Journal of the Endocrine Society, 2023, **7**, 1–16
https://doi.org/10.1210/jendso/bvad074
Advance access publication 7 June 2023
Research Article

Chronic Semaglutide Treatment in Rats Leads to Daily Excessive Concentration-Dependent Sucrose Intake

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Abstract

Context: The glucagon-like peptide-1 receptor (GLP-1R) agonist semaglutide (SEMA) produces 15% weight loss when chronically administered to humans with obesity.

Methods: In 2 separate experiments, rats received daily injections of either vehicle (VEH) or SEMA starting at 7 µg/kg body weight (BW) and increasing over 10 days to the maintenance dose (70 µg/kg-BW), emulating clinical dose escalation strategies.

Results: During dose escalation and maintenance, SEMA rats reduced chow intake and bodyweight. Experiment 2 meal pattern analysis revealed that meal size, not number, mediated these SEMA-induced changes in chow intake. This suggests SEMA affects neural processes controlling meal termination and not meal initiation. Two-bottle preference tests (vs water) began after 10 to 16 days of maintenance dosing. Rats received either an ascending sucrose concentration series (0.03-1.0 M) and 1 fat solution (Experiment 1) or a 4% and 24% sucrose solution in a crossover design (Experiment 2). At lower sucrose concentrations, SEMA-treated rats in both experiments drank sometimes >2× the volume consumed by VEH controls; at higher sucrose concentrations (and 10% fat), intake was similar between treatment groups. Energy intake of SEMA rats became similar to VEH rats. This was unexpected because GLP-1R agonism is thought to decrease the reward and/or increase the satiating potency of palatable foods. Despite sucrose-driven increases in both groups, a significant bodyweight difference between SEMA- and VEH-treated rats remained.

Conclusion: The basis of the SEMA-induced overconsumption of sucrose at lower concentrations relative to VEH controls remains unclear, but the effects of chronic SEMA treatment on energy intake and BW appear to depend on the caloric sources available.

Key Words: obesity, semaglutide, meal patterns, sucrose preference

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; FCM, food choice monitor; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; MRI, magnetic resonance imaging; SC, subcutaneous; SEMA, semaglutide; VEH, vehicle.

Obesity continues to be a critical, widespread public health concern [1–5]. By and large, lifestyle intervention strategies to treat obesity have minimal long-term success [6–8]. Bariatric surgery provides greater weight loss and a better chance for enduring results than other treatments [9, 10], but, for a variety of reasons, these surgeries are not universally feasible. Pharmacological interventions have been effective to a degree and can increase accessibility to treatment. However, historically, antiobesity drugs were unable to sustain more than a 10% loss of body weight (see reference [10] for a recent review). One of the more promising classes of pharmacotherapies for obesity are drugs that mimic the action of the endogenously occurring satiation-promoting incretin hormone, glucagon-like peptide-1 (GLP-1).

The GLP-1 analog semaglutide (SEMA) is the second long-acting GLP-1 receptor (GLP-1R) agonist, after liraglutide, to be approved for treatment of obesity [10]. Like liraglutide, SEMA was originally used at lower doses to manage type 2 diabetes mellitus and was recently approved at a higher dose for the treatment of obesity. Patients treated with SEMA achieve and maintain an average of ~15% body weight loss [11]. Clinical administration of SEMA, and other GLP-1 analogs, employs a dose escalation protocol to abrogate side effects and improve tolerance and compliance. Yet, notwithstanding some exceptions, much of what is known about the behavioral and neural effects of SEMA in preclinical studies is based on acute dosing. In fact, the exact SEMA-induced changes in ingestive behavior that contribute to weight loss are not well understood in humans or in preclinical models.

In rodents, administration of GLP-1 analogs, regardless of route, is thought to decrease the reward value of palatable food and to increase the satiating potency of caloric stimuli. In many such studies sucrose has been the prototypical stimulus, and many have reported reduced sucrose responding. In brief access testing where postingestive effects are minimized,

Received: 19 March 2023. **Editorial Decision:** 1 June 2023. **Corrected and Typeset:** 28 June 2023

GLP-1R agonism with Exendin-4 reduced licking and trial initiation when sucrose was the stimulus [12] and reduced progressive ratio responding for sucrose pellets [13–17], although exceptions exist (eg [18]). The only preclinical data regarding the effect of chronic SEMA treatment on palatable food intake of which we are aware demonstrated that it specifically decreases chocolate intake without any effect on the intake of chow [19]. Accordingly, we sought to emulate the clinical use of SEMA and investigate the effects of a dose escalation and dose maintenance protocol on energy intake and body weight in rats. In particular, we wanted to test whether SEMA would decrease sucrose intake preference across a broad concentration range when assessed under chronic dosing conditions. Here, we rigorously characterize SEMA-induced changes in food intake and body weight in 2 cohorts of rats during a 10-day dose escalation protocol followed by 10 to 16 days at maintenance dosing prior to, and continued through, sucrose preference testing. In the second cohort (Experiment 2), detailed eating and drinking pattern analysis was conducted with our newly designed food and fluid choice monitor to determine the behavioral basis for the SEMA-induced changes in total energy intake. As will be shown, among other novel effects revealed, SEMA treatment led to a striking and replicable 1.5 times to 2.8 times increase in the intake of low- to mid-range concentrations of sucrose solution compared with vehicle-treated rats; a result inconsistent with the suggestion that SEMA treatment universally decreases the reward value of sucrose and increases the satiating potency of all palatable energy sources based on acute dosing experiments.

Materials and Methods

Vivarium and Animal Housing

Rats were maintained in a vivarium where light cycle (12 hours:12 hours), temperature (21-22° C), and humidity were automatically controlled. Rats were singly housed in standard or modified polycarbonate cages with wood chip bedding, and ad libitum access to standard rodent chow (5001, LabDiet, St. Louis, MO) and deionized water throughout both experiments, unless specifically noted otherwise. Rats had access to either a Rattle-A-Round (Otto Environmental, Greenfield, WI) or stainless-steel nest for environmental enrichment. All procedures were approved by the Florida State University Animal Care and Use Committee (protocol #202100021).

Drugs

The rats received subcutaneous (SC) injections of vehicle (VEH, 44 mM sodium phosphate dibasic, 70 mM NaCl + 0.007% Tween 20) or SEMA (Novo Nordisk, Bagsvaerd, Denmark) in VEH given at approximately the same time each day throughout dose escalation, dose maintenance, and 2-bottle preference testing phases. SEMA dosing was escalated over 10 days from 7 µg/kg body weight to 70 µg/kg body weight, diluted such that injection volume was consistently 1 mL/kg body weight. After the 10-day dose escalation period, the 70 µg/kg body weight dose was maintained throughout the dose maintenance and sucrose preference testing periods.

Data Representation and Analysis

Body weight, energy intake, food and water intake, and meal pattern data are graphed as $\log_{10}$-transformed ratios to baseline (BASE) measures using GraphPad Prism 9. The application of the ratio to BASE measure allows each rat to serve as its own control and standardizes the data. The log transform was performed so that factor increases would be symmetrical with factor decreases; as such fold-change in either direction are represented by equal spacing on the y-axis. As appropriate, data were analyzed in Systat 13 using 2-way repeated measures analysis of variance (ANOVA) (treatment × day (or concentration)). When the ANOVA identified a significant interaction, post hoc pairwise testing between treatment groups was performed and Bonferroni correction applied to *P* values. In the case of sucrose, additional pairwise comparisons between concentrations within each drug group were conducted. For each outcome of interest in each experiment, statistics for each experimental phase (ie, dose escalation, dose maintenance, 2-bottle preference testing, postdrug monitoring) were treated separately.

Methods: Experiment 1

Experiment 1: subjects

Sixteen Sprague–Dawley rats (8 males, 8 females, Charles River, Raleigh, NC), approximately 22 weeks old at BASE had an average BASE bodyweight of 674.8 ± 31.3 g for the males and 320.8 ± 10.9 g for the females. Prior to this study, the rats had 20 days of palatable cafeteria diet exposure that ended 2 months prior to BASE.

Experiment 1: experimental design and timeline

The experimental timeline is detailed in Fig. 1.

Experiment 1: food and water intake measurements

Standard rodent chow was given to rats in food hoppers that hung on the inside of the cages. Hoppers were filled, vigorously shaken to remove any small pellet pieces, weighed, and the "on" weight recorded before being placed into the cage. Each day thereafter, the hopper was carefully removed, any visible crumbs in the cage were collected and an "off" weight (hopper with remaining food plus any crumbs) was recorded. The hopper was then refilled as needed, shaken vigorously to allow any small pieces to fall out and the new "on" weight recorded. Intake was determined by subtracting the hopper's "off" weight from the "on" weight. The rats were given deionized water in standard 500-mL glass water bottles with identical stoppered sipper tube tops. The filled bottles were weighed and carefully placed onto the cage top. Each day, the bottles were carefully removed from the cage top and the "off" weight was recorded. Water bottles were refilled as needed, the new "on" weight for the water bottle was recorded, and it was carefully placed onto the cage lid. Water intake was determined by subtracting the "off" weight from the "on" weight. Any sipper top with a propensity for dripping was replaced and no adjustments were made for water loss during use as this was thought to be evenly shared across subjects.

Experiment 1: 2-day 2-bottle preference tests

Over a period of 16 days (23 hours/day), the rats were given 2 fluid-filled glass bottles each day. The bottles were handled as described for water intake measurements with the addition that all nonwater solutions were replaced daily from freshly prepared solutions. The positions of fluid bottles were swapped daily to prevent side bias. All sucrose solutions

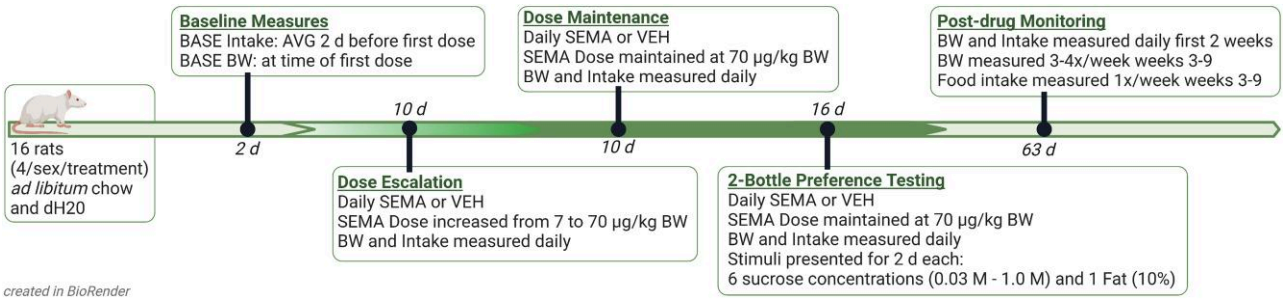

Figure 1. Experiment 1 timeline. The food and water intake measures from the 2 days immediately preceding dose escalation were averaged to determine the BASE food and water intake for each rat. Body weight measured immediately before the first SEMA or VEH injection was used as BASE body weight. Body weight, food intake, and water intake were measured at approximately 2 hours after lights on each day when drug or VEH injections were given. The dose escalation protocol is detailed under "Drugs," the method for food and fluid intake measurements under "Experiment 1: Food and Water Intake Measurements," and the preference testing approach in "Experiment 1: 2-day 2-bottle preference tests", with the order, concentration, and energy density of each test stimulus detailed in Table 1. BASE, baseline; BW, bodyweight.

were given in 500-mL bottles with an accompanying 500-mL water bottle. The fat solution and accompanying water were given in 250-mL bottles. For the first 2 days, both bottles contained deionized water. From the third to fourteenth day, a series of sucrose solutions, ranging from 0.03 to 1.0 M and given in ascending order was offered in 1 of the 2 bottles. On days 15 to 16, the rats were given a commercial nondairy creamer (10% fat, Publix, Lakeland, FL) containing ~10% fat from coconut oil (Table 1). The nondairy creamer also contained 1 g carbohydrate from corn syrup solids and modified starch, leading to 77% kcal from fat. The nondairy creamer was chosen as an alternative to Intralipid, which was in short supply at the time of the study. Energy and volume preference scores were calculated daily for each sucrose concentration and the 10% fat as follows: sucrose or 10% fat consumed (mL or kcal)/total consumed from all sources. For each solution, the average daily preference or intake from the 2-day exposure is reported and was used for statistical analyses.

Experiment 1: data notes

On a few occasions, mishandling of food hoppers or fluid bottles led to data loss. One water intake measure was lost due to spillage prior to measuring "off" weights. Two food intake measures were not acquired due to errors in hopper handling. In cases of lost food or water measurement, the average intake from the day before and day after the lost data were used in statistical analyses. On the second day of 1.0 M sucrose preference testing, the sucrose bottle of 1 male rat receiving SEMA injections was spilled before an "off" measurement could be collected. Data for that rat at that concentration is based on 1-day intake. One male rat receiving SEMA dislodged and removed the stopper from the water bottle on the second day of 10% fat preference testing, thus preventing calculation of a volume preference score for that day. For that rat, the data for that day were excluded from volume-based preference calculations and 1-day volume preference was used.

Methods: Experiment 2

Experiment 2: subjects

Sixteen male Sprague–Dawley rats (Charles River, Raleigh, NC) served as subjects for Experiment 2. Rats were ~19 weeks old with an average body weight of 611.8 g (±7.34 g) immediately prior to the first SEMA or VEH injection.

Experiment 2: experimental design and timeline

The timeline for Experiment 2 is detailed in Fig. 2.

Experiment 2: food and fluid intake measurements in the 5-item food choice monitor

Beginning 4 days prior to the first SEMA or VEH injection and continuing through the final SEMA or VEH injection, rats were continuously housed in our newly fabricated custom 5-item food choice monitor (FCM). The design and function of the FCM are fully described by Blonde et al [20]. Briefly, the FCM allows continuous monitoring of changes in weight of up to 5 food jars and licks to 2 fluid bottles. Food jars are arranged in a linear array along 1 long side of a modified standard polycarbonate rat cage. Access to the food jars is provided via a custom stainless-steel hood fitted with dividers to prevent mixing of foods and discourage sitting in the hood. The food jars themselves are placed into hoppers that catch spillage and the hopper is placed upon a load beam that collects the weight measurement 10 times per second. These jar weights are then assembled into 10-second bins and weight instability is used to identify eating bouts. In the present experiment, rats were given only 1 food choice, thus, only 1 food jar was used and access to the other jars was blocked by placing an upside-down food jar into the unused hoppers. Opposite the food jar access hood on the other long side of the cage are 2 lick blocks which flank a stainless-steel nest that provides environmental enrichment for the rat. The lick blocks provide access to 2 fluid bottles while monitoring licks including contact time with the spout and time between licks. Each day, food jars and fluid bottles are cleaned and refilled or replaced as needed and positions are rotated to prevent location bias. During this daily maintenance, jars are manually weighed to collect "on" and "off" weights which serve to verify data collected by the apparatus and provide backup data in the event of apparatus failure. For this experiment, data collection occurred for 21 hours each day with the other 3 hours being used to monitor rat body weight and health, deliver SEMA or VEH injections, maintain and weigh food jars and fluid bottles, and change cages. Custom software was used to analyze the apparatus-collected food intake and licking data and combine feeding bouts into meals separated by at least 900 seconds without intake. The number of meals, time of each feeding or drinking bout, licks to fluid bottles, mass of food consumed, rate of licking or eating, as well as summaries for

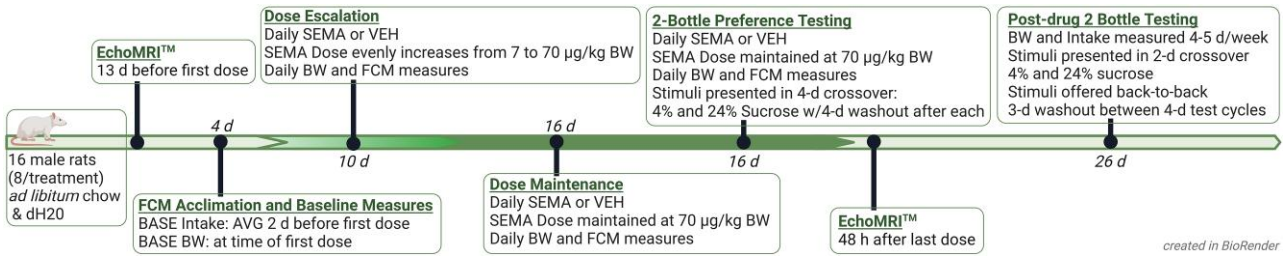

Figure 2. Experiment 2 timeline. Food intake, water intake, and meal pattern data for each rat were averaged for the last 2 days of the FCM acclimation period to arrive at individualized BASE measures. Body weight measured immediately before the first SEMA or VEH injection served as BASE. At times during Experiment 2, rats were transferred to another facility for EchoMRI™ measurements (detailed in "Experiment 2: EchoMRI™ Body Composition Measurements") or into a behavioral testing room for meal pattern monitoring (detailed in "Experiment 2: Food and Fluid Intake Measurements in the 5-Item Food Choice Monitor"). In all cases, the same light:dark cycle was maintained throughout the transfers. After 4 days of acclimation to the FCM cages, SEMA, or VEH injections began and were given approximately 4 hours after lights on each day. The dose escalation protocol is detailed in "Drugs." Rats underwent 2-bottle preference testing during SEMA or VEH treatment (described in "Experiment 2: 4-Day 2-Bottle Preference Tests"). After the post-treatment EchoMRI™, which required transfer to another facility, rats were returned to the home vivarium and allowed 24 hours of reacclimation before the start of post-treatment sucrose preference testing, described in detail in "Experiment 2: Post-SEMA 2-Bottle Preference Tests." BASE, baseline; BW, bodyweight; FCM, 5-item food choice monitor.

the entire period, the light period, and the dark period were calculated for each rat daily.

Experiment 2: 4-day 2-bottle preference tests

After rats had completed 16 days at the 70 ug/kg maintenance dose of SEMA, sucrose preference testing began. Rats in each treatment group were evenly divided and received either 4% or 24% sucrose for 4 days (21 hours/day) in a crossover design, with a 4-day washout period after each sucrose concentration. The sucrose concentrations were chosen based on data obtained during Experiment 1 and reflect 1 concentration at which SEMA-treated rats displayed excessive intake compared to VEH rats and 1 concentration where intake of SEMA and VEH rats was similar. During preference testing, food and fluid measures continued as described above (see "Experiment 2: Food and Fluid Intake Measurements in the 5-Item Food Choice Monitor") except that when sucrose was available both fluid bottles were emptied and refilled with fresh sucrose solution or deionized water each day. Preference scores were calculated as described in "Experiment 1: 2-Day 2-Bottle Preference Tests".

Experiment 2: post-SEMA 2-bottle preference tests

Postdrug, rats were returned to standard cages with food hoppers. Body weight, food, and fluid measurements occurred as described in Experiment 1 ("Experiment 1: Food and Water Intake Measurements") where daily "on" and "off" weights were manually collected and the difference between on and off weights determined intake. During the first week postdrug, beginning approximately 48 hours after the second echo magnetic resonance imaging (EchoMRI™) measurement, rats were tested with 1 day (23 hours) each at 4% and 24% sucrose in a crossover design with half the rats receiving each concentration on the right or left side of the cage top. Beginning in the second week, rats received each concentration for 2 days (23 hours/day) with half of the rats receiving 4% sucrose first and the other half receiving 24% sucrose first and bottle positions swapped daily such that each rat received each sucrose concentration in both bottle positions during each testing cycle. Sucrose preference scores were calculated as described in "Experiment 1: 2-day 2-bottle preference tests".

Experiment 2: EchoMRI™ body composition measurements

Thirteen days prior to the first SEMA or VEH injection and 48 hours after the last SEMA or VEH injection, rats underwent EchoMRI™ body composition measurements (EchoMRI™ LLC, Houston, TX). The day before measurements, rats were transferred to the Florida State University facility housing the EchoMRI™ machine. The next morning, each rat was placed into the machine for body composition analysis. To obtain the measurements, rats were placed into a tube and held in position by placement of a stopper. The tube was placed into the EchoMRI™ machine and all measurements were completed in 90 seconds or less. Upon completion of measurement, the tube was immediately removed from the machine and the rat removed from the tube and returned to its home cage. Rats were returned to the home vivarium after measurements were completed.

Experiment 2: data notes

On Day 8 of the dose maintenance period meal pattern data was not collected for half of the rats in each treatment group due to equipment user error. For these rats on this day, total intake was determined from manually collected food jar and water bottle weights. Meal number, meal size, and consumption rate were averaged from the day before and the day after the data loss to provide values for statistical analyses. On the second day of postdrug sucrose testing (PD5) we switched scales and suspect weights differed by approximately 1 g from the other scale. Because this difference was relatively small and affected all rats equally, we did not alter or exclude data collected on this day with the exception of water intake. Due to very low water intake, the scale difference led to some rats having negative water intake. These negative values were replaced with zeros for preference calculations. On the final day of post-SEMA sucrose preference testing, the sucrose bottle of 1 rat that had been treated with SEMA was spilled prior to obtaining an "off" measurement. For that rat in that 2-day concentration test, instead of a 2-day average, 1-day intake is reported and used in statistical analysis.

Results

SEMA Reduced Intake, Meal Size, Meal Eating Rate, and Body Weight During Dose Escalation

In both experiments, rats receiving SEMA quickly decreased their energy intake to 60 - 65% of BASE during dose escalation

Table 1. Experiment 1 schedule of preference testing solutions

Testing days	Test solution	kcal/mL
1-2	0.0 M sucrose	0.00
3-4	0.03 M sucrose	0.04
5-6	0.06 M sucrose	0.08
7-8	0.1 M sucrose	0.14
9-10	0.3 M sucrose	0.41
11-12	0.6 M sucrose	0.82
13-14	1.0 M sucrose	1.37
15-16	10% Fat	1.17

(Fig. 3 and Table 2; Supplementary Figs. S1, S2 and Tables S1, S2 [21]). We found in Experiment 2 that this decrease in energy intake was driven by a decrease in powdered chow meal size (Fig. 4B). Meal duration decreased in SEMA rats and they had a slower within meal eating rate compared with VEH rats but the number of meals per day did not differ (Fig. 4A-4D and Table 3). The reduction in energy intake consistently produced about 10% loss of body weight by SEMA rats at the end of dose escalation and treatment groups differed in body weight by the third day of drug dosing (Fig. 3 and Table 2; Figs. S1, S2A, S2B, S3A and Tables S1, S2 [21]). Not surprisingly, in line with food intake, SEMA rats also decreased their water intake during dose escalation (Table 2; Figs. S1B, S3B and Tables S1, S2 [21]). The number of calories, volume of water consumed, and body weight increased slightly during this period in VEH rats (Fig. 3 and Table 2; Figs. S1, S2, S3 and Tables S1, S2 [21]).

Body Weight Loss Became Asymptotic Despite an Increase in Chow Intake During SEMA Dose Maintenance

Once the maintenance dose was reached, SEMA rats in both experiments began to increase their chow consumption, averaging 80% to 90% of BASE energy intake during the Dose Maintenance phase (Fig. 3A and 3B and Tables 2 and 3; Figs. S1A, S2A, S3A and Tables S1, S2 [21]). Experiment 2 revealed that meal duration of SEMA rats began to increase during Dose Maintenance (Fig. 4C and Table 3) leading to an increased meal size. Nevertheless, the eating rate of SEMA rats remained lower than VEH rats. Meal number remained equal between groups. Despite the increased food intake in SEMA rats, in both experiments, there remained a 16% difference in body weight between SEMA and VEH rats at the end of the dose maintenance period (Fig. 3A and 3B and Tables 2 and 3; Figs. S1A, S2A, S3A and Tables S1, S2 [21]). The increase in food intake by SEMA rats was accompanied by an increase in water intake (Figs. S1B, S3B and Tables S1, S2 [21]). As expected, in both experiments, during dose maintenance, water intake of VEH rats remained stable and food intake and body weight gradually increased (Fig. 3 and Table 2; Fig. S1 [21]).

Rats Receiving SEMA Showed Excessive Intake of and Preference for Lower but not Higher Sucrose Concentrations with Concomitant Increases in Total Energy Intake and Body Weight

In Experiment 1, at low- to mid-range sucrose concentrations (0.03 M through 0.3 M), SEMA rats consumed, on average,

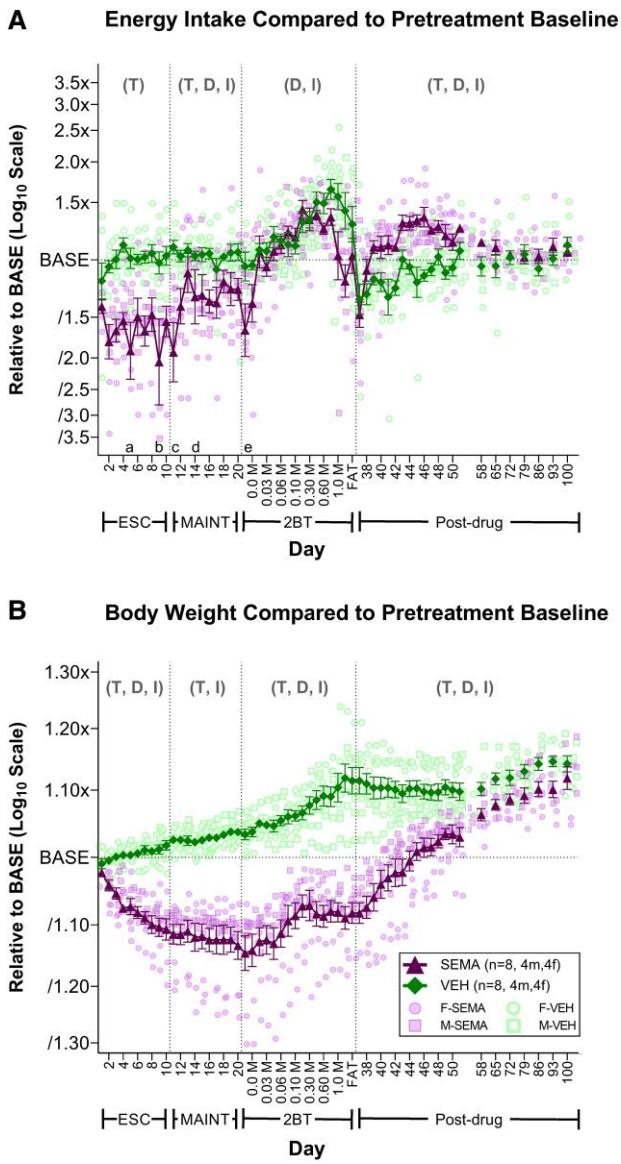

Figure 3. Experiment 1: impact of SEMA on energy intake and body weight (relative to pretreatment). Drug groups are shown in the foreground as group mean ± SE $\log_{10}$: ratio to baseline (BASE) with individual rats shown in the background. Groupwise, SEMA rats are shown with filled triangles and VEH with filled diamonds. Individually, SEMA rats are shown as filled and VEH as open symbols, with males as squares and females as circles (A) Energy intake shown as $\log_{10}$: ratio to baseline (BASE) energy intake. At BASE, SEMA rats had average energy intake of 89.25 ± 9.6 kcal (males 112.1 ± 7.2 kcal; females 66.4 ± 5.6 kcal) and VEH rats had average energy intake of 89.67 ± 11.9 kcal (males 116.8 ± 12.2 kcal; females 62.6 ± 4.9 kcal). In 5 instances individual rat data are below the range of the y axis: a = /6.3x; b = /12x; c = /8x; d = /4x; e = /4.8x. (B) Body weight shown as $\log_{10}$ (ratio to baseline body weight). At BASE, SEMA rats had average bodyweight of 492.0 ± 64.9 g (males 661 ± 22.1 g; females 323 ± 10.8 g) and VEH rats had average body weight of 503.5 ± 76.4 g (males 688.5 ± 63.0 g; females 318.5 ± 20.8 g). Bonferroni-corrected pairwise comparisons showed that, relative to BASE, body weight of treatment groups differed beginning on Day 3 and continuing through Day 49 (body weight differs through Day 93 before Bonferroni correction). Two-way repeated measures ANOVAs were run separately for each experimental phase (ESC, dose escalation phase, MAINT, dose maintenance phase, 2BT, 2-bottle preference testing phase) with significant effects and interactions designated at the top of the graph (T, treatment effect; D, day effect; I, treatment × day interaction) and detailed in Table 2.

Table 2. Experiment 1 statistics

Measure	Phase	Treatment	Day	Interaction
Energy intake[a]	ESC	**F (1, 14) = 24.044, *P* < .001**	F (9, 126) = 1.038, *P* = .413	F (9, 126) = 1.131, *P* = .346
	MAINT	**F (1, 14) = 8.254, *P* = .012**	**F (9, 126) = 2.201, *P* = .026**	**F (9, 126) = 2.698, *P* = .019**
	2BT	F (1, 14) = 3.894, *P* = .069	**F (15, 210) = 11.697, *P* < .001**	**F (15, 210) = 3.059, *P* = .013**
	PD	**F (1, 14) = 10.208, *P* = .006**	**F (21, 294) = 10.417, *P* < .001**	**F (21, 294) = 4.692, *P* < .001**
Relative energy Intake (kcal/g BW)[a]	ESC	**F (1, 14) = 14.620, *P* = .002**	F (9, 126) = 1.087, *P* = .377	F (9, 126) = 0.932, *P* = .500
	MAINT	F (1, 14) = 1.833, *P* = .197	**F (9, 126) = 2.118, *P* = .033**	**F (9, 126) = 2.835, *P* = .005**
	2BT	F (1, 14) = 0.296, *P* = .595	**F (15, 210) = 10.492, *P* < .001**	**F (15, 210) = 2.889, *P* < .001**
	PD	**F (1, 14) = 24.732, *P* < .001**	**F (21, 294) = 8.373, *P* < .001**	**F (21, 294) = 5.719, *P* < .001**
Chow intake[a]	2BT	**F (1, 14) = 37.753, *P* < .001**	**F (15, 210) = 38.847, *P* < .001**	**F (15, 210) = 2.080, *P* = .012**
Body weight[a]	ESC	**F (1, 14) = 64.227, *P* < .001**	**F (8, 112) = 9.103, *P* < .001**	**F (8, 112) = 25.667, *P* < .001**
	MAINT	**F (1, 14) = 62.724, *P* < .001**	F (9, 126) = .433, *P* = .915	**F (9, 126) = 3.397, *P* = .001**
	2BT	**F (1, 14) = 63.484, *P* < .001**	**F (15, 210) = 16.548, *P* < .001**	**F (15, 210) = 2.363, *P* = .004**
	PD	**F (1, 14) = 29.683, *P* < .001**	**F (21, 294) = 42.444, *P* < .001**	**F (21, 294) = 23.044, *P* < .001**
Water intake[a]	ESC	**F (1, 14) = 7.664, *P* = .015**	F (9, 126) = 1.103, *P* = .365	F (9, 126) = 0.835, *P* = .586
	MAINT	F (1, 14) = 0.437, *P* = .519	F (9, 126) = 1.351, *P* = .217	**F (9, 126) = 3.475, *P* = .001**
	2BT	Many rats w/0 mL intake		
	PD[b]	**F (1, 14) = 12.058, *P* = .004**	**F (14, 196) = 2.478, *P* = .003**	F (14, 196) = 1.338, *P* = .188

Measure	Phase	Treatment	Concentration	Interaction
Sucrose intake	2BT	**F (1, 14) = 14.411, *P* = .002**	**F (6, 84) = 89.561, *P* < .001**	**F (6, 84) = 24.445, *P* < .001**
10% fat intake	2BT	F (1, 14) = 0.952, *P* = .346		
Sucrose pref (vol)	2BT	F (1,14) = .016, *P* = .901	**F (6,84) = 149.031, *P* < .001**	F (6,84) = 0.400, *P* = .877
Sucrose pref (kcal)	2BT	**F (1, 14) = 31.964, *P* < .001**	**F (5, 70) = 563.858, *P* < .001**	**F (5, 70) = 13.442, *P* < .001**
10% fat pref (vol)	2BT	F (1, 14) = 0.010, *P* = .920		
10% fat pref (kcal)	2BT	F (1, 14) = 0.799, *P* = .387		

P-values ≤.05 are bolded.
Abbreviations: 2BT, 2-bottle preference testing phase; ESC, dose escalation phase; MAINT, dose maintenance phase; PD, postdrug monitoring phase.
[a]Two-way ANOVA on $\log_{10}$ (ratio to baseline).
[b]Water intake only monitored for first 2 weeks PD.

1.5 time to 2.5 times the volume consumed by VEH rats. At the 2 higher sucrose concentrations and for the 10% fat, intake did not differ between SEMA and VEH rats (Fig. 5 and Table 2; Fig. S4 [21]). Given the fluids available, the rats in both groups consumed the test solutions almost exclusively. Thus, when compared with water, preference generally approached unity in both groups and dipped only slightly for 1.0 M sucrose and for 10% FAT (Table 2; Fig. S4 [21]). Alternatively, given the energy sources available (chow and sucrose), SEMA rats consumed a greater proportion of their daily energy intake from sucrose at the 4 middle concentrations (0.06-0.6 M) compared with VEH rats. Energy source preference did not differ between the groups at the other sucrose concentrations and for 10% fat (Fig. 5C and Table 2).

The results of sucrose preference testing in Experiment 2 were on par with Experiment 1 demonstrating that the effects observed were independent of the order of presentation of sucrose concentrations. Compared to VEH rats, SEMA rats ingested more than double the volume of 4% sucrose while consumption of 24% sucrose was equal between the groups (Fig. 6A and 6B and Table 4). Compared with VEH, SEMA rats had extended meal duration when consuming 4% sucrose and there was a significant interaction between treatment and concentration affecting both sucrose meal number and sucrose meal size. However, the fact that post hoc pairwise comparisons did not reveal significant differences between the treatment groups in meal number and meal size suggests it was a combination of increased meal size and meal number that produced the elevated intake of 4% sucrose by SEMA rats (Fig. 6E-H and Table 4). Both groups preferred both concentrations over water by ≥87% on average (Fig. 6C and Table 4). Energy source preference compared to chow did not differ between the groups for 24% sucrose, with all rats averaging nearly half their kcal intake from sucrose across test days (Fig. 6D and Table 4). However, while SEMA rats consumed 25% to 33% of their daily energy intake from 4% sucrose, VEH rats consumed only about 12% of their daily energy intake from the low sugar concentration (Fig. 6D and Table 4).

In both SEMA and VEH rats in Experiment 1, chow intake decreased in both groups during 2-bottle preference testing (Fig. S4 [21]), but total energy intake dramatically increased (Fig. 3A and Table 2; Fig. S1A [21]). SEMA rats consumed as much as 45% above their BASE energy intake and VEH rats exceeded their BASE intake by 67%, leading to weight gain in both groups (Fig. 3 and Table 2; Fig. S1A [21]). However, in SEMA rats, maximal energy intake occurred during 0.3 M sucrose preference testing while in VEH rats, maximum energy intake occurred later, during testing of 1.0 M sucrose (Fig. 3A and Table 2). At the conclusion of preference testing, weight loss of SEMA rats had eroded to about 9% of BASE but, since VEH rats gained an additional 8% BASE body weight over the same period, a ~17% difference in body weight between SEMA and VEH rats remained (Fig. 3B and Table 2).

In Experiment 2, an analysis of the mean values for the 4 days at each sucrose concentration and the 8 washout days reveals SEMA rats consumed less energy than VEH rats during washout days; when sucrose was available, energy intake did not differ between the groups (Fig. S5 and Table S2 [21]). If we consider energy intake relative to body weight, it did not differ (Fig. S5A and Table S2 [21]). Rats in both groups drank little

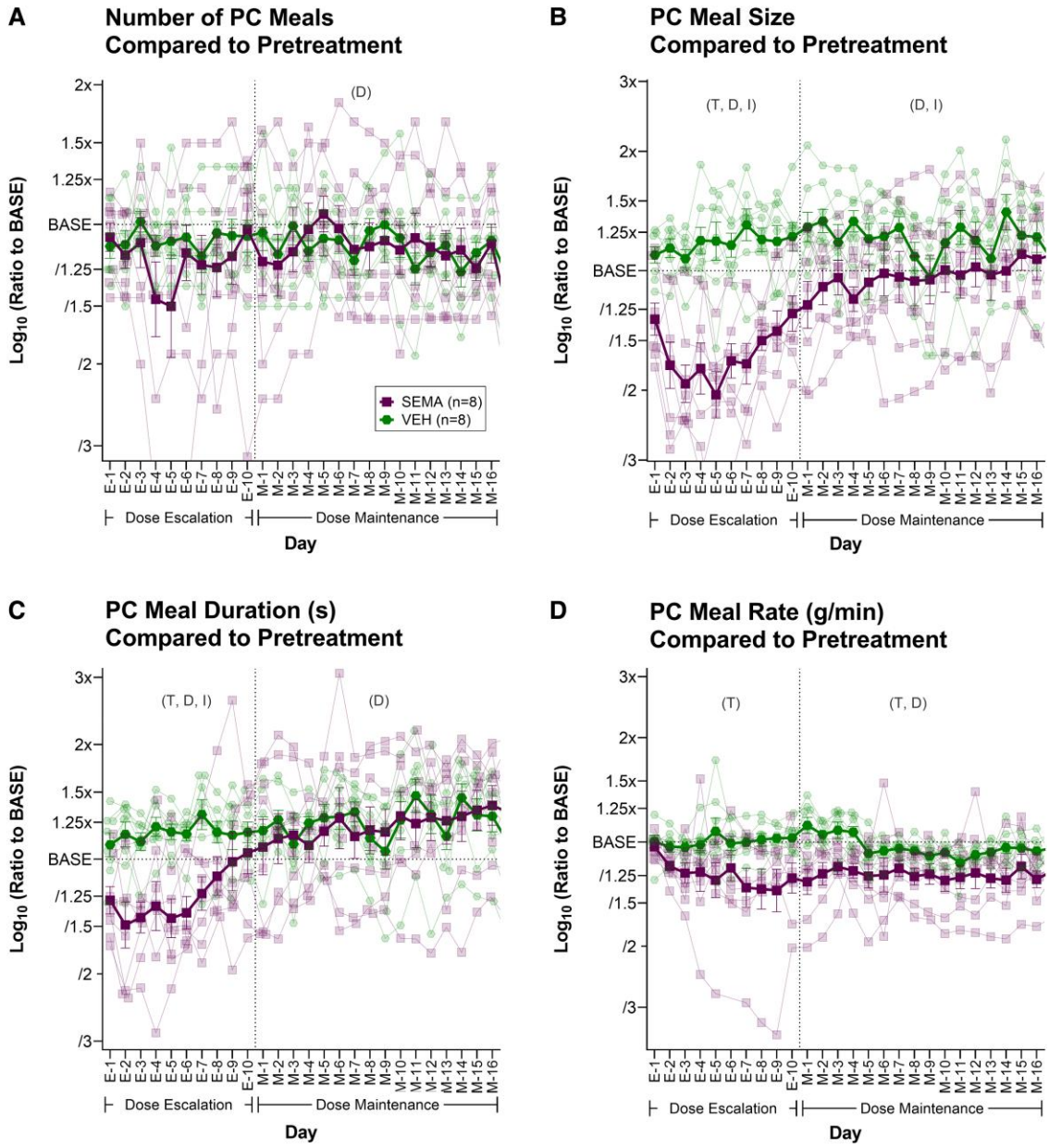

Figure 4. Experiment 2. Impact of SEMA dose escalation and dose maintenance on powdered chow (PC) meal patterns. Drug groups are shown in the foreground as group mean ± SE $\log_{10}$: ratio to baseline (BASE) with individual rats shown in the background. Groupwise, SEMA rats are shown with filled squares and VEH with filled hexagons. (A) Number of powdered chow meals/day shown as $\log_{10}$:ratio to baseline (BASE) meal number (VEH BASE = 8.3 meals/day; SEMA BASE = 9.0 meals/day). (B) Powdered chow meal size (g) shown as $\log_{10}$: ratio to baseline (BASE) meal size (VEH BASE = 3.1 g; SEMA BASE = 2.8 g). (C) Powdered chow meal duration (s) shown as $\log_{10}$: ratio to baseline (BASE) meal duration (VEH BASE = 12.23 min; SEMA BASE = 10.91 min). (D) Powdered chow meal rate (g/min) shown as $\log_{10}$ (ratio to baseline meal rate) (VEH BASE = 0.253 g/min; SEMA BASE = 0.262 g/min). Two-way repeated measures ANOVAs were run separately for each experimental phase with significant effects and interactions designated at the top of the graph (T, treatment effect; D, day effect; I, treatment × day interaction) and detailed in Table 3.

to no water when sucrose was available (Fig. S5 and Table S2 [21]). Consistent with Experiment 1, SEMA and VEH rats showed excess energy consumption when sucrose was available, and both groups gained body weight during sucrose testing, with VEH rats reaching almost 10% above BASE and the weight loss of SEMA rats eroding from ~10% to 6.5% BASE (Fig. S5 and Table S2 [21]). Still, the difference in body weight between SEMA and VEH persisted at about 16% (Fig. S5 and Table S2 [21]).

Rats in both treatment groups exhibited some variation in powdered chow meal patterns during sucrose preference testing (ie, main effects of day without group interaction). However, except for eating rate, there were no effects of treatment on powdered chow meal patterns during sucrose preference testing (Fig. S5 and Table S2 [21]).

Energy Intake and Body Weight Rapidly Increased and Sucrose Preference Became Similar to VEH in SEMA Rats During the Post-Treatment Period

In Experiment 1, when SEMA and VEH injections ceased at the conclusion of preference testing, energy intake of VEH

Table 3. Experiment 2 powdered chow meal pattern statistics during dose escalation and maintenance

Measure	Phase	Treatment	Day	Interaction
Number of meals[a]	ESC	F (1, 14) = 1.162, $P = .299$	F (9, 126) = 1.090, $P = .375$	F (9, 126) = 0.786, $P = .629$
	MAINT	F (1, 14) = 0.001, $P = .976$	**F (15, 210) = 1.821, $P = .033$**	F (15, 210) = 1.488, $P = .112$
Meal size[a]	ESC	**F (1, 14) = 45.090, $P < .001$**	**F (9, 126) = 2.754, $P = .006$**	**F (9, 126) = 2.492, $P = .012$**
	MAINT	F (1, 14) = 3.064, $P = .102$	**F (15, 210) = 1.825, $P = .033$**	**F (15, 210) = 2.489, $P = .002$**
Meal duration[a]	ESC	**F (1, 14) = 16.956, $P = .001$**	**F (9, 126) = 2.241, $P = .023$**	F (9, 126) = 1.940, $P = .052$
	MAINT	F (1, 14) = 0.038, $P = .848$	**F (15, 210) = 3.182, $P < .001$**	F (15, 210) = 1.457, $P = .124$
Meal rate[a]	ESC	**F (1, 14) = 6.961, $P = .019$**	F (9, 126) = 1.087, $P = .377$	F (9, 126) = 1.643, $P = .110$
	MAINT	**F (1, 14) = 9.546, $P = .008$**	**F (15, 210) = 1.979, $P = .018$**	F (15, 210) = 1.492, $P = .110$

P-values ≤.05 are bolded.
Abbreviations: ESC, dose escalation; MAINT, dose maintenance.
[a]Two-way ANOVA on LOG_{10} (ratio to baseline).

rats decreased to below BASE for about a week (Fig. 3A and Table 2; Fig. S1A [21]). SEMA rats were consuming ~10% above their BASE energy intake within 3 days and further increased to ~20% above BASE during the second week. After this steep increase, the energy intake of SEMA rats gradually decreased to BASE levels over ~5 weeks (Fig. 3A and Table 2; Fig. S1A [21]). In both groups, water intake trended on par with energy intake during the postdrug period (Table 2; Fig. S1B [21]). Not surprisingly, weight regain by the SEMA rats occurred rapidly and they reached BASE body weight within 2 weeks of the cessation of SEMA injections. However, since VEH rats lost only a small amount of weight after sucrose and fat testing concluded, SEMA rats continued to weigh less than VEH rats for more than 8 weeks (Fig. 3B and Table 2).

As in Experiment 1, in Experiment 2, energy intake by SEMA rats was elevated during the postdrug period, most notably when considered relative to body weight (Fig. 7C; Fig. S6C and Table S1 [21]). Still, the groups remained different in body weight during the postdrug period and uncorrected pairwise comparisons revealed that the groups differed until late in the fourth week (PD24) postdrug (Fig. 7D; Table S1 [21]). We should note, though, that after Bonferroni correction, there are not significant differences in body weight after 2 weeks (PD15, Fig. 7D; Table S1 [21]).

In Experiment 2, only during the first post-treatment sucrose testing cycle, the SEMA rats drank more sucrose compared with the VEH group (Fig. 7A; Fig. S6A and Table S3 [21]). However, preference for both sucrose concentrations, whether compared to water or chow, did not differ between SEMA and VEH rats at any time during the post-treatment testing (Fig. 7A and 7B; Fig. S6A and B and Table S3 [21]).

SEMA Reduced Body Fat but not Lean Mass

Pretreatment EchoMRI™ analysis found that SEMA rats had 10.4% (±1.15%) body fat on average and were not different from VEH rats (10.7% ± 0.85% body fat) (Fig. 8A and Table 5). At the start of the postdrug period, body fat of SEMA rats had decreased to 7.6% (±0.78%) while VEH rats had increased to 13.6% (±1.49%) of total mass (Fig. 8A and Table 5). Thus, at post-treatment, the percentage of body fat was different between groups and in both treatment groups differed from pretreatment (Fig. 8A and Table 5). Looking at absolute mass (ie, grams), in SEMA rats, fat mass decreased from pre- to post-treatment, but lean mass did not change. VEH rats increased in both lean

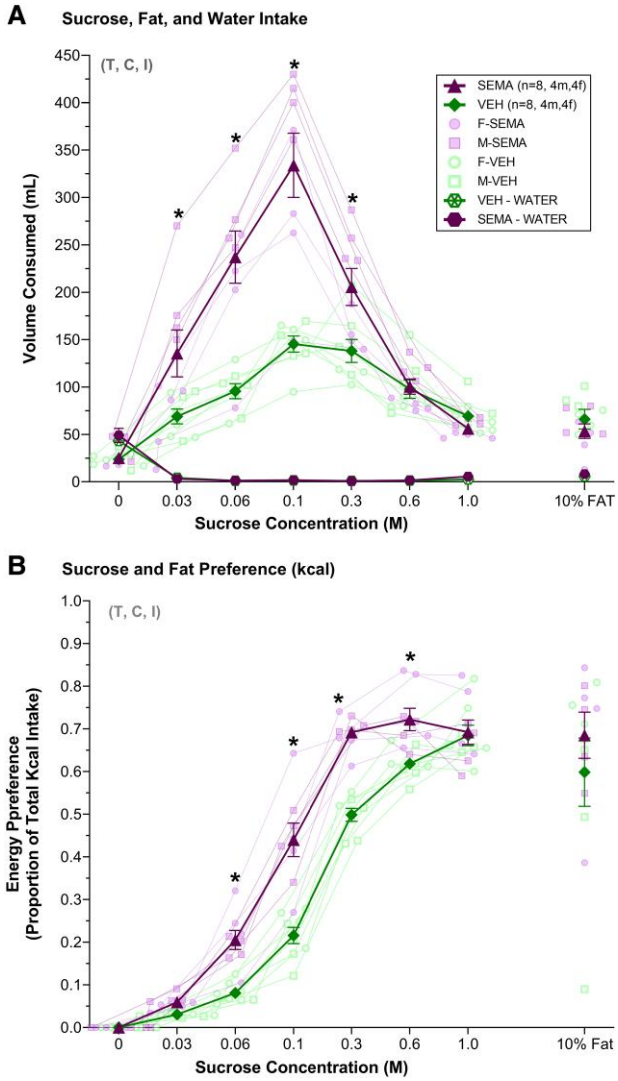

Figure 5. Experiment 1. Effect of long-term SEMA treatment on sucrose intake and preference during 2-bottle preference testing. Drug groups are shown in the foreground as group mean ± SE with individual rats in the background. (A) Two-day average sucrose, fat, and water intake. (B) Sucrose and fat energy source preference vs chow shown as the proportion of total kcal intake. Significant effects are indicated at the top of each graph (T, treatment effect; C, concentration effect; I, treatment × concentration interaction) and detailed in Table 2. Significant pairwise comparisons are marked with an asterisk (Bonferroni corrected).

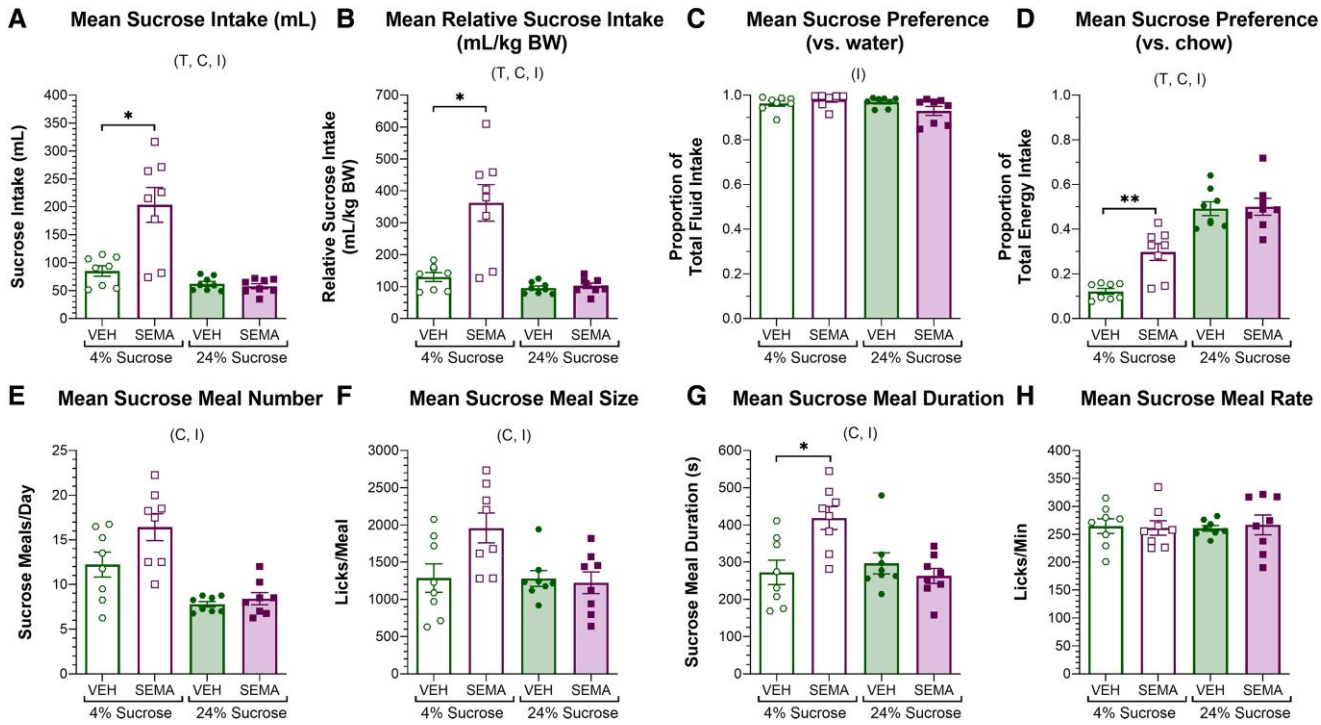

Figure 6. Experiment 2. Impact of long-term SEMA treatment on sucrose intake, preference, and meal patterns. Drug groups are shown by the bars as group mean ± SE with individual rats shown within each bar. Groupwise, SEMA rats are shown with squares and VEH with hexagons. Filled symbols represent data from testing of 24% sucrose and open symbols are 4% sucrose. (A) Mean sucrose intake (mL). (B) Relative sucrose intake (mL/kg bodyweight [BW]). (C) Sucrose preference vs water shown as proportion of total fluid intake from sucrose solutions. (D) Sucrose preference vs chow shown as proportion of total energy intake from sucrose solutions. (E) Mean number of sucrose meals/day. (F) Mean sucrose meal size (licks). (G) Mean sucrose meal duration (s). (H) Mean sucrose drinking rate (licks/min). Significant effects found using 2-way repeated measures ANOVAs are designated at the top of each graph (T, treatment effect; C, concentration effect; I, treatment × concentration interaction). When ANOVAs identified a significant interaction, pairwise comparisons were conducted between treatment groups at each concentration and within treatment group across concentrations. Significant pairwise comparisons (Bonferroni corrected *P* values) are denoted as follows: $*P < .05$, $**P < .01$, $***P < 0.001$) and are detailed in Table 4.

and fat mass from pre to post treatment (Fig. 8B and 8D and Table 5).

Discussion

Here, we monitored body weight, body composition, and energy and fluid intake in rats receiving SEMA using a dose escalation protocol prior to maintenance at our maximal dose. In humans, up-titration of dose is used to lessen the severity of SEMA-induced side effects and we chose to mimic this approach to increase translatability of our results. Existing research using SEMA in preclinical models does not often include a dose escalation protocol and evidence of drug-induced malaise, in addition to changes in food intake, have been reported [14]. Indeed, the reason that the dose is incrementally escalated clinically is in an effort to minimize gastrointestinal side effects [22–24]. Accordingly, the incorporation of dose escalation procedures into studies of the effect of SEMA may be important from the standpoint of clinical relevance. Moreover, the comparison of the metabolic, behavioral, and neural consequences of acute vs chronic SEMA administration can more completely characterize potential adaptations associated with prolonged GLP-1R agonism.

The Trajectory of Weight Loss During our Dose Escalation Protocol is Similar to Clinical Results

In Experiments 1 and 2, the 9% to 10% weight loss of our SEMA rats is consistent with reports of weight loss in humans after 16 to 20 weeks of treatment that included dose escalation [11, 25, 26]. The similarity in body weight loss suggests our dose escalation protocol is a good match for the one used clinically.

Evidence for Adaptation to SEMA

At the conclusion of dose escalation, when SEMA rats were maintained at our chosen maximum daily SEMA dose (70 µg/kg body weight), in both experiments, their energy intake increased (as did their water intake, see supplemental discussion [21]). That a continually increasing SEMA dose was required to maintain the greater reduction in energy intake seen during dose escalation compared to dose maintenance suggests there may be adaptation to the effects of SEMA over time. It remains unclear if our rats adapted to the on- or off-target effects of SEMA since there is evidence in humans and rodents that SEMA may cause malaise with acute dosing and during dose escalation [11, 14, 22, 24–27], a common side effect with administration of GLP-1 analogs generally [28–35]. Accordingly, the increase in food intake observed during Dose Maintenance could have, in part, represented the resolution of malaise which subsided over time once the SEMA dose became consistent.

Interestingly, even though chow intake progressively increased and approached VEH levels during dose maintenance, body weight did not follow the same trajectory but rather appeared to approach asymptote below BASE. Compared with VEH, the body weight of our SEMA rats at the end of dose

Table 4. Experiment 2 sucrose intake, preference, and meal pattern statistics (during SEMA or VEH treatment)

Measure	Treatment	Concentration	Interaction
Sucrose intake	**F (1,14) = 9.480, *P* = .008**	**F (1,14) = 34.811, *P* < .001**	**F (1,14) = 18.482, *P* < .001**
Relative sucrose intake	**F (1,14) = 12.709, *P* = .003**	**F (1,14) = 32.221, *P* < .001**	**F (1,14) = 18.963, *P* < .001**
Sucrose preference (mL vs dH_2O)	F (1,14) = 0.602, *P* = .451	F (1,14) = 3.405, *P* = .086	**F (1,14) = 5.658, *P* = .032**
Sucrose preference (kcal vs chow)	**F (1,14) = 5.617, *P* = .033**	**F (1,14) = 174.975, *P* < .001**	**F (1,14) = 15.076, *P* = .002**
Sucrose meal number	F (1,14) = 3.474, *P* = .083	**F (1,14) = 55.667, *P* < .001**	**F (1,14) = 4.624, *P* = .049**
Sucrose meal size	F (1,14) = 2.887, *P* = .111	**F (1,14) = 6.359, *P* = .024**	**F (1,14) = 6.169, *P* = .026**
Sucrose meal duration	F (1,14) = 3.503, *P* = .082	**F (1,14) = 6.147, *P* = .027**	**F (1,14) = 11.456, *P* = .004**
Sucrose meal rate	F (1,14) = 0.008, *P* = .931	F (1,14) = 0.007, *P* = .934	F (1,14) = 0.256, *P* = .621

P-values ≤.05 are bolded.

maintenance in both experiments resembled the difference between SEMA and VEH seen in clinical trials. It is also notable that dose maintenance was the only experimental phase for which the effects of SEMA differed between absolute and relative energy intake. Although the main effect of SEMA on kcal intake per gram body weight vanished during dose maintenance, weight loss was maintained or modestly increased, implying that energy expenditure is maintained during SEMA treatment [19, 36].

The Behavioral Bases for SEMA-Induced Changes in Chow Intake During Dose Escalation and Dose Maintenance

Daily food intake is the outcome of behavior and does not reflect the manner in which it was achieved. In this sense, meal pattern analysis has been indispensable for understanding how the brain controls the actions of the animal resulting in the amount of energy consumed over a day (eg, [37–49]). It is quite clear that the course of chow intake in SEMA-treated rats over the dose escalation and dose maintenance phases were mediated primarily by meal size and not meal number. Thus, SEMA was mainly affecting processes that control the termination, rather than initiation, of meals, with respect to chow ingestion. The reduced within-meal eating rate in SEMA-treated rats suggests a blunting of the general motivational state of the animals when chow was being ingested; whereas the reduced meal size suggests that the threshold for satiation decreased in rats receiving SEMA treatment. It is noteworthy, that as daily chow intake approached VEH levels during dose maintenance in Experiment 2, so did meal size. However, the SEMA-induced decrease in within-meal ingestion rate relative to VEH, for chow (but not sucrose—see below), persisted throughout all the drug treatment phases of the experiment. This suggests a dissociation of the effects of the drug on the threshold for satiation vs the within meal excitatory drive for chow intake. The neural bases for these effects remains to be investigated, but the behavioral results presented here provide a functional road map for such an effort.

SEMA Did not Reduce Preference for Sucrose or Fat Solutions

After 10 to 16 days of dose maintenance, we began testing preference for sucrose (concentrations up to 1.0 M) and, in Experiment 1, a single 10% fat solution. Surprisingly, in light of the existing literature showing SEMA reduces preference for palatable foods [19], we found that rats receiving SEMA, compared to VEH rats, ingested an equal or greater volume of all test solutions, with significant overconsumption of the low- to mid-range sucrose concentrations, leading to substantive increases in energy intake by the SEMA rats. VEH rats also increased energy intake during preference testing and the rats in both groups gained body weight. As SEMA rats reduced intake at the higher sucrose concentrations in Experiment 1, their body weight gain modestly reversed. Although it appeared that SEMA rats began to lose weight once testing of sucrose concentrations above 0.3 M was started in Experiment 1, the duration of SEMA treatment after sucrose preference testing was not sufficient for us to determine to what extent, if any, weight loss in SEMA rats would have been restored with continued treatment.

The SEMA rats displayed equal or greater preference for sucrose and fat solutions, whether calculated as a function of volume relative to water or as a proportion of energy intake relative to chow. Strikingly, in Experiment 1, for concentrations ranging from 0.06 to 0.6 M, the SEMA rats consumed a higher percentage of their total daily energy intake from sucrose compared with the VEH rats. Those concentrations were presented in ascending order. In Experiment 2, the 4% and 24% concentrations were presented in a counterbalanced crossover design and this did not change the overconsumption or preference displayed by SEMA rats. Meal pattern analysis suggests that neither meal size nor meal number solely accounts for the increase. The findings in Experiment 2 rule out an effect of stimulus delivery order as a factor in our results and suggest that decreases in stimulus palatability, a hypothesized consequence of GLP-1R agonism [10], may not explain all SEMA-induced intake reductions of normally preferred foods.

It is also worth noting that when SEMA treatment ceased in Experiment 2, other than an effect of treatment on sucrose intake during the first postdrug week, there were no differences in sucrose intake or preference (vs water or chow) over the probe tests in the following 3-week period. It seems, therefore, that SEMA must be on-board for the effects on sucrose intake described here to be manifest. Thus, there is no long-term change in sucrose intake and preference once chronic treatment is terminated.

Potential Mechanisms Underlying SEMA-Induced Over-Consumption of Low to Midrange Sucrose Solutions

The remarkable increase in sucrose intake and preference induced by SEMA was unexpected. In considering the

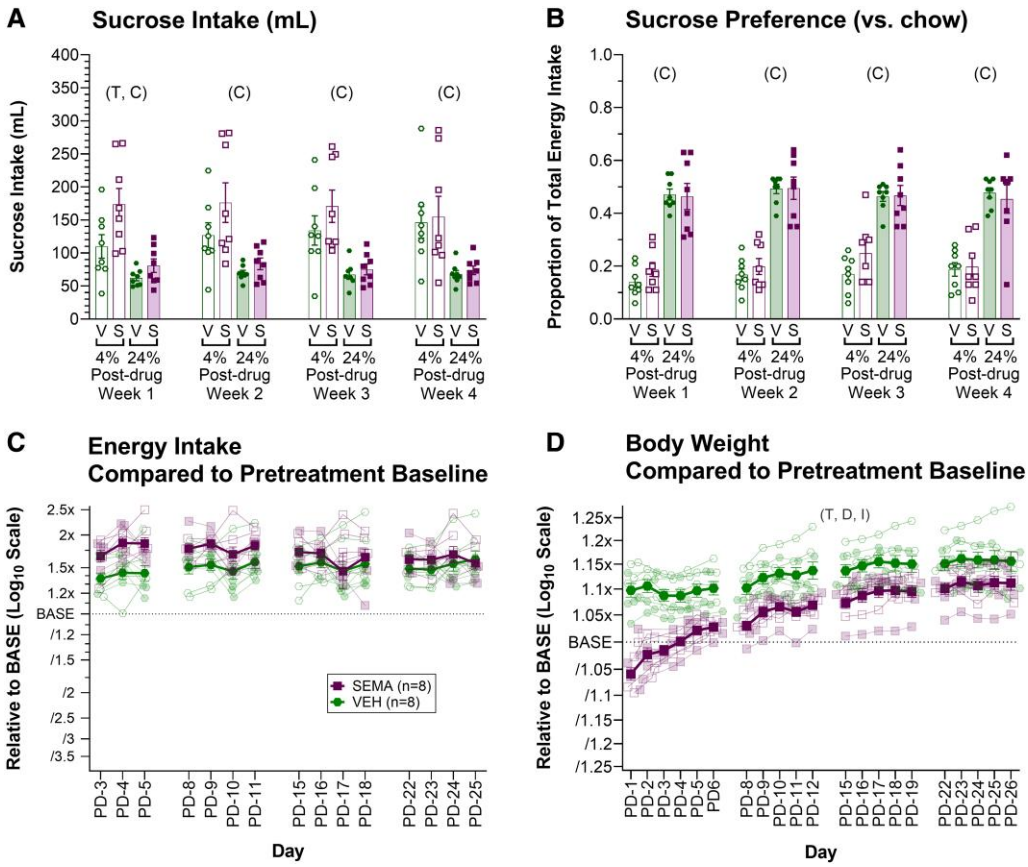

Figure 7. Experiment 2. Impact of cessation of SEMA treatment on sucrose intake, sucrose preference, energy intake, and body weight. Groupwise, SEMA rats are shown with squares and VEH with hexagons. Individual rats are shown in the background with their group symbol. In panels A and B, filled symbols denote results from 24% sucrose and open symbols designate 4% sucrose results. Two-way repeated measures ANOVA were run separately on each week postdrug and significant effects and interactions are indicated at the top of the graphs (T, treatment effect; C, concentration effect; I, interaction) and statistical details may be found in Table 4. (A) Sucrose intake (mL) shown as the average daily intake for each concentration during the named week. (B) Sucrose energy preference vs chow shown as the weekly average daily proportion of total energy intake from sucrose for each concentration during the named week. In panels C and D, drug groups are shown in the foreground as group mean ± SE $\log_{10}$: ratio to baseline (BASE) with individual rats shown in the background. (C) Energy intake shown as $\log_{10}$: ratio to baseline (BASE) energy intake (VEH BASE = 81.7 kcal/day; SEMA BASE = 82.0 kcal/day). (D) Body weight shown as $\log_{10}$ (ratio to baseline body weight) (VEH BASE = 612.7 g; SEMA BASE = 610.8 g). Two-way repeated measures ANOVAs were run on the postdrug preference testing period as a whole. Significant effects and interactions are designated at the top of the graph (T, treatment effect; D, day effect; I, treatment × day interaction) and detailed elsewhere (Table S3 [21]).

mechanism of action leading to this outcome, we believe that excitatory signals promoting sucrose intake could be amplified by the drug or that inhibitory signals discouraging sucrose intake could be blunted, or both. One possibility is that sensory-discriminative taste signals in either the peripheral or central gustatory system were potentiated by treatment such that the intensity of sucrose was greater in SEMA rats. For instance, GLP-1R KO mice have been shown to be less responsive to the nonnutritive sweetener sucralose as well as to low, but not high, concentrations of sucrose in a brief access licking test; licking of other prototypical taste stimuli was unaffected [50, 51]. Of course, GLP-1Rs were missing globally in a variety of tissues including the brain, making it difficult to unequivocally determine the origin of the effect. However, isolated taste cells release GLP-1 in response to application of sweet tastants, GLP-1Rs are expressed intragemmally in gustatory afferent nerve fibers, and circulating GLP-1 increases firing in gustatory nerves [51], leaving open the possibility that GLP-1 acts to enhance peripheral taste signals related specifically to sweeteners and thus may be a target for SEMA.

Another possibility, not mutually exclusive with others, is that SEMA treatment selectively enhanced the reward value of lower sucrose concentrations. Although the literature is equivocal, an effect of acute GLP1-R agonism on motivated behavior is supported by findings of altered dopamine signaling in reward-relevant brain regions including the ventral tegmental area [52, 53], amygdala [54], and nucleus accumbens [55–57], but not always [58]. GLP1-R signaling has been implicated in a variety of behavioral studies investigating motivation and reward. In some cases, studies using acute and chronic dosing have reached similar conclusions and GLP1-R agonism has been found to reduce alcohol intake [16, 57, 59, 60], drug seeking or self-administration [18, 30, 61–66], and palatable food intake [13, 19, 52, 67–73]. However, others using prolonged dosing protocols find no difference in the relative amounts of palatable foods eaten on a choice diet [74, 75]. The effects of GLP-1R agonism on sucrose responding have been the subject of numerous studies. With acute dosing, some have found responding to sucrose is reduced [12–16, 53, 72, 76, 77], unchanged [62], or both, depending on the experimental conditions [78, 79]. To our knowledge, there are no reports of GLP1-R agonism *increasing* sucrose intake or responsiveness. In one study using long-term dosing of liraglutide, rats were trained to expect sucrose

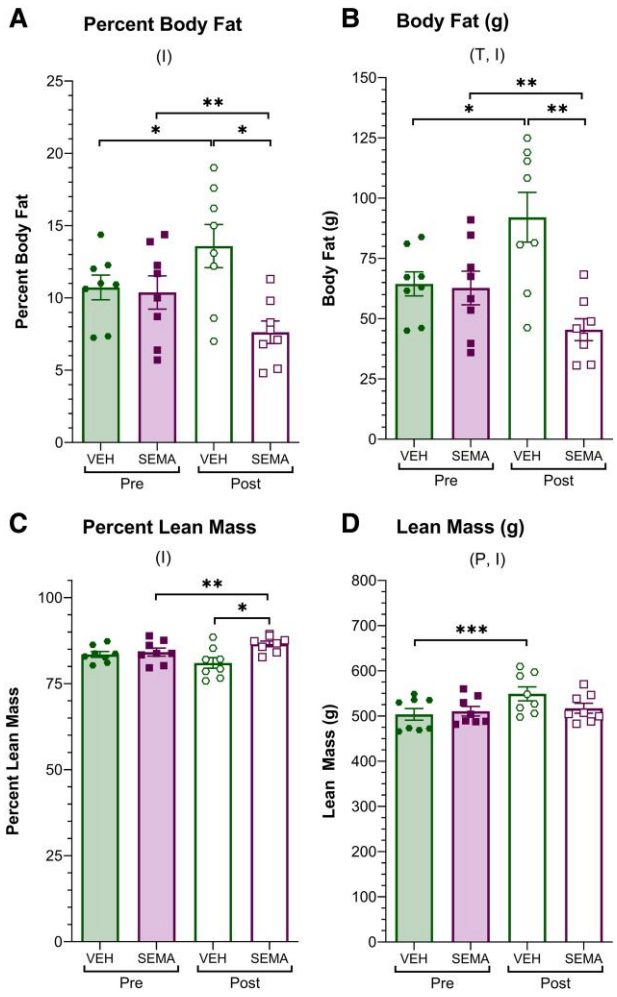

Figure 8. Experiment 2. Effects of SEMA on body composition. Drug groups are shown by the bars as group mean ± SE with individual rats shown within each bar. Groupwise, SEMA rats are shown with squares and VEH with hexagons. Filled symbols represent data from pretreatment EchoMRI™ and open symbols are post-treatment EchoMRI™ results. (A) Percent body fat (fat mass (g)/body weight (g). (B) Fat mass (g). (C) Percent lean mass (lean mass (g)/body weight (g). (D) Lean mass (g). Two-way repeated measures ANOVAs were run and significant effects and interactions are designated at the top of the graph (T, treatment effect; P, pre/posteffect; I, treatment × pre/postinteraction). When ANOVAs identified a significant interaction, pairwise comparisons were conducted between treatment groups at each time point and within treatment group across time points. Significant pairwise comparisons (Bonferroni corrected *P* values) are denoted as follows: $*P < .05$, $**P < .01$, $***P < .001$) and are detailed in Table 5.

pellet delivery after a tonal cue except for when the tone was preceded by a light cue. There were no effects of liraglutide to decrease responsiveness after 4 days of treatment, but after 12 days, rats receiving liraglutide showed fewer nose pokes only during trials when the nonrewarded light cue was presented. This finding suggests that, rather than modulating the reward value of sucrose, longer-term GLP-1R agonism enhanced the effectiveness of a learned inhibitory cue; this form of learning is thought to be hippocampus dependent [17]. Importantly, this inhibitory effect was not observed until after 12 days of treatment, suggesting the effects of liraglutide are dependent, in part, on the duration of treatment (ie, acute vs chronic). In sum, it appears that GLP-1R agonism does not universally reduce reward-based responding.

It could also be that the reduced weight state of our SEMA rats played a role as motivation is modulated by physiological state in humans and rodents (eg [80–82]). In fact, leptin, a long-term energy balance signal, modulates the ability of GLP-1R agonism to reduce food intake [83]. Consistent with the idea of modulation by physical state, humans treated with liraglutide for 12 weeks experience both significant weight loss and, if controlled for body mass index (BMI), show increased activation in brain areas associated with food reward, which the authors posit may be a factor in eventual weight loss plateaus [84]. Although it remains to be tested, it is feasible that the reduced body weight of our rats elevated the reward potency of the sucrose solutions such as to overcome SEMA-induced suppressive effects on intake until the concentration became sufficient to induce greater satiation. As an important postscript to this possibility, it would not make sense to run pair-fed controls in an experimental design such as ours. There is a difference interpretively between an increase in intake in an animal that has restricted food access vs an animal fed ad libitum. In fact, because chow intake rebounds over time in rats chronically treated with SEMA, one would have to provide less food to control rats than the experimental rats to achieve the same body weight target.

One caveat to a mechanism of action that involves SEMA modulation of taste-related sensory discriminative or reward processes is the fact that the drinking rate within sucrose meals did not differ between concentrations, nor between SEMA- and VEH-treated rats. However, it is possible that, although there is room for an increase in the licking rate based on the known interlick interval for rats, under these test conditions in this design, a functional ceiling may have been reached. It would thus be instructive to conduct explicit psychophysical tests of taste sensitivity in rats chronically treated with SEMA and likewise test such animals with behavioral approaches designed to assess taste-related motivation and affect, such as the oromotor/somatic taste reactivity paradigm, the progressive ratio task, and the brief access taste test.

In general, GLP-1 analogs are thought to augment satiation processes (eg [14, 19, 85–88],) which makes our results showing that SEMA-treated animals increased energy intake during 2-bottle testing all the more surprising. Ghidewon and colleagues [14] found that acute administration of SEMA (10 nmol/kg, SC) additively decreased food intake when administered in combination with a mixed-nutrient preload (Ensure) to 18-hour fasted rats during the first 60 minutes after food availability. Although semaglutide administration (10 nmol/kg, SC) alone did not affect activity in agouti-related peptide (AgRP) neurons, it enhanced the inhibition of these cells caused by cholecystokinin (CCK, 30 µg/kg, IP) in food restricted mice. These findings support the view that SEMA enhances naturally occurring satiation signals [14] and our finding that SEMA rats reduce chow meal size during dose escalation further supports this mechanism, at least during early SEMA treatment. Therefore, a simple explanation for the overconsumption we observed during preference testing could stem from the suggestion that liquid energy sources are less satiating than solid ones [89]. For example, DiMeglio and Mattes found that humans consuming 450 kcal of jellybeans or soft drinks nearly perfectly compensated for their jelly bean intake but failed to compensate for the beverage calories, leading to excess intake [90] and suggesting liquids less effectively induce satiation. In a case where consumption does not strongly produce satiation, the effects of SEMA could be

Table 5. Experiment 2 body composition statistics

Measure	Treatment	Pre–Post	Interaction
Fat mass (%)	F (1,14) = 4.477, $P = .053$	F (1,14) = 0.014, $P = .908$	**F (1,14) = 35.976, $P < .001$**
Lean mass (%)	F (1,14) = 4.146, $P = .061$	F (1,14) = 0.007, $P = .937$	**F (1,14) = 27.258, $P < .001$**
Fat mass (g)	**F (1,14) = 6.716, $P = .021$**	F (1,14) = 2.001, $P = .179$	**F (1,14) = 38.208, $P < .001$**
Lean mass (g)	F (1,14) = 0.507, $P = .488$	**F (1,14) = 49.945, $P < .001$**	**F (1,14) = 27.813, $P < .001$**

P-values ≤.05 are bolded.

severely impaired. Consistent with this perspective, the extended sucrose meal duration and effects on meal size suggest reduced satiating potency of 4% sucrose in SEMA rats.

However, it is possible that the enhanced satiation uncovered by Ghidewon et al does not explain our results since they employed acute SEMA dosing and our preference testing occurred after prolonged treatment. In agreement with human findings, their acute dosing paradigm produced malaise in rats [14]. Thus, malaise may be a factor in the SEMA-enhanced satiation Ghidewon et al observed and limit the generalizability of their findings to studies occurring after long-term SEMA treatment. In fact, our finding that the chow meal size of SEMA rats became similar to VEH rats during dose maintenance suggests broad enhancement of satiation may be a transient effect of SEMA associated with early dosing.

Other physical properties differ between the liquid and solid stimuli that could have contributed to the results of this study. In particular, the energy density of liquids tends to be lower than solid stimuli. Indeed, in our study, as intake of liquid kcal increased, SEMA and VEH rats reduced intake of chow, which has more than double the energy-density of our liquid taste stimuli, and the effect was greater in SEMA rats. The extended sucrose meal duration and effects on meal size in Experiment 2 support the suggestion that there is a difference in the satiation response to lower concentration sucrose solutions vs higher concentrations compared to VEH-treated rats. Future investigations should aim to determine if our effects were driven by energy density, the form of the food, and/or some other mechanism.

Extended Weight Recovery Supports Evidence that SEMA Promotes Fat Loss

When SEMA treatment ceased at the conclusion of preference testing in Experiment 1, energy intake of SEMA treated rats exceeded BASE by 10% within 3 days and ultimately remained above BASE for about 5 weeks. In Experiment 2, the provision of sucrose during the postdrug period caused VEH and SEMA rats to have elevated energy intake, which did not differ between the groups after week 3. In Experiment 1, without sucrose available, it took approximately 8 weeks after SEMA treatment stopped for SEMA and VEH rats to no longer differ in body weight. With sucrose available, SEMA rats had body weight lower than VEH rats for only about 2 weeks. While the rapid and sustained increases in energy intake in both experiments indicate SEMA suppression of intake is quickly eliminated after drug cessation, the contrasting time for recovery of body weight between Experiment 1 and Experiment 2 suggests that the extended durability of SEMA-induced weight loss may depend to some extent on the availability and consumption of palatable food or beverage choices.

Some SEMA research has found that this GLP-1 analog specifically promotes fat loss, preserving lean mass [19], ultimately resulting in a greater lean mass:fat mass ratio, and, after adjustment for lean mass, prevents SEMA-induced changes in energy expenditure compared to controls [19, 36]. In Experiment 2, our EchoMRI™ analysis agrees with these earlier reports.

Limitations of the Present Study

One limitation of the present report is our inability to statistically assess sex differences in Experiment 1 due to a lack of power. There is existing literature suggesting sex differences exist in the effects of GLP-1 or its analogs (eg, references [71, 77, 79, 91]). For the most part and speaking relatively, the changes in food intake and exceptional preference for sucrose of our male and female rats were similar during dose escalation and dose maintenance. Comparison of body weight changes finds similar results in male and female SEMA rats relative to VEH throughout the present study. Although the energy intake of SEMA to VEH rats by sex becomes somewhat less similar during sucrose testing and the postdrug period, both males and females in Experiment 1 displayed similar qualitative changes in their responses to the treatment. However, larger sample sizes could reveal differences in the magnitude of the response in males vs females.

Another potential limitation of this work is that our results may be specific to the body composition of our rats at the start of the experiments. Apart from the 20-day palatable diet exposure of rats in Experiment 1, previously mentioned, animals in both experiments were maintained on chow. We purposely chose not to maintain rats with a high energy diet to avoid the interpretive complexity of dissociating effects due to body composition from those due to exposure to a diet high in fat. Still, the male rats in both experiments were quite heavy (over 600 g; see Fig. 3 caption for specific weight ranges in Experiment 1 and "Experiment 2: Subjects" for Experiment 2) and obesity in the rat is not defined. Although poor diet quality has been associated with the development of obesity in humans, a recent study employing covert, objective measurements found differences in total energy intake but no difference in relative macronutrient intake between people with class 3 obesity (BMI > 40) and people with moderate overweight (average BMI 27) [92]. A second factor contributing to obesity in humans is lack of physical activity, and a sedentary lifestyle was well-modeled in our rats. Even so, it would be instructive to investigate the results of SEMA treatment in rats that had been maintained on other diet models.

Conclusions

The findings in the experiments presented here expand considerably on what is known about the response to SEMA in preclinical models with potentially increased translatability since our model employed a clinically relevant dose escalation protocol. We found that SEMA treatment yielded anticipated

reductions in energy intake and body weight in 2 cohorts of chow-maintained rats. Consistent with other findings, SEMA administration promoted fat loss while maintaining lean mass. Along with adding confirmatory results to existing findings, our meal pattern analyses, which spanned the entirety of 42 days of SEMA treatment, revealed that meal size serves as the mediator of SEMA-induced changes in chow intake suggesting that the drug acts on neural processes responsible for termination, as opposed to initiation, of meals. We also report a persistent reduction in the rate of chow consumption, implying that there is an effect of SEMA on the motivation of rats to consume their standard diet. Contrary to expectations based on earlier reports, SEMA rats in both experiments consumed, in some cases, more than 2.5 times the volume of low- to mid-range sucrose solutions compared to VEH treated rats. Further investigation is required to determine if the apparent stimulus-dependent results we obtained during preference testing are driven by energy density of the stimuli, liquid vs solid foods, effects of GLP-1R activation on taste responding, changes in reward valuation, or some other factor. In both experiments, energy intake increased rapidly once treatment ceased, and the durability of SEMA-induced weight loss seems to depend on the post-treatment diet. The findings presented here suggest that the effects of SEMA on eating and drinking and the long-term maintenance of body weight loss depend on the caloric sources available.

Acknowledgments

We would like to acknowledge that NNC0113-0217 (semaglutide) was provided by Novo Nordisk Compound Sharing. We also thank Florida State University Laboratory Animal Resources for their exceptional support, the Florida State University Psychology Department Electronics Shop for maintaining our testing equipment, and Victor Oliveira for assistance with data collection.

Funding

This research was funded by NIH R01-DK106112 to A.C.S. and the Florida State University Chemosensory Training Program (NIH T32 DC000044) to C.R.C. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Support was also provided by Florida State University Bridge Funds (042617) to A.C.S.

Author Contributions

C.R.C., G.D.B., C.W.L.R., and A.C.S. conceived and designed research; C.R.C., G.D.B., V.N., H.B., and B.K. performed experiments; C.R.C., G.D.B., and A.C.S. analyzed data; C.R.C. and A.C.S. interpreted results of experiments; C.R.C. prepared figures; C.R.C. drafted manuscript; C.R.C., G.D.B., V.N., H.B., B.K., C.W.L.R., and A.C.S. edited and revised manuscript; C.R.C., G.D.B., V.N., H.B., B.K., C.W.L.R., and A.C.S. approved the final version of manuscript.

Disclosures

C.l.R. reports grants from the Irish Research Council, Science Foundation Ireland, Anabio, and the Health Research Board. He serves on advisory boards of Novo Nordisk, Herbalife, GI Dynamics, Eli Lilly, Johnson & Johnson, Gila Therapeutics, and Boehringer Ingelheim. C.l.R. is a member of the Irish Society for Nutrition and Metabolism outside the area of work commented on here. He was the chief medical officer and director of the Medical Device Division of Keyron in 2011. Both are unremunerated positions. ClR was a previous investor in Keyron, which develops endoscopically implantable medical devices intended to mimic the surgical procedures of sleeve gastrectomy and gastric bypass. The product has only been tested in rodents and none of Keyron's products are currently licensed. They do not have any contracts with other companies to put their products into clinical practice. No patients have been included in any of Keyron's studies and they are not listed on the stock market. C.l.R. was gifted stock holdings in September 2021 and divested all stock holdings in Keyron in September 2021. He continues to provide scientific advice to Keyron for no remuneration. A.C.S. is a member of the scientific advisory board of Gila Therapeutics. No other authors have any disclosures.

Data Availability

Some or all datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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