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Sham Feeding of Sucrose Increases the Ratio of 3,4-Dihydroxyphenylacetic Acid to Dopamine in the Hypothalamus

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SMITH, G P , K A BOURBONAIS, C JEROME AND K J SIMANSKY *Sham feeding of sucrose increases the ratio of 3,4-dihydroxyphenylacetic acid to dopamine in the hypothalamus* PHARMACOL BIOCHEM BEHAV 26(3) 585–591, 1987 —Recent pharmacological experiments suggested that central dopaminergic (DA) mechanisms are necessary for the normal eating response to sweet stimuli To test this hypothesis, we measured the ratio of dihydroxyphenylacetic acid (DOPAC) to dopamine (DA) in forebrain DA terminal fields during the sham feeding of sucrose (1–40%) by rats after 17 hr of food deprivation After 9 min of sham feeding 10% or 40% sucrose, DOPAC/DA increased in the hypothalamus, but not in other forebrain regions including the n accumbens, amygdala, and pyriform cortex. This increase in hypothalamic DOPAC/DA did not occur after 9 min of sham feeding 1%, 1 25%, or 2 5% sucrose The increased DA metabolism required that sham feeding of 10% or 40% sucrose be maintained for longer than 3 min, because no increase of DOPAC/DA was observed in any forebrain region after 3 min of sham feeding These results are strong evidence that hypothalamic DA mechanisms are activated by the sham feeding of sucrose solutions and they support the hypothesis that central DA mechanisms are necessary for the normal eating response to sweet stimuli

Dopamine metabolism Sham feeding Sucrose Positive reinforcement Sweet taste Self-stimulation Eating Dopac/DA ratio

THE possibility that central catecholamine mechanisms are involved in the eating response to sucrose was first suggested by Sorenson *et al* [19] They observed decreased 24 hr-intake of 8% sucrose in rats after intracisternal 6-hydroxydopamine (6-OHDA) This was not simply a motor deficit because the 6-OHDA rats had normal 24 hr water intakes and normal water intakes in 1 hr after 24 hr of water deprivation. Sorenson *et al.* suggested that the deficit was due to noradrenergic damage produced by 6-OHDA, but Breese *et al.* [3] obtained evidence favoring dopamine (DA) by demonstrating that 6-OHDA treatments that produced marked DA damage, but little NE damage, decreased the 24 hr intake of 5% sucrose and of 0 03% saccharin about 50%. Despite the decrease in intake, 6-OHDA rats showed a clear preference for 5% sucrose or for 0.03% saccharin over water. Breese *et al* concluded that the DA damage was necessary for the decreased intake of sucrose and of saccharin, but they did not exclude some role for NE Similar results were obtained after neonatal treatment with 6-OHDA [18].

Recent acute pharmacological experiments with DA receptor antagonists are consistent with the importance of central DA for the normal ingestive response to sucrose and other sweet stimuli. Xenakis and Sclafani [24] showed that low doses of pimozide, a reasonably specific antagonist of DA receptors, decreased the 30-min intake of a mixture of 0.2% saccharin and 5% glucose. Sanberg *et al* [15] observed this effect of pimozide on sucrose intake. More recently, Deupree and Hsiao [5] reported a similar inhibition of sucrose with haloperidol, another DA receptor antagonist.

All of these investigators inferred a dopaminergic mediation of the stimulating effect of sucrose or other sweet stimuli on eating from a decrease in short term or 24 hr intakes. This is reasonable, but not compelling. A decrease in intake can also be the result of an increase in the postingestive satiating effect of sucrose or of the mixture of saccharin and glucose This possibility was tested by using sham feeding to minimize or abolish the postingestive satiating effect of sucrose [1, 7, 10, 12, 16, 22]. Geary and Smith [6] showed that pimozide decreased the 30-min intake of sham fed sucrose in 17 hr deprived rats. Since the dose of pimozide (0 25 $mg \cdot kg^{-1}$) did not appear to decrease consummatory behavior nonspecifically, this result provided crucial evidence against the possibility that pimozide decreased intake by enhancing the potency of postingestive satiating effects and supported the hypothesis that central DA mechanisms are necessary for the normal stimulating effect of sucrose on eating.

If central DA mechanisms are necessary for the normal positive reinforcing effect of sham fed sucrose, the hypothesis predicts that central DA turnover will increase during the sham feeding of sucrose We tested this hypothesis by measuring the ratio of dihydroxyphenylacetic acid (DOPAC) to dopamine (DA) in dopaminergic terminal fields of the

forebrain during sham feeding of sucrose solutions We report here that the ratio of DOPAC/DA increased significantly in the hypothalamus as a function of the concentration of sucrose sham fed A preliminary report of this work has appeared [4]

METHOD

All experiments were performed on adult male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 240 to 600 g at the beginning of the study All rats were housed and tested in individual, hanging, wire mesh cages on a 12 12 hr light/dark cycle, with lights on at 0700. They were maintained on pellets (Purina Rat Chow No 5001, Ralston Purina, St Louis, MO) and tap water.

Surgery

All rats, with the exception of the control groups, were implanted with chronic, stainless steel, gastric cannulas The surgical procedure has been described in detail previously [25] Rats were allowed 9–12 days to recover before the testing procedure began.

Testing Procedure

Rats were adapted to the following protocol Solid food, but not water, was removed at 1700 At 0930 on the following day, the gastric cannula of each rat was opened and the stomach was flushed several times with 5 ml 0.15 M NaCl until the drainage was clear A plastic drainage tube protected by a flexible, stainless steel spring was attached to the open cannula; this drainage tube passed through a slot running the length of the cage floor, allowing the rat to move about freely A plastic collecting pan was placed under each cage to collect gastric contents A sham feeding test was considered invalid and the data were discarded if gastric contents did not begin to drain out of the collection tube within 15 sec after sham feeding began and if the volume of gastric drainage collected was less than the volume of sucrose ingested. When these criteria are met, the drainage of ingested sucrose is complete or nearly so [7, 10, 16]

After the drainage tubes were attached, the rats were returned to their cages and at 1000 were presented with a sucrose solution which was available from a graduated cylinder mounted on the front of the cage The rats were allowed to sham feed the sucrose solution for 30 min, intakes were measured and the sucrose was then removed The contents of each collecting pan were measured, drainage tubes were removed, and the cannulas were closed Rats were replaced in their cages and given solid food until 1700 Rats were usually tested 5 days each week

Sucrose solutions were made by dissolving crystalline sucrose (Fisher Scientific Co , Pittsburgh, PA) in distilled water immediately before the test All sucrose solutions were at room temperature when offered to the rat After the rats had become familiar with sham feeding 10% or 40% sucrose, each rat was given 1 of 5 concentrations of sucrose (1 0%, 1.25%, 2.5%, 10% or 40%) in a series of 30-min tests (mean=8 8 tests, range=3–12)

On the final test day, rats were weighed and then allowed to sham feed their assigned sucrose solution for 9 min (Experiments 1, 2 and 3) or for 3 min (Experiment 4). Intakes were recorded and the rats were immediately decapitated The control rats (n=23) that had not been implanted with the gastric cannulas and never sham fed, but had been on the same 17 hr food deprivation schedule, were decapitated at the end of the deprivation period

Regional Dissection

The brains of all the rats were removed and transferred to a chilled operating stage where regional dissections were performed with the aid of a Zeiss operating microscope (Carl Zeiss, Inc., West Germany) The regions obtained were olfactory tubercle, frontal cortex, amygdala-pyriform lobe, nucleus accumbens, hypothalamus, and striatum

The frontal cortex tissue was obtained with the brain resting on its ventral surface. A Tissue Slicer Blade (No 6727-C20, A H Thomas Co , Philadelphia, PA) was inserted into the dorsal cortical surface at approximately a 30° angle from the vertical. The blade was lowered through the frontal lobe to the ventral surface so that it passed on a diagonal plane in front of the genu of the corpus callosum Any portion of the olfactory bulbs that adhered to the ventral surface of this block of tissue was removed

Then the brain was turned over so that it rested on its dorsal cortical surface The olfactory tubercles were pinched off the base of the brain with fine microdissection forceps

The accumbens nuclei were approached by dissecting along the trajectory of the anterior limb of the anterior commissure. First, the anterior commissure was located by gentle dissection along the midline from the ventral surface Then, using the transverse sections of the anterior limbs of the anterior commissure (formed as a result of the frontal cortex section) as the lateral boundaries, one pincer of fine forceps was inserted along the midline to the depth of the anterior commissure and the other pincer was drawn through the tissue in an arc that was bounded posteriorly by the anterior limb of the anterior commissure The depth of this pinched-off section extended less than 1 mm dorsal to the anterior commissure Thus, in addition to the accumbens, this section included some lateral and medial septal tissue and the vertical limb of the diagonal band

The hypothalamic tissue block was approached by removing the optic nerves and optic chiasm, and the meningeal tissue Then an incision was made with a fine scissors along the medial edge of both optic tracts This incision was extended posteromedially along the caudal edge of the mamillary bodies. The anterior face of the hypothalamic block was made by a frontal cut at the level of the optic tract. The dorsal boundary of the hypothalamic block was made by a horizontal incision at the dorsal edge of the descending columns of the fornix

The brain was next placed on its lateral surface so that the pryiform lobe was accessible The anterior cut of this tissue block was made in the frontal plane where the lateral olfactory tract enters the ventral surface of the brain at the level of the optic chiasm. The posterior cut was made in the frontal plane of the posterior edge of the mamillary body Both the anterior and posterior cuts extended from the rhinal sulcus down to the lateral edge of the optic tract A cut was made along the lateral edge of the optic tract to connect the anterior and posterior cuts Then the block of pyriform lobe containing the amygdaloid nuclei, the entorhinal cortex, and the primary olfactory cortex was separated from the brain by dissecting along the rhinal sulcus and peeling the lobe out and down This dissection was done in a way that excluded the ventral portion of the hippocampus. When one pyriform lobe had been removed, this procedure was repeated on the other side

TABLE 1

HYPOTHALAMIC CONCENTRATIONS OF DOPAC AND DA, AND RATIOS OF DOPAC/DA AFTER 9 MINUTES OF SHAM FEEDING DIFFERENT CONCENTRATIONS OF SUCROSE

Group	n	Intake (ml)	DOPAC (ng mg^{-1} tissue)	DA (ng mg^{-1} tissue)	DOPAC / DA
			Experiment 1		
Control	12	0	0 16 ± 0 02	0 60 ± 0 04	0.28 ± 0 02
1–1 25%	9	9 6 ± 2 4	0 23 ± 0 02	0 83 ± 0 08	0 30 ± 0 04
10%	26	16 0 ± 1 1†	0 20 ± 0 01	0 58 ± 0 03	0 35 ± 0 02
40%	16	18 2 ± 1 2†	0 23 ± 0 01	0.47 ± 0 03*	0 50 ± 0 03*
			Experiment 2		
Control	5	0	0 20 ± 0 06	0 72 ± 0 07	0 27 ± 0 05
2 5%	6	7 8 ± 2 2	0 12 ± 0 02	0 54 ± 0 05†	0 22 ± 0 02
10%	6	19 2 ± 2 0†	0 17 ± 0 01	0 53 ± 0 01†	0 32 ± 0 02
40%	6	22 8 ± 2 2†	0 33 ± 0 02†	0 68 ± 0 04	0 48 ± 0 02*
			Experiment 3		
Control	6	0	0 15 ± 0 01	0 64 ± 0 04	0 23 ± 0 04
2 5%	6	4 3 ± 0 8	0 19 ± 0 02	0 60 ± 0 01	0 28 ± 0 01
10%	6	9 8 ± 2 2†	0 23 ± 0 03†	0 74 ± 0 04	0 30 ± 0 02†
40%	6	16 8 ± 2 3†	0 23 ± 0 01†	0 66 ± 0 06	0 36 ± 0 02*

Data are mean ± SE *$p<0$ 01, †$p<0$ 05 compared to Control by Tukey's HSD test after a significant treatment effect by one-way ANOVA

The striatum was the final structure to be removed. Each striatum was approached through the lateral ventricle and pinched off with microdissection forceps

Sample Preparation

The tissue samples were weighed and stored on dry ice until they were extracted later the same day. Two extraction procedures were used In Experiments 1 and 2, samples were extracted in 0.1 M perchloric acid (PCA), dithiothreitol was used as antioxidant and dihydroxybenzylamine hydrochloride (DHB) was the internal standard In Experiments 3 and 4, samples were extracted in 0 4 M PCA and $Na_2S_2O_5$ was the antioxidant

A series of internal standards was prepared at this time from individual stock solutions of each standard (1 mg·ml^{-1} in 0.01 N hydrochloric acid) diluted with 0 4 M PCA so that all compounds were present at a concentration of 1 ng·mcl^{-1}. The standards were 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxybenzylamine hydrochloride (DHB), homovanillic acid (HVA), and dopamine (DA, 3-OH tyramine-HCl). All salts and free bases were from the Sigma Chemical Co. (St. Louis, MO) An additional dilution containing only DHB, used for spiking tissue samples, was also prepared in this fashion Stock solutions were stable for 4 months at 0–5°C, dilutions were made on the day of the extraction.

All tissue samples and standards were centrifuged for 20 min at 15,000 rpm, 4°C. Aliquots (250–300 mcl) were transferred to "WISP" mini vials and stored at −40°C until analysis, which was performed as soon as possible

Micro-Lowry protein analyses were performed on some of the perchloric acid-precipitated tissue pellets according to the procedure of Lowry *et al.* [11]. The protein, expressed as a percentage of tissue weight, of the various regions was: hypothalamus=8.4±0.4% (n=50), amygdala pryiform lobe=7 7±0.3% (n=47), nucleus accumbens=9 7±0.6% (n=50), frontal cortex=7.2±0.2% (n=50), olfactory tubercle=9.2±0.2% (n=52), and striatum=8 3±0.3% (n=47). Since the protein concentration was reproducible within a region and the range of concentrations between regions was not large, we have presented our data in terms of mg wet tissue

Neurochemical Measurements

The chromatographic system used in Experiment 1 consisted of a Hewlett-Packard model 1081 A pump (Hewlett-Packard, Paramus, NJ) linked to a "WISP" Automatic Sample Processor, model 710A (Waters Assoc., Milford, MA) Electrochemical measurements were made by a Brinkman Metrohm VA-detector E611; sensitivity was set at 30 nAmp/V, and potential was set at +700 mV

The mobile phase buffer used was a 0.1 M phosphate buffer prepared from $Na_2H_2PO_4$ H_2O (J T Baker Chemical Co , Phillipsburg, NJ) pH adjusted to 5.0, containing 0 1 mM EDTA (Fisher Scientific Co.) and 0.35 mM 1-heptane sulfonic acid (sodium salt, Eastman Kodak, Co., Rochester, NY) Buffer solutions were prepared fresh daily and filtered through a 0 2 μ polycarbonate membrane filter, and degassed by sonication The analytical column used was a μ-Bondapak C-18, 10 μ particle size stainless steel reverse-phase column (Waters Associates) protected by a CO:PELL ODS pre-column (Whatman, Inc , Clifton, NJ) packed with 30-38 μ glass beads with chemically bonded octadecyl (C18) groups

The chromatographic system used in Experiments 2, 3 and 4 was the same except that electrochemical measure-

TABLE 2

REGIONAL RATIOS OF DOPAC/DA AFTER 9 MINUTES OF SHAM FEEDING DIFFERENT CONCENTRATIONS OF SUCROSE

Group	Striatum	Olfactory Tubercle	Amygdala-Piriform Cortex	Frontal Cortex	Nucleus Accumbens
			Experiment 1		
Control	0 17 ± 0 01 (11)	0 19 ± 0 01 (12)	0 29 ± 0 02 (12)	0 69 ± 0 05 (11)	0 17 ± 0 01 (11)
1–1 25%	0 13 ± 0 01 (8)	0 18 ± 0 01 (9)	0 28 ± 0 03 (9)	0 96 ± 0 09* (6)	0 23 ± 0 03 (6)
10%	0 14 ± 0 01 (26)	0 18 ± 0 01 (26)	0 36 ± 0 05 (26)	0 74 ± 0 05 (16)	0 18 ± 0 02 (19)
40%	0 14 ± 0 01 (16)	0 16 ± 0 01 (16)	0 36 ± 0 03 (15)	0 75 ± 0 03 (16)	0 17 ± 0 04 (6)
			Experiment 2		
Control	0 16 ± 0 00 (6)	0 20 ± 0 01 (4)	0 22 ± 0 02 (6)	0 32 ± 0 03 (6)	0 27 ± 0 01 (4)
2 5%	0 16 ± 0 02 (6)	0 20 ± 0 01 (5)	0 19 ± 0 01 (5)	0 24 ± 0 05* (4)	—
10%	0 15 ± 0 01 (6)	0 18 ± 0 01 (6)	0 21 ± 0 04 (6)	0 30 ± 0 02 (6)	0 26 ± 0 03 (4)
40%	0 16 ± 0 00 (6)	0 20 ± 0 01 (6)	0 17 ± 0 01 (6)	0 40 ± 0 03 (6)	0 28 (2)
			Experiment 3		
Control	0 13 ± 0 01 (6)	0 16 ± 0 01 (6)	0 35 ± 0 07 (6)	0 59 ± 0 02 (6)	0 24 ± 0 01 (6)
2 5%	0 13 ± 0 01 (6)	0 15 ± 0 01 (6)	0 30 ± 0 04 (6)	0 55 ± 0 04 (6)	0 22 ± 0 03 (5)
10%	0 12 ± 0 01 (6)	0 16 ± 0 01 (6)	0 33 ± 0 03 (6)	0 54 ± 0 02 (6)	0 24 ± 0 01 (6)
40%	0 12 ± 0 01 (6)	0 16 ± 0 01 (6)	0 31 ± 0 04 (6)	0 59 ± 0 04 (6)	0 26 ± 0 01 (6)

Data are mean ± SE The number in parentheses is the number of brains from which the mean value was calculated

*$p<0$ 05 compared to Control by Tukey's HSD test after a significant effect by one-way ANOVA

ments were made using a Bioanalytical Systems LC-4B detector and the buffer was that of Wagner *et al* [21].

Statistics

Statistical analyses were performed using one-way ANOVA followed by Tukey's HSD test

RESULTS

The ratio of hypothalamic DOPAC/DA increased significantly as a function of sucrose concentration that was being sham fed for 9 min (Table 1) In all 3 experiments the ratio was largest when 40% sucrose was ingested. In Experiment 3, sham feeding of 10% sucrose also produced a significant increase in the ratio Note that in Experiments 2 and 3, the significant increase in the DOPAC/DA was the result of a significant increase in the DOPAC concentration (Table 1)

The hypothalamus was the only dopaminergic terminal field that showed a significant increase in the DOPAC/DA that was correlated with the concentration of the sucrose sham fed (Table 2). There were two significant differences in DOPAC/DA in the frontal cortex measurements, but the changes were in opposite directions and, thus, were judged to be biologically insignificant

Homovanillic acid (HVA) was also measured in Experiment 3. Neither the concentration of HVA nor the HVA/DA ratio changed significantly during the sham feeding of any of the sucrose solutions in any of the brain regions measured, including the hypothalamus (Table 3).

It was possible that the failure to observe increased DOPAC/DA throughout the dopaminergic terminal fields of the forebrain was the result of sampling after 9 min of sham feeding. We investigated this possibility by repeating the experiment and measuring DOPAC/DA in the same regions after 3 min of sham feeding the sucrose solutions Under these conditions, neither the hypothalamus nor any of the other forebrain dopaminergic terminal fields showed a significant increase in DOPAC/DA (Table 4). We have not yet evaluated the effect of longer periods of sham feeding on DOPAC/DA in this experimental preparation.

TABLE 3

REGIONAL CONCENTRATIONS OF HVA AND RATIOS OF HVA/DA AFTER 9 MINUTES OF SHAM FEEDING DIFFERENT CONCENTRATIONS OF SUCROSE (EXPERIMENT 3)

Group	Striatum	Olfactory Tubercle	Amygdala-Piriform Cortex	Frontal Cortex	Nucleus Accumbens
			HVA Concentration (ng mg^{-1} tissue)		
Control	0 89 ± 0 9 (6)	0 54 ± 0 06 (6)	0 10 ± 0 04 (4)	0 13 (2)	0 64 ± 0 10 (6)
2 5%	0 88 ± 0 05 (6)	0 65 ± 0 06 (6)	0 12 ± 0 02 (4)	0 13 ± 0 02 (5)	0 51 ± 0 05 (5)
10%	1 17 ± 0 07 (6)	0 57 ± 0 06 (6)	0 08 ± 0 01 (6)		0 69 ± 0 10 (6)
40%	1 07 ± 0 04 (6)	0 52 ± 0 03 (6)	0 08 ± 0 01 (6)		0 52 ± 0 05 (6)
			Ratio of HVA/DA		
Control	0 05 ± 0 00	0 05 ± 0 00	0 12 ± 0 04	0 61	0 07 ± 0 01
2.5%	0 06 ± 0 01	0 06 ± 0 01	0 21 ± 0 03	0 66 ± 0 10	0 07 ± 0 01
10%	0 05 ± 0 00	0 06 ± 0 01	0 13 ± 0 02		0 09 ± 0 01
40%	0 06 ± 0 00	0 05 ± 0 01	0 11 ± 0 02		0 08 ± 0 01

Data are mean ± SE The number in parentheses is the number of brains from which the mean value was calculated

TABLE 4

HYPOTHALAMIC CONCENTRATIONS OF DOPAC AND DA, AND RATIOS OF DOPAC/DA AFTER 3 MINUTES OF SHAM FEEDING DIFFERENT CONCENTRATIONS OF SUCROSE

Group	n	Intake (ml)	DOPAC (ng/mg tissue)	DA (ng/mg tissue)	DOPAC / DA
Control	6	0	0 21 ± 0 03	0 78 ± 0 07	0 26 ± 0 03
2 5%	6	0 8 ± 0 2	0 12 ± 0 01	0 54 ± 0 02*	0.26 ± 0 01
10%	6	6 0 ± 0 9*	0 14 ± 0 02	0 65 ± 0 05	0 21 ± 0 03
40%	6	7 0 ± 0 5*	0 16 ± 0 01	0 81 ± 0 05	0 20 ± 0 01

Data are mean ± SE *$p<0$ 01 compared to Control by Tukey's HSD test after a significant treatment effect by one-way ANOVA

DISCUSSION

The ratio of DOPAC/DA increased significantly in the hypothalamus as a function of the sucrose concentration that was being sham fed for nine minutes. To the extent that the DOPAC/DA is a valid index of dopamine release [14] under these experimental conditions, this is the first evidence that hypothalamic dopaminergic synaptic activity increases as a function of the concentration of sucrose ingested This suggests that it is the concentration of sucrose at oral receptors that is the critical stimulus for this increase in hypothalamic dopaminergic activity The fact that this was observed in sham feeding rats in which the postingestive effects of sucrose were minimized supports this interpretation. If the concentration of sucrose in the mouth is the critical stimulus, then apparently it must act for a certain minimal length of time because the hypothalamic DOPAC/DA ratio did not increase after three minutes of sham feeding Of course, this temporal requirement and the failure to measure increases of DOPAC/DA in other DA terminal fields could be the result of the failure of the electrochemical measurement technique to detect very small neurochemical changes and/or the use of regional dissection instead of smaller, more homogeneous samples of tissue such as those obtained by the punch technique.

In attempting to interpret these results, it is useful to emphasize that the sham feeding of sucrose is a form of oral self-stimulation It is functionally similar to intracranial self-stimulation (ICSS) in that both procedures deliver an unconditioned, positive reinforcing stimulus (sucrose or electrical stimulation of neurons and fibers), as a consequence of emitting a motor response (licking and swallowing in sham feeding, lever press or other operant movement in intracranial self-stimulation) Our interest in this form of oral, self-stimulation derives from three facts (1) it uses an unconditioned stimulus, sucrose, that is a natural, unconditioned reward in the sense that there are oral receptors that respond to sucrose and that sucrose is a nutrient; (2) the

positive reinforcing effect of sucrose is a direct function of the concentration of sucrose across a broad range of concentrations [13]; and (3) this form of self-stimulation is a natural sequence of consummatory movements

Considering sham feeding as a form of oral self-stimulation is useful because it reminds us of the interpretative problems that have plagued intracranial self-stimulation, particularly when rate of responding was used to measure the positive reinforcing potency of the intracranial stimulus [19]. In our experiments, there are three candidates for correlation with increased hypothalamic DOPAC/DA—ingestive movements such as licking and swallowing, the sensory intensity of sucrose, and the hedonic intensity (positive reinforcing potency) of sucrose. The intake data (Tables 1 and 4) may be considered evidence against ingestive movements because in Experiments 1 and 2, the DOPAC/DA ratio was significantly larger than control after 40% sucrose, but not after 10% sucrose even though the 9 min intakes after 40% and 10% sucrose were not different. But intakes are crude measures of responding—lick rates may show a closer correlation with DOPAC/DA.

The other two candidates, the sensory intensity and the hedonic intensity of sucrose, are direct functions of sucrose concentration and, thus, both could be correlated with increased hypothalamic DOPAC/DA ratio. Since the present experiments were descriptive in intent, they do not permit a choice among these three possible correlations, let alone determining the causal nature of the correlation.

An additional problem for interpretation is the possibility that the increase of hypothalamic DOPAC/DA is a result of a conditioned effect of sham fed sucrose. Certainly the training sessions prior to decapitation provided an opportunity for conditioned effects to occur. The only relevant evidence is that of Simansky *et al.* [17] who recently demonstrated an increase of hypothalamic DOPAC/DA in rats that did not eat, but were exposed for one hour to the stimuli associated with the eating of nearby rats. That particular conditioned response could not occur in these experiments, however, because the control rats were decapitated prior to the sham feeding test

Despite these interpretive problems, our experiments provide the first evidence of an increase of hypothalamic dopaminergic metabolism during the ingestion of a food stimulus, such as sucrose. In this respect, they extend recent reports of increased DA metabolism during access to and ingestion of food [2, 8, 9] by correlating a phase of eating, the maintenance of eating after ingestion has begun, the concentration of the food stimulus, and the increase of DA metabolism in a specific DA terminal field. From this perspective, the results strongly support the hypothesis that central DA mechanisms are active during the ingestion of sucrose [3,6] and they are consistent with the DA hypothesis [23] of the positive reinforcing effect of food stimuli that maintains eating during a meal

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