Sham feeding corn oil increases accumbens dopamine in the rat

Nu-Chu Liang, Andras Hajnal, and Ralph Norgren.

Department of Neural and Behavioral Sciences, College of Medicine, The Pennsylvania State University, Hershey, PA 17033, USA.

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Corresponding Author
Nu-Chu Liang
Department of Neural and Behavioral Sciences
College of Medicine
The Pennsylvania State University
500 University Drive
Hershey, PA 17033
Phone: (717) 531-7796
Fax: (717) 531-6916
E-mail: nzl105@psu.edu

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Abstract

Both real and sham feeding of sucrose increase dopamine (DA) overflow in the nucleus accumbens (NAc). Fat is another constituent of foods that is inherently preferred by humans and rodents. We examined the affect of sham feeding corn oil in rats that were food and water deprived overnight. Rats were implanted with guide cannulas aimed at the NAc as well as gastric fistulas. On alternate days, they were trained to sham lick 100% corn oil or distilled water (dH₂O) for 20 min in the morning. Twenty-minute microdialysis samples were taken before, during, and after sham licking. Dopamine and monoamines were analyzed by reverse-phase high performance liquid chromatography (HPLC) with coulometric detection. The results show that DA release in the NAc was significantly increased during sham licking of corn oil compared with the prior baseline (157.5 ± 18.8%, n = 12). During sham licking of dH₂O, DA release in the NAc was not changed (93.0 ± 4.0%, n = 15). This experiment demonstrates that sham feeding corn oil releases accumbens DA in a manner similar to ingestion of sucrose. Although both stimuli may have an olfactory component, sucrose is a gustatory, and 100% corn oil appears to be a trigeminal stimulus. Thus, these data support the hypothesis that different sensory modalities produce reward using the same or closely related substrates in the forebrain.
Although the exact role of mesolimbic dopamine (DA) in reward remains controversial, considerable
evidence demonstrates that both natural (26, 27, 35) and non natural (36) rewards release DA in this
system. Dopamine neurons in the ventral tegmental area (VTA) project predominantly to the nucleus
accumbens (NAc) (3, 22). Intake of palatable foods such as chocolate (35) and shortcake (14, 15) results
in an increase of extracellular DA in the NAc. Among the constituents of these foods, sucrose has been
tested most because it has inherent rewarding properties. In our laboratory, we have demonstrated that
both real (4, 5) and sham (7) feeding of sucrose increase DA overflow in the NAc. During sham feeding,
sucrose solution is drained out from the stomach by a gastric fistula and so the postingestive effects of
sucrose are excluded. Thus, the result of the sucrose sham feeding experiment indicates that the
orosensory effects of sucrose alone are sufficient to increase extracellular levels of NAc DA.

Fat is another macronutrient that appears to be inherently preferred by both humans and rodents. Rats
prefer 25%, 50% and 100% corn oil emulsions to water. Using corn oil emulsions, Smith and colleagues
demonstrated that sham intake of corn oil is an inverted-U function of concentration in both preweanling
(1, 29) and adult (17, 29) rats. Furthermore, systemic application of the DA receptor antagonists
SCH23390 and raclopride dose dependently decrease the intake of corn oil emulsions without affecting
the latency to sham feed or producing obvious motor impairment (33, 34). These results support the
hypothesis that the rewarding effects of oral corn oil are mediated by central dopaminergic activity, but
do not specify the site of the effect. A more direct support for this hypothesis requires measuring
mesolimbic DA levels during orosensory stimulation with corn oil. Therefore, in the present study, we
used microdialysis in combination with reverse-phase HPLC to investigate DA levels in the medial shell
of NAc during sham feeding of 100% corn oil emulsion. Some of these data were presented at the
A total of 43 male Sprague-Dawley rats (275-325 g, Charles River, Wilmington, MA) were used in five iterations of this study. They were individually housed on a 12:12-h light-dark schedule with ad libitum tap water and standard laboratory diet [Rodent diet (W) 8604; Harlan Teklad, Madison, WI]. For the implantation of gastric fistulas and microdialysis cannulas, the subjects were food deprived overnight, then treated with atropine sulfate (0.15mg/kg, ip) and, 20 min later, anesthetized with pentobarbital sodium (50mg/kg, ip). Each rat was fitted with a stainless steel gastric fistula and bilateral, 21-guage stainless steel guide cannulas aimed above the posterior medial NAc [A 1.0 mm, L 1.0 mm from the bregma, and V 4.0 mm from the skull; Ref.(23)]. The design and implantation of the gastric cannulas are described elsewhere (28). The guide cannulas were fixed to the skull using stainless steel screws (Fillister head 1-72 × 1/8”, Small Parts, Inc, FL) and dental acrylic. All the procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine, and comply with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

After 7-10 days recovery, the rats were transferred to individual hanging cages that had a longitudinal slot in the floor, and placed on an 18-h food and water deprivation regimen. One hour before the sham licking training session (7:30 AM), the stomach was flushed with lukewarm water. A flexible tube was screwed in the gastric fistula and passed through the slot to drain solutions. On alternative days, the subjects received dH2O or 100% corn oil emulsion [100 ml corn oil blended with 0.75 ml tween-80 (Sigma-Aldrich Inc, St Louis, MO)] for 20 min (9:00 – 9:20 AM). They were then allowed 2-3 hr (12:00 – 15:00) real intake of normal powder chow and dH2O. Each rat had 6 to 10 training trials with dH2O and 100% corn oil emulsion. They were then transferred to one of the six microdialysis chambers and
received the same training regimen for 4-7 more days. On the last day of training in the chamber, the concentric microdialysis probes with 2-mm active membrane were implanted bilaterally in the medial shell of the NAc through the guide cannulas. The active membrane of the probes consisted of cellulose tubing (20-kDa cutoff, 0.2-mm OD × 2-mm length; Spectrum, Ranch Dominguez, CA; see ref. 5 for details). The probes were perfused with artificial cerebrospinal fluid [aCSF; in mM: 145 NaCl, 2.7 KCl, 1.2 CaCl₂, 1.0 MgCl₂, and 2.0 Na₂HPO₄ in HPLC-grade water (Fisher Scientific, Pittsburgh) adjusted to pH 7.4] through a microdialysis swivel (375/D/22QE; Instech Laboratories, Plymouth Meeting, PA) at a rate of 1.0 µl/min using microsyringe pumps (Model A99; Razal Scientific Instruments, Stamford, CT). On the test days, 20-min dialysis samples were taken before, during, and after sham licking. Because of the limits of the microdialysis probes, each subject had at most three test days. At the end of the experiment, the rats were sacrificed with an overdose of pentobarbital sodium (150 mg/kg ip), then perfused transcardially with 0.9% saline solution followed by 10% formalin. The brains were frozen, serially sectioned at 50 µm, and stained with cresyl violet to verify placement of the microdialysis probes. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) from microdialytic samples (20 µl) were analyzed by reverse-phase HPLC with coulometric detection (ESA CoulArray system, ESA, Inc., Chelmsford, MA, Analytic cell: Model 5014B, electrode 1: -175 mV; electrode 2: +175 mV; guard cell: Model 5020: +300 mV). The chromatograms were recorded and analyzed off line by ESA data system on a PC.

After exclusions for poor placement (n = 4), malfunctioning probes (n = 20), and inadequate fistula drainage (n = 7), data from 12 rats with a total of 15 probes were included in the results. The probes excluded for location were in the rostral shell of NAc (β+2.50 mm, n=2), lateral to the NAc core (β+1.20 mm, n=1), and dorsal to the core (β+1.70 mm, n=1). During sham licking of dH₂O and corn oil,
dopamine overflow at these sites was unchanged or increased somewhat (data not shown). Because the subject numbers were small, the results were not analyzed further. The successful probes were located between 1.00 mm to 1.40 mm anterior to β in the medial shell of NAc (Fig. 1). Sham intake of both dH2O and corn oil emulsion increased during training. There was a significant trial effect and a stimulus×trial interaction [F(5, 110)=13.64, p<0.001; F(5, 110)=7.29, P<0.001]. The dH2O intake reached peak on the third training trial (20.08 ± 3.18 ml / 20 min) and then decreased in the following trials. In contrast, corn oil emulsion intake increased continuously with mean intake reaching 22.33 ± 1.89 ml / 20min by the sixth trial. On the first trial in the microdialysis chamber, both dH2O and corn oil emulsion intakes decreased significantly compared with the prior training trial (dH2O: 16.92 ± 3.41 ml vs. 7 ± 1.11 ml, t-test, p<0.02; corn oil emulsion: 22.33 ± 1.89 ml vs. 12.47 ± 2.43 ml, t-test, p<0.005). Although the dH2O intake was smaller than the corn oil emulsion intake on the first trial in the chamber, the difference was not quite significant (t-test, p=0.053). During the dialysis tests, however, sham intakes of corn oil and dH2O were statistically identical (corn oil vs. dH2O: 13.97 ± 2.08 ml vs. 11.73 ± 1.39 ml; t-test, p=0.37).

For neurochemistry data analysis, the raw results from the chromatograms were converted to a percentage of the mean value of the 3 baseline samples taken before the sham licking sessions. These normalized data for DA, DOPAC and HVA were analyzed by separate two-way ANOVAs (stimulus × sample/time), followed by post hoc Newman-Kuels tests when justified.

The results showed that sham licking corn oil stimulated accumbens DA flux, while licking dH2O did not. The percent increase in DA overflow, however, was not correlated with the volume of oil consumed (r=-0.17). Two-way ANOVAs (stimulus × sample) revealed that there were stimulus [F(1, 25)=10.17,
p<0.004], sample [F(8, 200)=2.85, p<0.006], and stimulus×sample [F(8, 200)=2.52, p<0.02] effects.

After 20-min of sham corn oil intake, DA levels were significantly higher than baseline and higher than DA levels after dH2O intake (corn oil vs. dH2O: 157.5 ± 18.8% vs. 93.0 ± 4.0%, p<0.001; Fig. 2).

Dopamine levels continued to be significantly higher for 20 minutes after oil intake ceased (corn oil vs. dH2O sample 5: 141.9 ± 21.7% vs. 96.0 ± 5.4%, p<0.05). Two-way ANOVAs demonstrated that DOPAC and HVA levels also were higher than baseline after sham licking of corn oil. There were stimulus [DOPAC: F(1, 25)=8.98, p<0.007; HVA: F(1, 25)=8.53, p<0.008] and sample [DOPAC: F(8, 200)=2.67, p<0.009; HVA: F(8, 200)=5.89, p<0.001] effects in both cases, but no interaction between stimulus and sample [DOPAC: F(8, 200)=1.17, p=0.32; HVA: F(8, 200)=1.08, p=0.38].

This experiment has demonstrated that sham licking 100% corn oil increases DA and its metabolites in the NAc. The design controlled for licking behavior because the rats received dH2O and corn oil on alternative days and ingested similar amounts. The DA activation during licking corn oil, therefore, was unlikely to result from differential oromotor activity. Because the rats were sham feeding to minimize gastrointestinal feedback, the nutritive component in the corn oil should not contribute to the increased DA overflow in the NAc. The results support the hypothesis that the oral sensory properties of corn oil drive accumbens dopaminergic activity.

Sham licking of a gustatory stimulus, sucrose, stimulates accumbens DA overflow as a function of concentration (7). The effects of 0.3M sucrose and 100% corn oil on DA overflow in the NAc did not differ -- 156.05 ± 11.78% and 157.5 ± 18.8%, respectively, ref. 6. Behaviorally, rats prefer 100% corn oil to 10% sucrose [± 0.24 M, Ref.(33)]. Theoretically, if both the behavioral and neurochemical indexes reflected the same underlying reward mechanisms, the measures would match. In fact, it is unlikely that
either NAc DA overflow or preference reflect reward similarly because the construct cannot be defined precisely, especially in neural terms (20). Nevertheless, the results from the present study and the sucrose studies (4, 5, 7) support the hypothesis that the reward produced via different sensory modalities are mediated by the same or closely related substrates in the forebrain.

Anatomical studies have demonstrated direct and indirect connections between the forebrain gustatory relays and the mesolimbic DA areas, including the NAc and the ventral tegmental area (VTA) (3, 13, 19, 22). Those forebrain and hindbrain connections provide possible substrates for DA activation in the accumbens, and may also be involved in the hedonic effects of taste (19). Hajnal and Norgren (2005) demonstrated that lesions in the secondary taste relay, the parabrachial nuclei (PBN), but not in the gustatory thalamus blunt the DA overflow during sucrose licking (6). This result implies that the hedonic information of the sucrose taste reaches the NAc via the PBN and the limbic forebrain circuits.

Dopamine flux occurs in other areas during tasks involving ingestive behavior but not always directly in register with oral stimulation. Dopamine release in the striatum occurred during operant learning for food reward but peaked around the lever press rather than reward consumption (18). Extracellular DA in the medial prefrontal cortex (MPC) was increased in response to presentation of a neutral stimulus, a plastic box, that had been associated with a palatable food. The same neutral stimulus, however, did not modify extracellular DA in the medial NAc (2). In the MPC, dopamine appears to be essential for attention and working memory related learning. In an eight arm radial maze task, MPC DA efflux increased in the absence of food reward (24). These and other studies indicate that increased accumbens DA release during sham intake is not just a general response to food, but one facet of the complex, highly orchestrated neural activity that accompanies rewarded behavior.
The sensory mechanisms by which corn oil is detected are not known. The best candidates are olfaction, taste, and the oral somatosensory system. Rats made anosmic by nasal instillation of ZnSO₄ still discriminated fats such as margarine and lard mixed with food (17). After olfactory bulbectomy, rats still preferentially ingest 0.5% and 1% corn oil (25). Anosmic mice can show conditioned place preference to 100% corn oil (30). Although preference for 1 and 3% corn oil is decreased in anosmic mice, their preference for higher concentrations 5 and 10% is not affected (30). These results suggest that an olfactory mechanism is not necessary for processing oral oil stimulation. Recent studies suggest that fatty acids are important for the gustatory recognition of fats. Rats can detect free fatty acids and acquire a conditioned aversion to them (16). In addition, a fatty acid transporter, CD36, is located on the taste cells (11). Although provocative, this evidence does not prove that taste is responsible for detecting oils or dietary fats. The main reason is that the dietary fats consist mainly of triglycerides, and triglycerides need to be digested first to become fatty acids. Although lingual lipase can hydrolyze triglycerides to free fatty acids (10), how effective this mechanism is for the gustatory recognition of dietary lipids remains unknown. In rats, addition of a potent lipase inhibitor diminished preference for a triacylglyceride solution. This effect, however, did not occur when the lipase inhibitor was added to a corn oil emulsion (10). Furthermore, rats with lesions in the secondary gustatory nucleus, the PBN, fail to learn aversions to taste stimuli but do learn to avoid 100% corn oil (21). Thus it is possible that the olfactory or the gustatory systems are not essential for processing the sensory and hedonic aspects of corn oil. This would leave the trigeminal system as the best candidate.

The intraoral trigeminal system, however, does not project directly to the limbic or DA systems. The mandibular branch of the trigeminal nerve innervates the anterior tongue, lower teeth, and much of the
intraoral mucosa (9, 31, 32). The maxillary branch distributes to the hard and soft palate. The axons of these nerves project to the mediadorsal principal and spinal trigeminal sensory nuclei as well as the nucleus of the solitary tract (NST) (9, 31, 32). In contrast to the anterior oral cavity, somatosensory information from the posterior oral cavity reaches the brain through the glossopharyngeal nerve. Tactile information detected by the glossopharyngeal nerve is also carried to the NST (8). There is little if any evidence of direct projections to the mesolimbic areas from the trigeminal system. The NST and the spinal trigeminal nuclei project strongly to the parabrachial nuclei. Thus it is possible that intraoral somatosensory activity reaches the ventral forebrain via the PBN (13, 32, 37). Electrophysiological confirmation of this possibility is lacking and, as mentioned above, behavioral evidence suggests that, at minimum, additional routes exist (21). The current experiment demonstrates that sham licking of corn oil releases accumbens DA much the same way as does sucrose ingestion. The central pathways that are critical for this effect can be determined using experiments parallel to those used to narrow the sucrose hedonic response down to the parabrachial ventral pathway (6).

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Figure legends

Figure 1. **Localizations of the microdialysis probes.** Microdialysis sites in the NAc are drawn in the sections of the rat brains’s left hemisphere. The active membranes of the probes (0.2 mm × 2 mm), depicted with gray bars, were located between 1.40 - 1.00 mm rostral to the bregma in the medial shell of the NAc based on the atlas of Paxions and Watson (23).

Figure 2. **Extracellular levels of DA (top), DOPAC (middle), and HVA (bottom) in the NAc before, during, and after sham licking of dH2O and corn oil.** Licking corn oil stimulated DA flux in the NAc. This effect lasted at least 20 min after the end of the corn oil bout. Sham licking of corn oil also increased DOPAC and HVA overall, but none of the post hoc comparisons was significant. *; significant post hoc tests for differences from baseline samples and from sham water intake, p < 0.05. #; significant difference in sample 5 when the rats ingested water in sample 4 compared with corn oil in the same period, p < 0.05)
Figure 1
Figure 2