Gustation as a factor in the ingestion of sweet and fat emulsions by the rat

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Abstract

This paper was written to pay honor to Professor Gerard P. Smith because of his strong influence on me to study the ingestion of sweet and fat mixtures. Three experiments are reported here, in which the laboratory rat was given an emulsion of a glucose + saccharin mixture with corn oil. In the first experiment, a two-bottle, 24-h test was given comparing the emulsion with water. Over 6 weeks, the concentration of the corn oil was gradually increased. When given only food and water, or the glucose/saccharin solution, the rats regulated their caloric intake and grew at a normal rate. In contrast, when the corn oil was present, the rate significantly increased their caloric intake, resulting in a marked increase in body weight. In the second experiment, a detailed analysis of the ingestion revealed that the rate of licking the emulsion during drinking bouts increased in a linear manner as the concentration of the corn oil was increased. In the third experiment, a conditioned taste aversion to the sweet/fat emulsion generalized to the fat more than to the sweet solutions. The implications for a gustatory input are discussed.

Keywords: Gustation; Ingestion; Sweet and fat emulsion

I am honored to be asked to make a tribute to Professor Gerard P. Smith in a special issue of Physiology and Behavior. Gerry Smith has been a colleague, a collaborator, and, most importantly, a true friend.

I first met Gerry in La Napoule, France, at the Second Benjamin Franklin/Lafayette Seminar at La Napoule organized by Eliot Stellar, Alan Epstein, and Stelio Nicolaidis in 1982. My first sight of Gerry was a memorable event.

I had received an invitation to this seminar from Alan Epstein. Most of my research up to that time had been devoted to olfaction and gustation, and, although I had known Professors Epstein and Stellar since the early 1960s, I was new to the feeding group. I did not know all of the “players” and I was quite intimidated by all of these experts on the topic of ingestion. Two things involving ones participation in the seminar had been made quite clear to me by Professor Epstein. The first was that you could not come to this meeting unless you agreed to be there on Sunday and stay through the day on Friday. After we got there, the second rule I remember was that you only entered the conference room by the back door. Never were you to come into a session by the side door. The lecture hall at Le Château de La Napoule was a dark cave with questionable acoustical qualities.

Enter Professor Gerard P. Smith. It was the middle of the second day when the forbidden side door burst open, and the Cote D’Azur sun flooded the room. In the middle of this sunlight stood Gerry Smith, as if surrounded by an aura. He had violated both of the firm rules. He was late in arriving, and he interrupted the ongoing meeting by coming in the forbidden door. My first thought was that Alan Epstein and Eliot Stellar would eat him alive. Instead, all of the participants rose to their feet and broke into a vigorous applause. My second thought was that this must be some pretty special person. I soon learned that Gerry Smith was indeed someone pretty special, and, in my mind, the aura that surrounded him that day in southern France has never diminished.

Meeting and talking with Gerry was fun from the beginning. It is even more pleasing that the conversation has lasted for over 20 years. After returning from France, I made the first of many trips to the Bourne Laboratory in White Plains, NY, and both Gerry Smith and Jim Gibbs have visited often in my laboratory at The Florida State University in Tallahassee, FL.

The conversations led to a strong collegiality and collaboration. Some years later, while Gerry and I were doing
sucrose taste tests with rats after injections of Raclopride, one of my undergraduate students, Julie Schumm, made the observation that the rats could "identify the concentration of the sucrose solution" when the shutter opened and before they took their first lick. Being somewhat skeptical about this observation, Gerry and I designed a series of experiments in which we studied the latency to the first lick when the shutter opened, allowing us to infer the capability of the rat to detect sucrose concentrations by sniffing, not licking, on the bottles. This led to a paper showing how rats could detect the odor of sucrose and even discriminate different concentrations of the sucrose solutions by olfaction. Along with Jodi Rhinehart-Doty and Julie Schumm, Gerry and I published these results in Chemical Senses [1].

In an essay that Gerry wrote about his meetings with Professor Curt Richter [2], he recalled, "I once asked him why he had never been interested in studying the ingestion of fats when he had spent so much time studying the effects of sugars. He just laughed and said that he never got around to fats because the sugars always gave such neat results." Gerry probably asked more than one person that question, but when he asked me, it started a chain of events that lasted many years.

During one of my visits to the Bourne Laboratory in 1989, I lectured on sweeteners ingested by the rat, especially the special mixture of glucose and saccharin [3–6]. It was then that Gerry asked me the same question that he had asked Richter: "Why limit your study to sweeteners, why not study fat?"

While preparing this essay, I was perusing my thick file on Gerry Smith. There, I found a letter I had written to him shortly after that visit where I said, "I talked with my technician, and as soon as I can get to the store we are going to hang some corn oil on a few cages and get some basic information about 'if and how' our rats handle it." We did that, and it led to several experiments, which I shall report in this essay.

We first blended the corn oil with a glucose and saccharin solution and made some interesting findings. In the first of three experiments, we gave 30 rats 56 continuous days of 24-h, two-bottle preference testing. After 7 days of baseline testing, where all rats were given their powdered food (Purina Chow) and distilled water in both drinking bottles, the 30 rats were divided into three groups of 10 each. Group 1 continued to receive only food and water for the next 7 weeks. Group 2 received food and a choice between water and a glucose + saccharin mixture (1.25 g of sodium saccharin and 30 g of glucose mixed in 1 l of distilled water, subsequently referred to as G + S) for the entire remaining 7-week period. Group 3 received the G + S solution during the second week of testing, and, after that week, corn oil was blended with the G + S (making G + S + F). During Week 3, the concentration of the corn oil was 2%; in Week 4, it was 4%; in Week 5, it was 8%; in Week 6, it was 16%; in Week 7, it was 32%; and finally, in Week 8, it was 64%. This G + S + F mixture was made by blending the G + S solution with the corn oil in a VITA-MIX blender for 2 min. Tween-80 was added to the mixture to inhibit separation. Each day, we measured the amount of powdered chow and fluid that each animal consumed. We measured the body weight of the 30 rats at the end of each week of testing.

The mean body weights for each of the three groups are plotted for the 8-week testing period in Fig. 1.

As can be seen, there were no differences between the WATER and the G + S groups, but the G + S + F group, gained about 140 g compared with a 60-g weight gain for the other two groups over the 6-week period. A repeated sampling of the same-subject ANOVA and subsequent paired tests indicated that a significant difference occurred on the fifth week of testing when the corn oil concentration reached 8%. The G + S rats in Group 2 took about 10 cal/day from the sweetened solution, but their food intake dropped about 10 cal/day, hence, their total caloric intake did not differ from the F + W group. In contrast, the G + S + F group, on the last 2 weeks of testing, took about 90 cal/day from the sweet/fat solution. They also decreased their food intake, but by only about 30 cal, resulting in a net increase of about 60 cal, hence, the large increase in body weight.

Fig. 1. Mean body weights are plotted as a function of the concentration of corn oil in the glucose + saccharin + corn oil emulsion. Starting on Week 3, 2% corn oil was added to the sweetened solution. The concentration of the corn oil was doubled at the beginning of each subsequent week, resulting in a 64% concentration on Week 8. The error bars are standard errors.
drinking in a sham-feeding test, and (e) electrophysiological recording from the Greater Superficial Petrosal Nerve [10], we have postulated that the rate of drinking during a bout in these 24-h tests is a good measure of the taste of sucrose by the rat. It seems as though the rat treats a drinking bout as a short-term test, and he adjusts the overall daily caloric intake by the bout duration and number.

In Experiment 2, we tested this within-bout rate of drinking as the concentration of corn oil was increased. With the G+S+F mixture, we tested eight additional rats in the "hotel" with a two-bottle, 24-h test with water versus the G+S+F emulsion. As with Group 3 in the first experiment, each week, the concentration of the corn oil blended with the G+S emulsion was doubled. As we had previously seen with sucrose, we observed here a similar increase in the rate of drinking during a bout when increasing the concentration of corn oil across the weeks of testing. This rate increase can be seen in Fig. 2.

The last experiment that I will describe in this essay (Experiment 3) is a conditioned taste aversion experiment, induced by a LiCl injection. The magnitude of the taste aversion was measured with single-bottle tests in a "Davis Rig" [11,12]. Briefly, the Davis Rig has eight drinking tubes that are aligned on a sliding metal platform that can be moved in either direction by a reversible motor. Access to any one of the eight tubes was possible by aligning the tube with a slot in front of the testing cage. A motor-driven shutter could open, giving the rat access to a particular drinking spout. The rig was connected to a Davis MS80, an eight-station programmable taste analysis system that records the amount of time that elapses between each lick (in ms), thereby recording the number of licks on each tube (Dilog Instruments).

In the present experiment [13], on conditioning day (after the 14 SD rats had previously been trained to lick the sipper tube), all of the bottles were filled with the G+S+F emulsion. This emulsion served as the conditioned stimulus in this taste aversion experiment. Following consecutive presentations of each of the eight tubes con-

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**Fig. 2.** Licks per second within a drinking bout are plotted as a function of the concentration of the corn oil in the glucose + saccharin + corn oil emulsion. Starting on Week 3, 2% corn oil was added to the sweetened solution. The concentration of the corn oil was doubled at the beginning of each subsequent week, resulting in a 64% concentration on Week 8.

The question was "why did the G+S+F rats fail to adjust their total caloric intake?" Is the G+S+F solution that much more palatable?

We have, over the years, developed a special set of testing cages that yielded moment-by-moment measurement of ingestive behavior, allowing us to quantify the number of ingestive bouts, the length of these bouts, and the rate of consumption during a bout [7,8]. At the Bourne Lab, this rig was later labeled as a "rat hotel". Using this hotel, we had previously reported that when given a 24-h, two-bottle preference test between sucrose and water, rats drink the sucrose within a bout at a rate that is directly proportional to the concentration of the sucrose [9,10]. Because this rate function is so similar with (a) licking in a short-term test, (b)
Fig. 4. On conditioning day, one group of rats received an injection of LiCl and another received isotonic saline after drinking a glucose + saccharin + corn oil emulsion. The data reported in this figure were taken on the second day following conditioning day. Each rat received eight 30-s presentations of a particular fluid, as illustrated in the figure. As can be seen, all four presentations containing the corn oil (F) were avoided by the conditioned group (LiCl) and avidly consumed by the control group (NaCl).

The mean number of licks for the LiCl- and NaCl-injected groups are reported in Table 1, along with the appropriate t tests.

In these brief exposure tests, the conditioned aversion to the G + S + F (corn oil) mixture generalized profoundly to all of the components of the mixture containing the corn oil (Presentations 2, 5, 7, and 8). There was no generalization to any of the sweetened solutions. We conclude that the salient feature of the glucose + saccharin + corn oil emulsion was the corn oil. Because these presentations were only 30 s in duration, we think it unlikely that postigestional factors influenced the outcomes. We conclude that the results from these ultra-short-term tests are the result of oral cavity factors, most likely, gustation. As mentioned earlier, differences in latencies to lick a particular solution when the shutter opened had been used as evidence that the rat could discriminate one solution from another on the basis of odor [1]. The latency to the first lick of the G + S + F mixture was the same for the LiCl- and NaCl-injected groups, indicating that it is unlikely that the rats could detect the G + S + F mixture on the basis of odor alone.

The extensive work of Gilbertson [14] and Gilbertson et al. [15,16] has provided even stronger evidence that the rat can taste corn oil as the result of the breakdown of the oil into its fatty acid components. We have initiated a series of experiments like those described above, substituting linoleic acid, a major fatty acid component of corn oil, for the corn oil in the G + S mixture. Preliminary results have shown that the rat can discriminate water from linoleic acid in concentrations as low as 10 μM. Offactory bulb ablations do not interfere with these discriminations, but bilateral sections of the chorda tympani nerve do. Evidence points to a gustatory discrimination.

Inspired by Gerry’s experiments with corn oil [17], we have completed studies similar to those reported above when mixing corn oil with sucrose [18]. When we finished the first experiment in that work, I reported to Gerry that rats conditioned with the mixture of sucrose + corn oil generalized the aversion to corn oil, but not to sucrose; Gerry raised his eyebrows and said, “let me tell you the experiment that will make me believe your results”. He suggested that we condition an aversion to sucrose in one group of rats and an aversion to corn oil in a second group. Then, we were to test both groups to see if their aversion

Table 1
The mean number of licks for the LiCl- and NaCl-injected groups are reported for each of the eight mixtures presented to the rats

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>G + S + F</td>
<td>GLUC</td>
<td>SACC</td>
<td>OIL</td>
<td>G + S</td>
<td>G + F</td>
<td>S + F</td>
</tr>
<tr>
<td>Mean licks by the LiCl group</td>
<td>203.9</td>
<td>2.8</td>
<td>214.6</td>
<td>208.9</td>
<td>2.6</td>
<td>211.4</td>
<td>1.0</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean licks by the NaCl group</td>
<td>201.7</td>
<td>195.8</td>
<td>199.7</td>
<td>182.3</td>
<td>199.8</td>
<td>202.00</td>
<td>170.7</td>
<td>148.7</td>
</tr>
<tr>
<td>t Tests between the groups</td>
<td>.27</td>
<td>.23</td>
<td>1.58</td>
<td>1.41</td>
<td>21.94</td>
<td>0.90</td>
<td>10.84</td>
<td>7.23</td>
</tr>
<tr>
<td>Values of P</td>
<td>&gt;.05</td>
<td>&lt;.01</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
<td>&lt;.01</td>
<td>&gt;.05</td>
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generalized to the sucrose + corn oil mixture. If we could show a stronger generalization in the group conditioned with corn oil, then we would be satisfied that the salient feature of the sucrose + corn oil mixture was the corn oil. We did that experiment (the third experiment in Ref. [18]), and, indeed, the generalization was stronger by far in the corn oil group. My students labeled that experiment the “Gerry Smith verification”.

When Gerry writes the sequel to “The controls of fat intake” [19], I hope our results will be considered. In their paper on these controls, Danielle Greenberg and Gerry Smith said, “In summary, the changed intake produced by different concentrations of corn oil emulsions is evidence for an orosensory system that responds to oils in a regulated and sensitive fashion. The nature of the neurological substrate for this response remains to be determined. We have proposed that tactile receptors of afferent fibers of the trigeminal nerve respond to a textural dimension of oils, but olfactory mechanisms activated retrogradely from within the mouth cannot be ruled out” [19]. We hope that they will add the possibility of gustatory factors to the neurological substrate.

Gerry’s influence on my life goes far beyond sweet and fat oil emulsions. In science, the sage advice and the careful critique have been invaluable over the past 20 years. Our initial science-related contacts have evolved into a deep and lasting friendship that has been a valuable part of both Liz’s life and mine. For all of these reasons, I will remain grateful for having known Professor Gerard P. Smith.

Acknowledgements

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References