastant versus control solution);**
  < 50% preference scores indicate avoidance of the tastant;
  > 50% preference scores indicate preference of the tastant
- A 2 X 6 mixed-design ANOVA was calculated to examine the between subject and within subject effects of injection and day on the preference scores. No significant main effect of day ($F_{(5,70)}$=.346, p > 0.05) or interaction of injection X day ($F_{(5,70)}$=.221, p > 0.05) were found. There was a significant main effect of injection ($F_{(1,14)}$=113.982, p > 0.01). Post-hoc one-way ANOVA compared the preference scores of the NaCl and LiCl injection groups for each testing day. **Values of p < 0.05 are reported as significant differences between injection groups and marked with red star symbols.**

DETECTION OF LINOLEIC ACID BY CTA



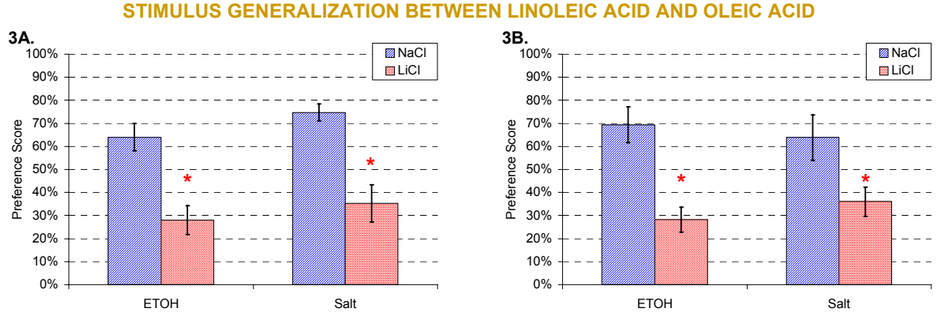
Compared to the NaCl group, the LiCl group significantly avoided 88µM linoleic acid regardless of whether the free fatty acid was dissolved in 5mM ethanol or as a sodium salt.

DETECTION OF OLEIC ACID BY CTA



Compared to the NaCl group, the LiCl group significantly avoided 88µM oleic acid regardless of whether the free fatty acid was dissolved in 5mM ethanol or as a sodium salt.

STIMULUS GENERALIZATION BETWEEN LINOLEIC ACID AND OLEIC ACID



Rats receiving a pairing of linoleic acid & LiCl injection avoid oleic acid on the subsequent testing day and vice versa.

Results

GUSTATORY DETECTION OF FREE FATTY ACIDS

- In a conditioned taste aversion test, rats could successfully detect and avoid oleic acid at concentrations equal to or greater than 66µM (Figure 2A).
- Rats not exposed to an aversive stimulus (NaCl-injection) preferred 66µM and 88µM oleic acid to the control solution in a 2-bottle test (Figures 2A, B, C).
- Rats were able to detect and avoid sodium linoleate in a similar manner as linoleic acid dissolved in 5mM ethanol (Figure 1B).
- Rats were able to detect and avoid sodium oleate in a similar manner as oleic acid dissolved in 5mM ethanol (Figure 2B).
- There was a stimulus generalization between linoleic acid dissolved in 5mM ethanol (conditioned tastant) and sodium linoleate (tested tastant) and vice versa (Figure 1C).
- There was a stimulus generalization between oleic acid dissolved in 5mM ethanol (conditioned tastant) and sodium oleate (tested tastant) and vice versa (Figure 2C).

GENERALIZATION OF FREE FATTY ACIDS

- There was a stimulus generalization between 88µM linoleic acid and 88µM oleic acid (Figure 3A).
- There was a stimulus generalization between 88µM oleic acid and 88µM linoleic acid (Figure 3B).

Conclusions

1. Rats can taste oleic acid, a secondary principle free fatty acid constituent of corn oil.
2. The rat detection threshold for oleic acid (>44µM; <66µM) as measured by conditioned taste aversion is similar to the detection threshold for linoleic acid, the principle free fatty acid constituent of corn oil.
3. Rats respond behaviorally to solutions that contain free fatty acids (linoleic and oleic) dissolved in 5mM ethanol and aqueous solutions that contain the sodium salt form of the free fatty acids (linoleate and oleate) in a similar manner suggesting that the salient taste is the fatty acid component.
4. In a conditioned taste aversive paradigm, rats indiscriminately avoid solutions containing either linoleic or oleic acid suggesting a similarity of taste perception between the two fatty acids.

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Our latest research on the taste of fat can be found at:
http://FatTaste.com

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