Role of the Gustatory System in Fatty Acid Detection in Rats

David W. Pittman

CONTENTS

4.1 Introduction ................................................................. 105
4.2 Innate Preference for Dietary Fat and Fatty Acids ........................................ 106
4.3 Fatty Acid Detection by Rats .................................................. 107
4.4 Gustatory Chemoreception of Fatty Acids ............................................... 114
4.5 Summary and Conclusions .................................................... 119

References ............................................................................... 120

4.1 INTRODUCTION

The prevalence of obesity has provided a strong incentive to understand the motivation to consume high-fat foods. Taste palatability of high-fat food is cited as the primary influence in the over consumption of dietary fat (Drewnowski, 1997; Drewnowski and Greenwood, 1983; McCrory et al., 1999, 2000; Warwick and Schiffman, 1990) and there is a strong positive correlation between obesity and daily high-level intake of dietary fat (McCrory et al., 1999; Miller et al., 1990, 1994). Traditionally, the sensory perception of dietary fat during ingestion has been characterized in terms of textural and postingestive effects. Certainly, dietary fat contributes to the mouthfeel of foodstuffs and can act as a positive reinforcer through fatty acid receptors found in the digestive tract that stimulate pathways leading to the natural reward centers of the brain. However, across the last decade, accumulating evidence from both molecular and behavioral research has begun to challenge the traditional perspectives on dietary fat perception by introducing the concept of chemoreception of dietary fat within the oral cavity, specifically through the gustatory system, as providing immediate and selective signals during dietary fat consumption. Like humans, rodents demonstrate a robust preference for high-fat foods (Greenberg and Smith, 1996; Smith et al., 2000; Warwick and Synowski, 1999; Warwick et al., 1990) and represent the most typical animal model in which the reception, transduction, and neural signaling of the peripheral gustatory system has been explored. This chapter reviews the behavioral evidence supporting a role of the gustatory system in the chemoreception of fatty acids, the principle chemical component of dietary fat.
4.2 INNATE PREFERENCE FOR DIETARY FAT AND FATTY ACIDS

Corn oil and mineral oil are the two prototypical oils respectively representing nutritive and nonnutritive stimuli in research examining dietary fat intake. Sham-feeding paradigms using a gastric fistula to eliminate postingestive feedback are useful in the analysis of orosensory influences on consumption. For example, sham-fed rats unanimously preferred corn oil over mineral oil in two-bottle forced choice tests (Mindell et al., 1990). The salient orosensory cue underlying this innate dietary fat preference does not appear to be textural in nature as rats systematically preferred corn oil to control solutions containing either mineral oil or xanthan gum when the viscous stimuli were presented either in isolation or mixed with other taste stimuli (Elizalde and Sclafani, 1990; Greenberg and Smith, 1996; Mindell et al., 1990; Ramirez, 1992; Smith, 2004; Smith et al., 2000; Takeda et al., 2001). When an aversion was conditioned to a solution of glucose, saccharin, and corn oil, the aversion did not generalize to either sweet stimulus, rather rats selectively avoided all test solutions containing the dietary fat during brief-access (30 s) trials which effectively eliminated postingestive feedback signals (Smith, 2004). Collectively, behavioral research suggests that a salient, nontextural, orosensory cue contributes to the detection and subsequent innate preference for dietary fat.

To effectively explore the chemoreception of dietary fat, we must first identify the constituent chemicals of dietary fat that could act as stimuli within the oral cavity. Vegetable oils representing prototypical dietary fats consist of triacylglycerides, the combination of three fatty acids with an ester. Linoleic acid (52%) and oleic acid (31%) are the principal fatty acids found in corn oil, the quintessential vegetable oil (Gunstone, 1995). Analysis of triacylglyceride hydrolysis by lingual lipase in the rodent oral cavity has shown that millimolar concentrations of dissociated fatty acids can be produced within 1–5 s of exposure to the lingual epithelium (Kawai and Fushiki, 2003). In support of orosensory detection of fatty acids as opposed to triacylglycerides, two-bottle forced choice tests show increased preferences for fatty acids such as oleic acid over triacylglycerides such as triolein, an oleic acid triacylglyceride (Fukuwatari et al., 2003; Tsuruta et al., 1999). Furthermore, the presence of orlistat, a potent lipase inhibitor, significantly reduces the preference for triacylglycerides but does not affect the preference for fatty acids themselves (Kawai and Fushiki, 2003). Conditioned taste aversions (CTAs) to a sucrose–corn oil mixture result in the generalized avoidance of a nonviscous sucrose–linoleic acid mixture, but do not generalize to a viscous sucrose–mineral oil mixture (Smith et al., 2000) providing additional evidence that the likely salient cue for fat detection is the chemoreception of fatty acids as opposed to textural cues. The identification of fatty acids, particularly linoleic and oleic acid, as the most likely candidates for the chemoreception of dietary fat has provided the benefit of conducting research using isolated, minute quantities of the fatty acids as chemical stimuli instead of complex lipids. Using fatty acids as stimuli instead of complex lipids allows discrete control over the concentration of the fatty acid stimulus and minimizes textural cues such as lubricity and viscosity, thus eliminating the necessity of vehicle solutions to mask textural cues.
One of the first studies to support the chemoreception of fatty acids by the gustatory system was conducted by Tsuruta and colleagues (1999) using short-term, two-bottle, forced-choice preference tests to demonstrate that rats prefer fatty acids to control solutions when textural cues were masked by xanthan gum in test and control solutions. Additionally, long-chain fatty acids such as linoleic acid were more preferred than medium-chain acids such as oleic acid suggesting that rats could detect and discriminate between fatty acid chemicals (Tsuruta et al., 1999). A similar innate preference for linoleic acid has also been confirmed in the C57BL/6J mouse strain (Gaillard et al., 2008; Laugerette et al., 2005; Sclafani et al., 2007). A specific role for gustatory sensitivity to fatty acids was first supported by evidence from the research of Fukuwatari and colleagues (2003) examining fatty acid preferences in anosmic rats. Anosmia was confirmed through conditioned odor aversion tests in rats having received intranasal zinc sulfate. Even though anosmia increased the threshold for demonstrating a preference for oleic acid suggesting a role of olfaction in the multimodal detection of fatty acids, anosmic rats were still able to detect and prefer concentrations of oleate ≥ 0.5% even when masked by the textural agent, xanthan gum. Thus, this research confirmed the contribution of a nontextural, nonolfactory orosensory cue, likely gustation, as an immediate sensory signal capable of influencing fatty acid consumption (Fukuwatari et al., 2003).

4.3 FATTY ACID DETECTION BY RATS

In addition to the innate preferential consumption of fatty acids, the orosensory detection of fatty acids has also been shown to stimulate the cephalic phase and secretion of pancreatic enzymes. Similar to innate preferences only for selective fatty acids, oral stimulation with linoleic and oleic acid enhanced pancreatic secretions in esophagostomized rats while oral application of long-chain fatty acid derivatives and middle-chain fatty acids did not elicit a pancreatic response (Hiraoka et al., 2003; Laugerette et al., 2005). Similar pancreatic responses elicited by oral stimulation with linoleic acid have also been replicated in the C57BL/6J mouse strain (Gaillard et al., 2008; Laugerette et al., 2005). The rapid cephalic response within 5 min of fatty acid stimulation suggests that orosensory cues of fatty acid ingestion not only contribute to enhanced consumption of dietary fat but also initiate digestive responses in preparation for fatty acid ingestion.

CTA methodology is a simple yet powerful paradigm used to identify the detection of sensory stimuli through pairing a conditioned stimulus (CS), typically a tastant, with an innately aversive stimulus termed the unconditioned stimulus (US). Often, a single pairing of the CS and US is sufficient for an association to be formed between the CS and the adverse effects created by the US. This association results in future avoidance of the CS until extinction of the learned association occurs following subsequent repetitive, unpaired exposures of the CS. After pairing the ingestion of a chemical stimulus as the CS with a known aversive US, such as the intraperitoneal injection of LiCl which induces gastric malaise in rodents, it is possible to test the ability of rats to detect and avoid the presence of the CS in subsequent behavioral testing. Dr. James C. Smith and colleagues (2000) were the first to employ CTA methodology to assess the detection of fatty acids in a paramount study examining
the similarity between corn oil and its main fatty acid component, linoleic acid. Following conditioned aversions to corn oil, female rats avoided 22 μM linoleic acid in a 1 h, two-bottle preference test and conversely, following a conditioned aversion to 22 μM linoleic acid, female rats avoided corn oil in a similar preference test (Smith et al., 2000). This experiment suggested that rats could detect linoleic acid and that linoleic acid and corn oil shared similar sensory perceptions such that avoidance of one stimulus generalized to the other. However, due to the 1 h testing duration, contributions of post-ingestive influences on the detection and avoidance of the fatty acid stimuli could not be discounted.

Recognizing the potential of CTAs to characterize the gustatory detection of fatty acids, we began a series of psychophysical experiments designed to measure the detection threshold and similarity between various fatty acids. Our first experiments utilized 1 h, two-bottle preference tests following a single pairing of either 88 μM linoleic acid or oleic acid dissolved in 5 mM ethanol as the CS with either a LiCl (US) or saline (control) injection. After conditioning a taste aversion, during the 1 h preference test session, male rats were able to detect and avoid both linoleic acid and oleic acid at concentrations ≥66 μM (McCormack et al., 2006). Using unesterified free fatty acids as taste stimuli required dissolution in a low concentration of ethanol (5 mM), therefore there was the possibility that an interaction between the free fatty acids and ethanol or another concomitant tastant such as sodium ions in saliva was acting as the detectable cue allowing avoidance of the CS solutions during the testing phase. However, in follow-up testing, when an aversion was conditioned to 88 μM linoleic acid in dissolved 5 mM ethanol, there was no generalized avoidance of either ethanol alone or NaCl concentrations in two-bottle preference testing between these solutions and appropriate controls (McCormack et al., 2006). These tests provided strong evidence that rats were demonstrating avoidance of the CS solutions based on cues from the free fatty acids themselves as opposed to interactions between the free fatty acids and the vehicle solution or saliva. In addition to the unesterified form of free fatty acids, fatty acids can also bind to a sodium ion to form sodium salt fatty acids such as linoleate and oleate for linoleic acid and oleic acid, respectively. Although linoleate and oleate are aqueous in water, thus eliminating the need to add ethanol to the taste stimuli, there is the additional presence of sodium ions in the fatty acid solutions. However, the presence of these sodium ions does not introduce a confounding taste variable as the concentration of sodium ions in the aqueous fatty acid solutions is in the subthreshold, micromolar concentration range far below the millimolar concentration of sodium necessary for salt detection in rodents (Slotnick et al., 1991). To ensure that rats respond equivocally to sodium salt fatty acids and unesterified free fatty acids dissolved in ethanol, we conditioned aversions to both linoleic and oleic acid in ethanol and linoleate and oleate solutions. In subsequent 15 min, two-bottle preference tests, rats demonstrated similar avoidance between the free fatty acids and sodium salt fatty acids regardless of the form of the fatty acid CS (McCormack et al., 2006).

Our series of two-bottle preference tests following a CTA to linoleic or oleic acid provided firm evidence that rats can both detect fatty acids and avoid consumption of fatty acids in short-term tests; however, measuring the amount of solution consumed during a 15 min, two-bottle test session did not allow discrete assessment of licking
Role of the Gustatory System in Fatty Acid Detection in Rats

behaviors nor could behavioral variables associated with the use of odor cues to avoid consumption of the fatty acids be measured. Additionally, although we believed that 15 min was probably not sufficient time to allow postingestive detection of fatty acids to influence consummatory behavior, we could not exclude postingestive cues as a potential contributor to the avoidance of fatty acids. Therefore, we designed a set of experiments measuring the licking responses to fatty acid stimuli during brief-access testing in an attempt to further isolate the orosensory contributions to fatty acid detection by rats. In these brief-access tests, fatty acid stimuli were presented to the rats in trials ranging from 8 to 30 s in duration following either a single CTA pairing or three consecutive days of CTA pairings of 88 μM linoleic or oleic acid with either saline or LiCl injections. This series of brief-access tests following 1 or 3 conditioning days revealed significant effects of the method of conditioning as well as the method of testing on the ability of the rats to demonstrate a conditioned avoidance to either linoleic or oleic acid.

Following a single conditioning trial using 88 μM linoleic acid, male rats demonstrated avoidance of linoleic acid at 88 and 176 μM during testing with 8 s stimulus durations; however, when the stimulus duration was extended to 30 s, rats were able to demonstrate avoidance of linoleic acid at concentrations as low as 44 μM (Pittman et al., 2006a, 2007). The generalized avoidance of oleic acid following a CTA to linoleic acid showed a similar effect of ultrashort versus brief-access testing with rats not demonstrating a generalized avoidance of oleic acid (up to 176 μM) in 8 s trials but avoiding both 88 and 176 μM oleic acid during testing with 30 s trials (Pittman et al., 2006a, 2007). In contrast to the avoidance of oleic acid in 15 min, two-bottle preference testing, following a single conditioning trial using 88 μM oleic acid male rats did not show conditioned avoidance of oleic acid at any concentration (44, 88, 176 μM) during testing with either 8 or 30 s stimulus trials (Pittman et al., 2006a, 2007).

Troubled by the incongruence of our preference testing and brief-access testing results for the detection of oleic acid, we considered two hypotheses: (1) oleic acid was not detectable through orosensory cues generated during brief-access trials; (2) a sufficient aversion was not conditioned with a single pairing of 88 μM oleic acid with a LiCl injection. Given that rats showed a generalized avoidance of oleic acid in 30 s stimulus trials following a CTA to linoleic acid, we believed that oleic acid was, in fact, detectable during brief-access testing but the weaker sensory saliency of oleic acid may have resulted in a weaker conditioned aversion than when linoleic acid was the CS. To test this hypothesis, we increased the conditioning phase of the experiment to three consecutive daily pairings of 88 μM oleic acid with LiCl injections prior to brief-access testing. Consistent with the results from our previous two-bottle preference testing of oleic acid, rats showed significant decreases in their consumption of the CS oleic acid on conditioning days 2 and 3 (see Table 4.1). In support of our hypothesis, following three consecutive CTA pairings, rats robustly avoided oleic acid at concentrations ≥50 μM in testing using 15 s stimulus durations (Pittman et al., 2007). Collectively, these experiments suggested that oleic acid was a weaker orosensory stimulus than linoleic acid supporting Tsuruta’s prior claims that rats show stronger innate preferences for linoleic acid than oleic acid (Tsuruta et al., 1999).
Fat Detection: Taste, Texture, and Post Ingestive Effects

Having characterized the responsiveness to fatty acids based on orosensory stimulation in male Sprague-Dawley rats, we expanded our research to assess the responsiveness to linoleic and oleic acid in female Sprague-Dawley rats as well as strains of obesity-prone and obesity-resistant rats. Regardless of strain, female rats consistently showed greater responsiveness to the fatty acid stimuli compared to male cohorts. Following 3 days of conditioning to 88 μM linoleic acid, the threshold for avoiding linoleic acid in brief-access testing using 15 s trials was 50 μM for male Sprague-Dawley rats and 20 μM for female Sprague-Dawley (Pittman et al., 2007). In follow-up tests, 4 days after the final conditioning day, females showed less evidence of CTA extinction avoiding 50 μM linoleic acid while the male threshold for avoidance had increased to 75 μM (Pittman et al., 2007). Female rats also showed less extinction of aversions conditioned to 88 μM oleic acid than their male counterparts by avoiding 75 μM oleic acid 4 days following conditioning compared to the male threshold for avoidance of 100 μM oleic acid. The results from our brief-access tests provided evidence that differences in the orosensory detection of fatty acids likely underlie an earlier report that female rats could detect lower concentrations of linoleic acid than male rats during 10 min preference testing to assess conditioned avoidance (Stratford et al., 2006).

Differences in dietary preferences for fat have been documented between obesity-prone (Osborne–Mendel) and obesity-resistant (S5B/Pl) rat strains such that obesity-prone rats prefer high-fat diets while obesity-resistant rats prefer high-carbohydrate diets (Gilbertson et al., 1998). Differences in the chemoreceptive sensitivity of taste receptor cells to fatty acids had been proposed as a contributing factor in these behavioral preferences for dietary fat. In vitro electrophysiological recordings showed that fatty acids produce greater inhibition of delayed rectifying potassium (DRK) currents in taste receptor cells collected from obesity-resistant rats than cells harvested from the obesity-prone rats (Gilbertson et al., 2005). Using our CTA methodology, we also found significant differences in the ability to detect and avoid fatty acids between the obesity-prone and obesity-resistant strains during brief-access testing. Within each sex, the obesity-prone rats showed conditioned avoidance to lower concentrations of linoleic acid compared to the obesity-resistant rats (Pittman et al., 2008). The obesity-prone rats also showed greater generalized avoidance of oleic acid than the obesity-resistant rats (Pittman et al., 2008). Within each strain of rat, the female rats consistently avoided lower concentrations of linoleic acid than their male cohorts (Pittman et al., 2008) confirming our results from tests of Sprague-Dawley rats. Furthermore, the

### TABLE 4.1

<table>
<thead>
<tr>
<th>Conditioning Day 1</th>
<th>Conditioning Day 2</th>
<th>Conditioning Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>13.6 ± 0.7</td>
<td>6.2 ± 0.9</td>
</tr>
<tr>
<td>NaCl</td>
<td>13.3 ± 1.3</td>
<td>14.5 ± 1.5</td>
</tr>
<tr>
<td>Statistics</td>
<td>tₙᵣ = 0.3, p = 0.74</td>
<td>tᵣ = 3.8, p &lt; 0.01</td>
</tr>
</tbody>
</table>

Differences in dietary preferences for fat have been documented between obesity-prone (Osborne–Mendel) and obesity-resistant (S5B/Pl) rat strains such that obesity-prone rats prefer high-fat diets while obesity-resistant rats prefer high-carbohydrate diets (Gilbertson et al., 1998). Differences in the chemoreceptive sensitivity of taste receptor cells to fatty acids had been proposed as a contributing factor in these behavioral preferences for dietary fat. In vitro electrophysiological recordings showed that fatty acids produce greater inhibition of delayed rectifying potassium (DRK) currents in taste receptor cells collected from obesity-resistant rats than cells harvested from the obesity-prone rats (Gilbertson et al., 2005). Using our CTA methodology, we also found significant differences in the ability to detect and avoid fatty acids between the obesity-prone and obesity-resistant strains during brief-access testing. Within each sex, the obesity-prone rats showed conditioned avoidance to lower concentrations of linoleic acid compared to the obesity-resistant rats (Pittman et al., 2008). The obesity-prone rats also showed greater generalized avoidance of oleic acid than the obesity-resistant rats (Pittman et al., 2008). Within each strain of rat, the female rats consistently avoided lower concentrations of linoleic acid than their male cohorts (Pittman et al., 2008) confirming our results from tests of Sprague-Dawley rats. Furthermore, the
female rats showed greater generalized avoidance of oleic acid as well as lauric acid, a fatty acid that male rats have not been able to detect (Pittman et al., 2008). Our results confirm an increased sensitivity of female rats to the presence of fatty acids and suggest that in both male and female rats, the obesity-prone strain may be more sensitive to detecting fatty acids than the obesity-resistant strain. These recently published findings represent the initial exploration of differences in fatty acid detection between males and females within specialized strains of rats. While our data suggest the potential for genetic influences on the ability to detect fatty acid, more research is necessary to fully characterize the differences in sensitivity and responsiveness to fatty acids between sexes and strains of rats and furthermore, to relate those behavioral differences to physiological mechanisms.

Through brief-access tests following conditioned avoidances to fatty acids, we had clearly identified the ability of rats to detect and avoid fatty acids on the basis of orosensory cues. We postulated that the salient orosensory cue was likely a gustatory signal based on the minimal textural cues associated with micromolar quantities of fatty acids and the lack of evidence to support a role of olfactory-mediated detection of the fatty acids such as differences in the latencies to lick between avoided and nonavoided stimuli during our testing sessions. If we entertain the theory that rats use a gustatory signal to detect fatty acids in our CTA experiments, then there is the question of whether gustatory signals induced by fatty acids are perceptually unique or perceptually similar to known taste qualities such as sweet, sour, bitter, or salty perceptions. In a previously unpublished study, we attempted to address this question by assessing the ability of rats to detect fatty acid to produce generalized avoidance of prototypical taste qualities. Water-restricted, adult, male Sprague-Dawley rats consumed 88 μM linoleic acid followed by an intraperitoneal injection of either 150 mM LiCl (n = 27) or saline (n = 27) at a dosage of 20 mL/kg body weight. On the following day, the licking responses of the rats were measured in an MS-160 Davis Rig (DiLog Instruments, Tallahassee) during randomly ordered, 30 s stimulus presentations of 88 μM linoleic acid, 0.2 M sucrose, 0.2 M NaCl, 0.01 M citric acid, 0.0002 M quinine-HCl, and water. All responses were standardized to the mean number of licks during water trials (licks stimulus/licks water). A mixed factorial ANOVA compared the effect of the between-subject variable, injection, on the repeated measures variable of stimulus and post hoc t-tests identified significant differences between the licking responses of the injection groups to specific stimuli. As expected, the rats responded differently to the fatty acid, sweet, salty, sour, and bitter stimuli with a main effect of stimulus type ($F_{(4,208)} = 38.1, p < 0.01$). There were both, a main effect of injection ($F_{(1,52)} = 5.2, p < 0.02$) and an interaction between stimulus and injection ($F_{(4,208)} = 6.6, p < 0.01$) indicating that the LiCl and saline-injected animals respond differently to some but not all of the stimuli. As shown in Figure 4.1, a CTA to linoleic acid was formed by the rats receiving the LiCl injection compared to the saline-injected rats ($t_{52} = 4.2, p < 0.01$). There were no other significant differences between the licking responses of the injection groups to the sweet, salty, sour, or bitter stimuli (all $t_{52} < 1.7, p > 0.09$). The lack of a generalized avoidance between linoleic acid and any of the representative tasters suggests that orosensory fatty acid detection is perceptually independent from sweet, salty, sour, or bitter taste qualities.
An alternative hypothesis to the gustatory detection of fatty acids following a CTA could be the use of olfactory cues to detect and avoid fatty acids during subsequent brief-access testing. In a preliminary, yet-to-be published study, we have attempted to test whether or not olfactory cues were sufficient to allow detection and avoidance of a stimulus tube during brief-access tests following a CTA to linoleic acid. Water-restricted, adult, female Long–Evans rats received two consecutive conditioning days receiving either 150 mM LiCl \((n = 5)\) or saline \((n = 6)\) injections \((20\, \text{mL/kg body weight})\) 20 min after consumption of 100 \(\mu\text{M}\) linoleic acid. Consumption of the linoleic acid CS did not differ between injection groups on day 1 (saline group, 9.5 ± 0.7 g; LiCl group, 9.2 ± 0.8 g); however, CS consumption significantly decreased for the LiCl-injected group compared to the saline-injected group on conditioning day 2 (saline group, 10.2 ± 0.8 g; LiCl group, 4.1 ± 0.5 g; \(t_9 = 6.8, p < 0.01\)), indicating successful formation of a CTA to linoleic acid. In order to assess the effect of fatty acid olfactory cues on avoidance of stimuli during brief-access testing, cotton gauze soaked in either distilled water or 100 \(\mu\text{M}\) linoleic acid was placed directly above and below each stimulus sipper tube in the MS-160 Davis Rig gustatory behavioral apparatus. Using this combination of stimulus solutions paired with fluid-soaked gauze to generate olfactory cues, we measured the licking responses to the following combinations of stimuli: (1) water stimulus + water olfactory cue, (2) water stimulus + linoleic acid olfactory cue, (3) linoleic acid stimulus + water olfactory cue. Each stimulus combination was presented once in five sets of randomly-ordered stimulus blocks. Stimulus presentations were 15 s in duration with 15 s interstimulus intervals between trials. Rats were given 30 s to initiate the first lick of a trial before the trial period was terminated.
The licking responses for each stimulus combination were averaged across the five sets of stimulus blocks and are shown in Figure 4.2. A mixed factorial ANOVA revealed no overall effect of injection group but a significant interaction between the stimulus type and injection group \( (F_{2,18} = 13.428, p < 0.01) \) meaning that overall, the LiCl- and saline-injected animals did not treat all stimuli differently, rather, the injection condition affected the licking response to specific stimuli. Post hoc \( t \)-tests revealed a significant difference between the licking responses to the linoleic acid stimulus + water olfactory cue combination \( (t_9 = 3.3, p < 0.01) \) for LiCl-injection \( (M = 22.8) \) and saline-injection \( (M = 63.8) \) groups. There was no significant difference in the licking responses to the water stimulus + linoleic acid olfactory cue between the LiCl-injected and saline groups. Within the LiCl-injected group, the licking response to the linoleic acid gustatory stimulus \( (M = 22.8) \) was significantly below the licking response to both water alone \( (M = 60.5, t_8 = 2.3, p < 0.05) \) and the water stimulus paired with linoleic acid olfactory cues \( (M = 66.3, t_8 = 2.9, p < 0.02) \).

In all the brief-access tests conducted in our laboratory, we assess the latency until the first lick in each trial as a measurement of olfactory influences on consummatory behavior. If olfactory cues are being used to detect and avoid conditioned stimuli, then longer latencies until the first lick would be expected for stimuli with lower lick responses than stimuli that are not avoided. Across all of our behavioral experiments, we have consistently measured no difference in the latency until the first lick between experimental groups. In the experiment described above, rats were given a 30 s window in which they must lick the stimulus tube in order to initiate a trial. Figure 4.3 displays the mean latencies until first lick across the three stimulus combinations for the two injection groups. Similar to the findings in our previous experiment, we found no significant difference in latencies between injection groups.

**FIGURE 4.2** Mean (±SEM) licks during 15 s trials to stimulus tubes containing linoleic acid or water paired with either water olfactory cues or linoleic acid olfactory following a CTA to linoleic acid. Stars represent significant \( (p < 0.01) \) differences in licks between injection groups.
studies, there was no effect of injection nor was there an effect of stimulus type on the mean latency until the first lick. On average, rats initiated a lick within the first 10 s of stimulus tube availability with a 95% confidence interval that the first lick would occur within a range of latencies from 7.1 to 12.1 s in duration.

4.4 GUSTATORY CHEMORECEPTION OF FATTY ACIDS

Three gustatory nerves transmit afferent neural signals from the rodent oral cavity to the first gustatory synapse in the nucleus of the solitary tract (NST). The greater superficial petrosal branch of the facial nerve innervates taste buds located in the nasal incisor ducts and palatal regions, the glossopharyngeal nerve innervates the taste buds of the circumvallate papilla on the posterior tongue, and the chorda tympani branch of the facial nerve innervates taste buds found in the fungiform papillae on the anterior tongue. The evidence from CTA studies implies a role for the gustatory system in the orosensory detection of fatty acids; therefore, we hypothesized that bilateral transection of a gustatory nerve could compromise the ability of rats to detect and avoid fatty acids following a CTA. In 2000, we presented the first evidence to support a role of the chorda tympani nerve in the detection of linoleic acid demonstrating that rats with intact chorda tympani nerves were able to form a CTA and subsequently avoid 28 μM linoleic acid in a 1 h, two-bottle preference test; however, rats with bilateral chorda tympani transections did not form CTAs to linoleic acid (Pittman et al., 2000). Using more refined, brief-access testing (8 and 30 s trials), we later confirmed a role for the chorda tympani nerve in

![FIGURE 4.3](image-url)
the detection of linoleic acid by providing evidence that rats with bilateral chorda tympani transections showed elevated thresholds for detecting linoleic acid compared to sham-operated controls (Harris et al., 2005). In these preliminary investigations, the transection of the chorda tympani nerve preceded the conditioning and testing phases of the experiment. Therefore, we conducted a final experiment to demonstrate that chorda tympani nerve transection could impair the detection of fatty acids following a known, preexisting CTA to either linoleic or oleic acid. Male and female Sprague-Dawley rats received CTAs to either 88 μM linoleic or oleic acid prior to nerve transection. Having confirmed a CTA to the CS, either linoleic or oleic acid, half of each subject group received bilateral chorda tympani nerve transections. Following three recovery days, brief-access tests with 15 s trials showed a persistent avoidance of linoleic acid in the sham-operated male (threshold ≥75 μM) and female (threshold ≥50 μM) rats, while bilateral chorda tympani transections eliminated any avoidance of linoleic acid up to 100 μM concentration for both male and female rats (Pittman et al., 2007). A similar effect of chorda tympani transection was observed for the conditioned aversions to 88 μM oleic acid with lesioned animals showing no avoidance of oleic acid (≤100 μM) and sham-operated male and female rats demonstrating 100 and 75 μM thresholds, respectively for oleic acid (Pittman et al., 2007). One other publication to date has confirmed a role of the chorda tympani nerve in the detection of fatty acids by rats demonstrating that bilateral chorda tympani nerve transection prior to conditioning an aversion to linoleic acid produced elevated thresholds for linoleic acid detection during longer, 10 min, two-bottle preference testing (Stratford et al., 2006).

A similar impairment in linoleic acid detection following gustatory nerve transection has recently been shown in the C57BL/6J mouse strain (Gaillard et al., 2008). This study showed that transection of the glossopharyngeal nerve reduced short-term (0.5 h) and long-term (48 h) innate preferences for linoleic acid and combined transections of both the glossopharyngeal and chorda tympani nerves eliminated the innate preference for linoleic acid. After conditioning a strong aversion to 2% (75 mM) linoleic acid in C57BL/6J mice, combined glossopharyngeal and chorda tympani transections eliminated the detection and avoidance of linoleic acid in subsequent 1 h, two-bottle preference tests. In addition to impaired consummatory responses related to the detection of linoleic acid, glossopharyngeal nerve transection also reduced pancreatic-bile flux following oral stimulation with linoleic acid. Combined glossopharyngeal and chorda tympani nerve transections resulted in even greater reductions in pancreatic-bile secretions in response to oral application of linoleic acid (Gaillard et al., 2008). Collectively, nerve transection research has shown that disrupting either the chorda tympani or glossopharyngeal afferent taste pathways impairs the ability of rodents to express innate ingestive preferences for fatty acids, to detect and avoid fatty acids following a CTA, and reduces the cephalic responses to oral stimulation by fatty acids. This is the strongest evidence to date supporting a role for fatty acid taste signals as immediate sensory cues capable of influencing ingestive behaviors.

Several molecular mechanisms have been proposed to be involved in the oral detection of fatty acids. Localized expression in gustatory cells and neurophysiological evidence supports a role for the lipid-binding protein CD36 (Fukuwatari
and the DRK channel (Gilbertson et al., 1997, 1998, 2005) as potential transduction mechanisms for fatty acids within the gustatory system. Furthermore, there is emerging evidence that a subset of G protein coupled receptors, specifically GPR120 (Matsumura et al., 2007) and GPR40 (Hansen et al., 2006), may also have the potential to transduce fatty acid stimuli within the gustatory system. Behavioral evidence also supports a role for CD36 in the detection of fatty acids utilizing a CD36 knockout (KO) mouse model in comparison with responses of C57BL/6J wild-type (WT) mice. Removal of the CD36 protein in the KO mice eliminated the innate preference shown by WT mice for 2% (75 mM) linoleic acid in 0.5 and 48 h preference tests (Laugerette et al., 2005). Also contrary to the preference of WT mice, CD36 KO mice showed indifference between consumption of a 5% linoleic acid-enriched solid diet compared to a paraffin oil-enriched diet in a 1 h meal preference test (Laugerette et al., 2005). Sclafani and colleagues (2007) provided independent confirmation that naïve CD36 KO mice show indifference to fatty acids and do not prefer soybean oil or Sefa Soyate oil at dilute concentrations as compared to WT mice which preferred the fatty acid stimuli. Sclafani et al. (2007) also demonstrated that postigestive exposure to fatty acids was sufficient to condition a learned preference for fatty acids and oils in the CD36 KO mice although this “rescued” preference was less than the innate preference for fat demonstrated by the WT mice. The most compelling evidence thus far that CD36 is involved in the transduction of fatty acids was recently published by Gaillard and colleagues. In this study, oral stimulation with linoleic acid induced c-fos activity in NST neurons compared to either a control xanthan gum solution or water stimulation in WT mice, and a lack of differential expression of c-fos between the viscous xanthan gum and water control solutions in the WT mice indicated that textural cues are not activating neurons within the gustatory zones of the NST (Gaillard et al., 2008). In contrast to the WT mice, CD36 KO mice showed no increase in the number of Fos-immunoreactive neurons between linoleic acid versus control solutions indicating that CD36 is a necessary protein for fatty acid activation of neurons within the gustatory zone of the NST in the C57BL/6J mouse (Gaillard et al., 2008).

The mechanisms of action for CD36 and DRK following fatty acid stimulation act to depolarize taste receptor cells, therefore, increasing the responsiveness of the taste receptor cells. As supported by nerve transection research and observed c-fos activation in the gustatory zone of the NST, in absence of other taste chemicals, the depolarization of taste receptor cells in response to fatty acid application appears sufficient to stimulate afferent neural signals in the gustatory nerves. Given the ability of fatty acids to depolarize taste receptor cells in theory, adding fatty acids to a solution of known taste chemicals should produce a larger afferent taste signal than the same concentration of taste chemicals in the absence of the fatty acids. To test this hypothesis, we characterized the licking responses of male, Sprague-Dawley rats across varied concentrations of both innately appetitive and aversive taste stimuli with and without fatty acids added to the taste solutions (Pittman et al., 2006b). Appetitive taste stimuli were defined as innately preferred tastants such as the sweet stimuli, sucrose and glucose. Whereas, aversive taste stimuli such as
sour, bitter, and salty tastants produce innate rejection behaviors and are naturally avoided unless animals are sufficiently motivated to consume the tastants such as due to thirst. Using a 23 h water-restriction paradigm and brief-access stimulus testing, rats are sufficiently motivated to consume low to moderate concentrations of salty (NaCl), sour (citric acid), and bitter (quinine-HCl) taste stimuli at a maximal lick rate similar to water; however, in spite of the thirst motivation, moderate to high concentrations of these aversive taste stimuli are not tolerated and thus licking responses decrease to near total avoidance of strong concentrations. In contrast, in similar brief-access testing procedures, increasing the concentration of the unadulterated, appetitive taste stimuli produces s-shaped concentration-dependent functions of licks per 20 s trial for water-replete rats. When either 88 μM linoleic or oleic acid was added to the appetitive solutions, the animals increased their licking responses resulting in upward shifts in the concentration-dependent functions (Pittman et al., 2006b). This characteristic change in licking behavior is reflective of an increase in the responsiveness to previously less preferred concentrations of the sweet stimuli. Conversely, when either 88 μM linoleic or oleic acid was added to the aversive stimuli, licking responses decreased, producing downward shifts in the concentration-dependent licking responses (Pittman et al., 2006b). In effect, the breakpoint of thirst-motivated tolerance of the moderate concentrations of aversive stimuli was lowered by the addition of fatty acids resulting in avoidance of previously tolerated concentrations of salty, sour, and bitter stimuli. The addition of fatty acids to the tastant solutions produced the greatest effects on middling concentrations of both the appetitive and aversive taste stimuli. These middling concentrations of tastants in absence of fatty acids were in the range of stimuli that elicited a transition in licking responses from tolerance to avoidance of aversive stimuli or in the case of appetitive stimuli, from minimal towards maximal licking responses. We interpret these characteristic changes in licking responses when fatty acids were indicative of an increase in the perceived intensity of the tastants at concentrations that in absence of fatty acids elicited neither minimal nor maximal licking responses. Figure 4.4 compares the magnitude of significant effects collapsed across concentrations for each of the taste stimuli tested in the original publication (Pittman et al., 2006b). On average, the addition of either linoleic acid or oleic acid elicited >60% more licks to appetitive stimuli, while the presence of fatty acids typically suppressed the licking responses to aversive stimuli by approximately 30%. These results support the hypothesis that fatty acid-induced depolarization of taste receptor cells may act to increase the afferent taste signals elicited by concomitant taste chemicals such that in the presence of fatty acids weaker concentrations of appetitive or aversive stimuli produce behavioral responses previously associated with higher concentrations of the tastants in absence of fatty acids.

In addition to assessing the effects of 88 μM linoleic or oleic acid on prototypical taste stimuli, we also measured the effect of adding a fatty acid mixture of 55 μM linoleic acid and 33 μM oleic acid. This 88 μM combined-concentration of fatty acids approximates the ratio of linoleic to oleic acid found in the triacylglycerides of corn oil, a prototypical dietary fat. As shown in Figure 4.4, this fatty acid mixture did not produce synergistic changes in licking responses, but elicited similar effects in magnitude on the middling concentrations of both appetitive
Fat Detection: Taste, Texture, and Post Ingestive Effects

and aversive stimuli as either 88 μM linoleic or oleic acid when tested separately (Pittman et al., 2006b). Although the lack of synergistic increase in the magnitude of the responses to the fatty acid mixture supports common reception mechanisms and molecular pathways within the gustatory system for both linoleic and oleic acid, in general, linoleic acid did produce more robust changes in licking behavior to the tastants than oleic acid. Similar to previous reports of lower spontaneous preference for oleic acid compared to linoleic acid and evidence that oleic acid is a less effective CS than linoleic acid, oleic acid produced changes in the licking responses to fewer tastant concentrations (31% of tested concentrations) than linoleic acid (48% of tested concentrations). However, when oleic acid did produce a significant change in the licking response to either appetitive or aversive stimuli, the magnitude of the effect was similar to linoleic acid as shown in Figure 4.4.

Research from other laboratories has corroborated the evidence supporting the ability of fatty acids to increase the perceived intensity of appetitive stimuli. Gilbertson and colleagues demonstrated that while neither 20 μM linoleic acid nor a subthreshold concentration of the non-nutritive sweetener, saccharin, produced spontaneous preference in isolation, when 20 μM linoleic acid was added to the subthreshold saccharin solution, an ingestive preference was elicited (Gilbertson et al., 2005). A similar effect on the preference of sucrose was reported by Stratford et al. (2006) with both 44 and 88 μM linoleic acid increasing the number of licks to 0.0375 M sucrose during brief-access 10 s trials.

FIGURE 4.4 The magnitude of significant effects by fatty acid stimuli on the licking responses to appetitive (sucrose and glucose) and aversive (NaCl, citric acid, and quinine) tastants as reported in Pittman et al. (2006b).
4.5 SUMMARY AND CONCLUSIONS

The ability to detect dietary fat during feeding represents an advantageous adaptation facilitating the ability to consume high-caloric foods and essential fatty acids. This chapter reviewed the recent evidence supporting the role of the gustatory system in providing an immediate sensory signal allowing the detection of the fatty acid components of dietary fat during consumption by rats. Initial research supported an innate preference for dietary fat utilizing methodology that controlled textural and olfactory cues, leading to the discovery that rats could detect the fatty acid components of dietary fat through orosensory cues likely mediated by the rodent gustatory system. Subsequent research began characterizing the ability of rats to detect fatty acids through orosensory cues. As detection thresholds and fatty acid discrimination was examined through CTA studies, greater female sensitivity in detecting fatty acids was identified along with differences between strains of rats such as obesity-resistant rats showing stronger aversions to fatty acids than obesity-prone rat strains. New evidence was presented in this chapter suggesting that orosensory signals generated by fatty acids are likely to be unique from sensations associated with the prototypical tastes of sweet, sour, salty, and bitter chemicals and that olfactory cues are most likely not sufficient to allow detection and avoidance of fatty acids following a CTA. Finally, research demonstrating fatty acid influences on the innate ingestive behavior for prototypical tastants and impairments in fatty acid detection following gustatory nerve transections and genetic KOs of specific fatty acid receptors in the gustatory system were discussed as quintessential evidence for the chemoreception of fatty acids within the gustatory system.

The ingestion of fatty acids, the chemical components of dietary fat, produces a multimodal sensory perception. Perhaps the adaptation of multiple sensory systems to detect fatty acids is not surprising, given the high-caloric value of dietary fat and the importance of ingesting essential fatty acids such as linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids. Historically, the predominant sensory perceptions associated with fatty acids have been somatosensory and olfactory orosensory signals as well as postingestive mechanisms allowing the detection of fatty acids and subsequent activation of the intrinsic reward system in response to dietary fat consumption. This chapter outlined a preponderance of recent evidence suggesting that the gustatory system also plays a significant role in the immediate detection of fatty acid consumption. Furthermore, the effect of fatty acids on the gustatory system appears to be sufficient to influence consummatory ingestive behavior of fatty acids alone or concomitantly mixed with tastants as well as being sufficient to stimulate cephalic responses in anticipation of fatty acid digestion. Recent reports of the detection of fatty acids on the basis of gustatory cues in humans (Chale-Rush et al., 2007a,b; Mattes, 2001a,b, 2005, 2007) has given increased importance to the development of an animal model in which we can explore the ability of fatty acids to elicit gustatory sensations capable of influencing dietary fat consumption. A comprehensive understanding of the chemoreception and neural coding of fatty acids by the gustatory system holds the promise of being able to manipulate high-fat foods in the future such that the palatability associated with high-fat content can be retained.
while reducing the high-caloric density of dietary fat that is contributing to the human obesity epidemic. This chapter provides a historical review of the research to date that has led to the development of a rodent model allowing the characterization of the taste of dietary fat.

REFERENCES


Role of the Gustatory System in Fatty Acid Detection in Rats


