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Concentration and state dependent reductions in corn oil intakes after glossopharyngeal nerve transections in rats

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HIGHLIGHTS

- GLx reduced the intakes of low and moderate fats in satiated rats.
- GLx prevented increases in the intakes of low and moderate fats in hungry rats.
- GLx reduced the intake of full fat in thirsty rats.

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ABSTRACT

Previous studies indicate a role for the glossopharyngeal nerve (GL) in the detection of dietary fats. The present experiments examined the effects of bilateral glossopharyngeal nerve transections (GLx) on the intake of low (4.8%), moderate (16%), and full-fat (100%) corn oil in non-deprived, food-deprived, and water-deprived rats. The rats had access to oils, 0.3 M sucrose, and water in a gustometer that measured number of licks and latency to the first lick during brief access trials. The behavioral measures were used as indices of the amount consumed and the motivation to ingest, respectively. After baseline intakes had stabilized, the rats received GLx or sham transections (Sham) and were then re-tested. Pre and post-surgery responses were compared to determine the impact of GLx on intake and the motivation to ingest. In non-deprived rats, GLx reduced the intake of 4.8% and 16% oils and decreased the motivation to ingest these oils. In food-deprived rats, GLx prevented increases in the ingestion of 4.8% and 16% oils and in the motivation to ingest these oils. In water-deprived rats, GLx reduced the intake of 100% oil and produced a general decrease in the motivation to consume low, moderate, and full-fat emulsions. These results indicate that GL is partially involved in corn oil intake and suggest an interactive effect of oil concentration with homeostatic state.

1. Introduction

Humans and rodents prefer fat containing foods [1–4]. When intake is tested using sequential 1-bottle tests or a 2-bottle simultaneous test, rodents consume more oil than an oil-free control solution [5–7]. Fat preference in rats occurs early during development: Pre-weaning pups show a preference for corn oil over water by post-natal day 14 [8,9]. By post-natal day 21, pups display a concentration-dependent oil intake that is maintained into adulthood [3,4,8,9]. This response function is evident when rats are tested for short-duration intake (5–30 min) or sham-fed via a gastric or an esophageal fistula [8–12]. Since these procedures minimize the amount of oil consumed and absorbed, fat preference is likely associated with orosensory stimulation rather than post-ingestive feedback. Studies examining olfactory involvement in fat intake have yielded mixed results. Most investigations, however, show that intakes of low and full fats are merely reduced, and not eliminated, in anosmic rodents [7,13–15].

When dietary fats are introduced into the oral cavity, they are converted rapidly into free fatty acids (FFAs) [16]. The conversion of dietary fat to FFAs is thought to be mediated by lingual lipase secreted by the von Ebner's glands [16–18]. Although this enzyme is present in various species including rodents, humans, and non-human primates, the amounts present differ across species [19]. Compared with humans, rats show much higher levels of lingual lipase activity [19]. Recent evidence indicates that CD36, a fatty-acid binding membrane protein, may act as a lipid sensor [20–22]. In rodents, CD36 is expressed in the posterior lingual papillae, primarily in taste buds within the circumvallate (CV) and to a much lesser extent those in the foliate [22]. Taste buds in the posterior tongue are innervated by the glossopharyngeal nerve (GL), whereas those in the anterior tongue are innervated by the chorda tympani nerve (CT) [23–25]. Application of FFAs to the posterior tongue

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elicits responses from GL, whereas application of FFAs to the anterior tongue does not elicit responses from CT [26,27]. Furthermore, CD36-null mice show neither a preference for FFAs nor GL responses to FFAs [26,27]. These findings indicate that GL may function to transmit the afferent signals for FFAs and perhaps for dietary fats.

The present study aims to examine the role of GL in maintaining corn oil intake. Rats were trained to lick water, corn oil emulsions (4.8%, 16%, and 100%), and 0.3 M sucrose from a gustometer before and after bilateral GL (GLx) or sham (Sham) transections. In order to minimize post-ingestive effects, a brief access (10 s per trial) test was used. The number of licks and the latency to the first lick were measured and used as indices of the amount consumed and the motivation to feed, respectively. To determine the impact of GLx on oil intake under different homeostatic states, the rats were tested under non-deprived, water-deprived, and food-deprived conditions in Experiments 1–3, respectively.

2. General methods

2.1. Subjects

Subjects were 60 naïve male Sprague–Dawley rats (Charles River, Wilmington, MA). The rats were kept in a vivarium maintained at 23 °C and on a 12:12 light:dark schedule with lights on at 7:00 AM. They were housed individually in hanging wire mesh cages and given ad libitum access to food (#2018 Harlan-Teklad) and water except where noted. All procedures used were approved by the Institutional Animal Care and Use Committee (IACUC) at Pennsylvania State University (Hershey).

2.2. Apparatus

Training and testing were conducted in a custom-made, automated gustometer housed within a sound attenuating booth (Model 252, IAC Acoustics, Bronx, NY). The gustometer was similar in design and function to a 'Davis Rig' [28]. It consisted of a Plexiglas box (12.5′ or 31.75 cm on each side) with a stainless steel mesh floor. The back wall of the box had a small opening (0.75′ × 1.25′, w × h; 1.90 × 3.17 cm) located at midline and 2.5′ (6.35 cm) above the floor. A shutter door behind the opening allowed access to 1 of 16 drinking bottles (B1–B16) with metal sipper tubes. The bottles were held in a rack mounted onto a motorized linear slide. The bottle position and door opening determined the stimulus type and duration, respectively, and were controlled using in-line controllers, a Power1401 interface, and Spike2 software (CED, UK). A contact circuit was incorporated into the gustometer to function as a lickometer [29].

2.3. Stimuli

Sucrose (Sigma-Aldrich, St. Louis, MO) was dissolved in distilled water to a concentration of 0.3 M. Corn oil was emulsified with Tween 80 (1.5 in 100 ml) and distilled water or with the surfactant alone. Three oil concentrations (vol/vol) were used: 4.8%, 16%, and 100%. The lowest oil concentration was chosen because it is isocaloric with 0.3 M sucrose and the higher concentrations were selected because they are highly preferred by rats [11].

2.4. Data acquisition

Licking data was acquired using a contact lickometer circuit (<0.5 μA). When the rat licked from a metal sipper tube, an electrical circuit between the tube, the stainless steel floor, and an ADC input channel of the Power1401 was completed. This contact was registered as a voltage change. The lickometer voltage signal was digitized, sampled at 5000 kHz, and acquired onto a host computer using Spike2 (CED, UK). Licking generated a voltage change that ranged between 50 and 300 mV, which equates to a current of 0.05–0.3 μA. The order of the drinking bottle and position of the door were also recorded. The number of licks and latency to the first lick were recorded.

2.5. Pre-surgical training and testing

Rats were trained to lick from the gustometer before the start of testing. The day prior to the start of spout training, the rats were water-deprived and given access to water for 1.5 h in the afternoon. All rats were water-deprived on the day prior to the start of spout training and on each spout training day. On training days, each rat was placed in the gustometer and given 32 water trials. Each stimulus trial lasted 10.0 s and consisted of the duration between the opening and closing of the shutter door. The inter-stimulus interval (ISI) was 5.0 s and consisted of the interval between the closing of the shutter door of a trial and opening of the shutter door of the next trial. After 3 training days, the rats were tested on the following days for brief access intake of water, oils, and sucrose. During each test, the rats had 32 water (W) trials and 8 trials each of 4.8%, 16% and 100% oil and 0.3 M sucrose (S). Presentation of the oils and sucrose was counter-balanced across days using a Latin square design. This procedure generated 4 different sequences which started as follows: Test 1 (B1–B16): W, S, W, 4.8%, W, 16%, W, 100%; W, S, W, 4.8%, W, 16%, W, 100%; Test 2 (B1–B16): W, 4.8%, W, 100%, W, S, W, 16%, W, 4.8%, W, 100%, W, S, W, 16%; Test 3 (B1–B16): W, 16%, W, S, W, 100%, W, 4.8%, W, 16%, W, S, W, 100%, W, 4.8%, and Test 4 (B1–B16): W, 100%, W, 16%, W, 4.8%, W, S, W, 100%, W, 16%, W, 4.8%, W, S. Within each test session, the starting sequence was repeated 4× to yield a total of 64 trials. Testing occurred until intakes stabilized, which took 5 days. The rats were tested under non-deprived, food-deprived, and water-deprived conditions in Experiments 1–3, respectively. Food-deprived rats were given 1.5 h access to food at least 2 h after testing, and water-deprived rats were given 1.5 h access to water at least 2 h after testing.

2.6. Surgery

At the end of pre-surgery testing, the rats were allocated into 2 groups matched on weight and intakes of 100% oil and sucrose. One group received bilateral GLx and the other group received sham transections. All rats were anesthetized with ketamine hydrochloride (125.0 mg/kg, ip) and xylazine hydrochloride (5.0 mg/kg, ip). The rat was placed supine and a midline incision was made in the neck. The salivary glands and musculature were retracted to expose the GL. The nerve was freed from the surrounding connective tissue sheath and approximately 3.0 mm of the nerve trunk was excised. The procedure was then repeated on the contralateral side. The wound was closed with nylon sutures. Sham rats were treated similarly except that the GLs were not transected. All animals were allowed 9 days to recover and were given ad libitum access to food and water during that period.

2.7. Post-surgical training and testing

After recovery, the rats were water-deprived and re-trained to lick water from the gustometer on 2 training days. They were then tested for brief access intake of water, oil, and sucrose across 8 consecutive test days. The training and testing procedures used were the same as those employed pre-surgery.

2.8. Histology

At the conclusion of each experiment, the rats were euthanized with euthasol (120.0 mg/kg, ip) and perfused transcardially with physiological saline and 10.0% buffered formalin. The tongue was removed and stored in formalin. The posterior tongue containing the CV was dissected and embedded in paraffin. The embedded tissue was cut into 10.0 μm sections, mounted onto slides, and stained with hematoxylin and eosin.