Neuroscience Applied 3 (2024) 103925

Contents lists available at ScienceDirect

Neuroscience Applied

journal homepage: www.journals.elsevier.com/neuroscience-applied

Research Articles

GLP-1 receptor agonist semaglutide reduces appetite while increasing dopamine reward signaling

Karlijn L. Kooij [a,b], Derek IJsbrand Koster [a], Emma Eeltink [a], Mieneke Luijendijk [a], Lisa Drost [a], Fabien Ducrocq [a], Roger A.H. Adan [a,b,c,*]

[a] UMC Brain Center, Department of Translational Neuroscience, University Medical Center Utrecht, Utrecht University, the Netherlands
[b] Altrecht Eating Disorders Rintveld, Zeist, the Netherlands
[c] Department of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Sweden

ARTICLE INFO

Handling Editor: Prof. A. Meyer-Lindenberg

Keywords:
Semaglutide
GLP-1 analog
Dopamine
Ventral tegmental area
Reward

ABSTRACT

Semaglutide, a glucagon-like peptide-1 receptor agonist, is an effective drug reducing body weight and decreasing motivation for palatable food. The mechanisms underlying its effects on food reward remain unclear. We aimed to determine the impact of semaglutide on food reward collection and dopamine-neuron activity in the ventral tegmental area (VTA) upon exposure to a cue-induced sucrose delivery task.

Pitx3-cre mice were injected with cre-dependent GCaMP6s virus into the VTA, to measure the activity of dopaminergic neurons in the VTA using *in vivo* fiber photometry. Mice were trained on a Pavlovian sucrose conditioning paradigm in which a 5-s cue signaled a 20% sucrose reward. Upon stable performance, semaglutide or vehicle was intraperitoneally injected during the task.

1 mg/kg semaglutide reduced the number of collected rewards and licks during the task. Semaglutide increased VTA dopamine neuron activity during sucrose collection but not during the cue. Lower doses of semaglutide (0.1 and 0.3 mg/kg) reduced chow intake but not sucrose intake nor VTA dopamine activity in the task.

Semaglutide reduces appetite but increases VTA dopamine signaling during reward collection. Semaglutide does not influence dopamine signaling during the presentation of food cues.

1. Introduction

Obesity is a global health issue and there remains a need for improved treatments. Recent promising treatment options are glucagon-like peptide 1 receptor (GLP-1R) agonists, which were initially developed for type 2 diabetes. Currently, two GLP-1R agonists, liraglutide and semaglutide, are approved for obesity treatment. Semaglutide is optimized for a longer half-life in humans (160 vs. 12 h) and full dipeptidyl peptidase-4 stability (Lau et al., 2015). In randomized clinical trials, semaglutide-treated patients with obesity lost twice as much weight as other GLP-1R agonists (Ahmann et al., 2018; O'Neil et al., 2018; Pratley et al., 2018).

GLP-1 is a hormone produced by intestinal enteroendocrine L-cells and a subpopulation of hindbrain nucleus tractus solitarious (NTS) neurons (Alvarez et al., 1996; Holst, 2007). L-cell GLP-1 is primarily released after a meal, correlating with meal size, and quickly metabolized by endopeptidases (Hayes et al., 2014; Holst, 2007). GLP-1 receptors (GLP-1Rs) are found in both the periphery and the central nervous system (CNS), and GLP-1 binding elicits diverse physiological and behavioral effects depending on the site of action (Hayes et al., 2014). In the periphery, GLP-1 enhances glucose-dependent insulin secretion, reduces gastric emptying and glucagon secretion (Gutniak et al., 1992; Matsuyama et al., 1988; Schirra et al., 1997). In the CNS, GLP-1R agonists suppress appetite via the hypothalamus (López-Ferreras et al., 2018; McMahon and Wellman, 1998) and the NTS (Hayes et al., 2009) to maintain energy balance.

GLP-1R agonists were found to alter reward-related behaviors. Semaglutide reduced food cravings and the preference for fatty, energy-dense foods in participants with obesity (Blundell et al., 2017; Masaki et al., 2022). In rats, subcutaneously injected semaglutide reduced motivation for sucrose in a progressive ratio task (Ghidewon et al., 2022) and diminished chocolate intake (Gabery et al., 2020). Similar to

* Corresponding author. UMC Brain Center, Department of Translational Neuroscience, University Medical Center Utrecht, Heidelberglaan 100, 3584, CG Utrecht University, the Netherlands.
E-mail address: r.a.h.adan@umcutrecht.nl (R.A.H. Adan).

https://doi.org/10.1016/j.nsa.2023.103925
Received 30 September 2023; Received in revised form 9 November 2023; Accepted 16 November 2023
Available online 22 November 2023

semaglutide, other peripherally administered GLP-1R agonists, exendin-4 and liraglutide, decreased the intake of alcohol, cocaine and, food and reduced motivation for these substances in a progressive ratio task (Dickson et al., 2012; Egecioglu et al., 2013a, 2013b; Sørensen et al., 2015; Vallöf et al., 2016). Additionally, these agonists abolished alcohol and nicotine-mediated conditioned place preference (Egecioglu et al., 2013a, 2013b; Vallöf et al., 2016).

The GLP1R agonist induced alterations in reward-related behaviors are hypothesized to be mediated via mesolimbic areas, including the ventral tegmental area (VTA). The VTA is a major source of brain dopamine to projections as the nucleus accumbens (NAc) and prefrontal cortex, is involved in food motivation and cue processing and responds to intake of rewarding substances (Morales and Margolis, 2017; Salamone et al., 2016; Schultz et al., 1997). The peripheral GLP-1 agonists exendin-4 and liraglutide reduced extracellular dopamine levels in the NAc after alcohol, nicotine and cocaine intake, measured per 20 min (Egecioglu et al., 2013a, 2013b; Sørensen et al., 2015; Vallöf et al., 2016). C-fos studies showed that peripherally injected semaglutide activated brain areas, such as the hypothalamus, NTS and the lateral septum, which are connected to VTA signaling (Gabery et al., 2020; Hansen et al., 2021). Additionally, exendin-4 delivered in the brains' ventricle reduced VTA dopamine neuron responses during a food cue (Konanur et al., 2020). Moreover, semaglutide suppressed alcohol-induced elevation of dopamine levels in the NAc (Aranäs et al., 2023). However, there is a gap in knowledge how GLP1-agonist-dependent suppression of appetite is linked to DA neuronal activity.

In order to address this we determined how peripheral semaglutide impacts VTA dopamine signaling on a second-to-second time scale during food reward collection. This is important to resolve as it clarifies which aspect of food reward seeking is affected by the drug. We determined whether peripheral semaglutide affected reward collection and VTA dopamine-neuron activity related to food-predictive cues and sucrose rewards in mice. We used a Pavlovian sucrose conditioning task in combination with *in vivo* fiber photometry and semaglutide intervention (0.1, 0.3 and 1 mg/kg) in a within-subject design. Semaglutide is hypothesized to decrease reward collection and VTA DA activity during cue presentation and reward consumption. Semaglutide was injected peripherally to mimic its clinical application.

2. Materials and methods

2.1. Animals

29 adult male pitx3-cre$^{+/-}$ mice (4 months old, bred in-house) (Smidt et al., 2012) on a C57BL/6J background, and 14 adult male C57BL/6J mice (3.5 months old, bred in-house, originated from Jackson laboratory strain 664) were used. The 14 C57BL/6J were used for the food intake experiments and were co-housed in pairs with ad libitum chow access in Type II cages (Tecniplast). The photometry mice were individually housed after surgery in Type II cages. After surgery recovery, these mice were food restricted to 90–95% of their ad libitum body weight (CRM(E), SDS diets). The rooms were temperature (21.5 ± 1.0 °C) and humidity (57.8% ± 3.9%) controlled under a reversed day-night cycle (lights off at 8.00 photometry & 12.30 for food intake). Non-invasive handling was used to move and connect the mice. The experiments were approved by the Dutch Central Animals Testing Committee (AVD1150020198686), considering mild discomfort for the food intake experiment and moderate discomfort for the photometry mice based on the surgery, individual housing and food restriction. Five mice in the photometry experiment did not survive the surgery. Further, no unexpected discomfort occurred.

2.2. Food intake

The food intake experiments were conducted in a cross-over design, with four experimental conditions (0.1, 0.3, 1 mg/kg semaglutide and vehicle) tested biweekly. The mice were twice habituated to the injection procedure and their test cage (type IIL cage 365 x 207 × 140 mm, enriched with tissues). On an experimental days, the chow was removed from the home cages during the light phase, 5.5 h before dark phase onset. Within the first hour of the dark phase, the animals were weighed and intraperitoneally injected (i.p.) (0.1 ml/10 g BW) with semaglutide or vehicle. The mice were placed solitary in a test cage with a full rack of chow. The food intake was monitored hourly until 4 h after injection, by weighing (Sartorius, CP3202 S) the cage lid including food rack without water bottle. After, the mice were placed in their home cage with their cage mate.

2.3. Surgery

For the photometry experiments, a surgery was performed to enable the photometry measurement in the VTA. The mice were anesthetized with ketamine (75 mg/kg, Narketan 10), dexmedetomidine (1 mg/kg, Dexdomitor) and lidocaine (7 mg/kg, B. Braun). When anesthetized, we shaved the skin and made an incision above the skull, whereafter phosphoric acid (ultra-etch 35%, Ultradent Products) was applied under stereotaxic apparatus (UNO BV). Bilateral craniotomies were made above the VTA to inject 0.5 µl of rAAV5-Syn-FLEX-GCaMP6s-WPRE-SV40 (5 * 10^8 genetic copies, Addgene) virus at coordinates AP -3.2 mm, ML ±1.5 mm with a 15° angle and DV -4.8. Bilateral optical fibers (ø400 µm, Thorlabs) were inserted at coordinates AP -3.2 mm, ML ±1.5 mm with a 15° angle and DV -4.7 and fixated with Superbond glue (Sun Medical Co.) and dental cement (Fuji PLUS-capsules, G.C. corporation). The anesthesia was antagonized with atipamezole (2.5 mg/kg, s.c., Alzane, Syva). Thereafter, the rats received saline (1 ml, s.c.) and carprofen (5 mg/kg, s.c. & 0.027 mg/ml in drinking water, Carprofelican, Dechra) to recover for seven days.

2.4. Behavioral task

fter one week of recovery, the mice were trained on a Pavlovian sucrose conditioning task in an operant cage (L 21.6, W 18.0 and H 12.7 cm, ENV-307W, Med-Associates (MA)), three to five times a week. The mice were thought that after a 5-s tone (65 dB, 4500 Hz, ENV-223AM, MA) and light (55 lux, warm white light, ENV-315, MA) cue, they could collect an 8 µl sucrose reward (20%, Van Gilse, Oud Gastel, The Netherlands) in the receptacle (ENV-303RMA-3, MA). To prevent that the sucrose was pick-up at another moment, the sucrose was only delivered if the mice licked the receptacle within 10 s after cue onset. The mice received 80 cues (trials) on a training day with an inter-trial interval of 35, 45, or 55 s. The task responses were recorded with the Med-PC software (version 4.0). During trainings the mice were connected to a habituation cable.

2.5. Experimental design and pharmacology

Each mouse was measured with the fiber photometry system twice bilaterally during the training phase to determine the hemisphere with the strongest signal. This hemisphere was chosen for the semaglutide recordings. Eventually 10 mice were recorded on the right hemisphere and 13 mice on the left hemisphere.

After three to four weeks training the mice reach a stable training performance and are ready for the intervention phase of the experiment. As the mice keep on performing stable on the task, interventions washout and the photometry signal remains stable, the mice could be used in

different experiments, reducing the number of experimental animals needed. This resulted in 13, 28 and 31 weeks of training for the mice, aged 8.5, 12 and 13 months, in the 1, 0.3 and 0.1 mg/kg experiment. For the semaglutide experiment the mice were measured weekly with fiber photometry during the sucrose conditioning task. On a measurement day, the mice performed 20 baseline trials without intervention, whereafter they received a semaglutide (1 mg/kg, Novo Nordisk, n = 16) or vehicle i.p. injection and continued the task after 15 min for another 60 trials. Semaglutide was dissolved in 50 mM sodium phosphate, 70 mM sodium chloride and 5 mM sodium acetate (pH 7.3). After the task, home cage chow intake was monitored for 2 h. The conditions were blinded for the experimenter and offed in a cross-over design. The order was determined pseudo-randomized with Excel. The experiment included two measurements, with at least two washout training sessions in between. Four mice needed an extra measurement one week later due to an inaccurate amount injected or a technical failure of the photometry system. In a separate batch of mice (n = 7), we tested lower doses of semaglutide in separate experiments. The conditions were similar as before, except semaglutide (0.1 and 0.3 mg/kg) or vehicle was injected 15 min before the sucrose conditioning task, without the 20 trials of baseline before.

2.6. Fiber photometry

We used a 2-site photometry system of Doric lenses to measure the GCaMP6s′ green fluorescent protein (GFP) emitted light as an indirect measurement of neuronal activity together with an isobestic channel to control for non-GFP fluoresces (e.g., movement). Blue (465 nm (nm), 60 ± 5 μw) and purple (405 nm, 30 ± 3 μw) light emitting diode (LED) light was sent via a mini cube and patch cord (ø 400 μm, Doric) to the mouse. The emitted light was sent to a photodetector (AC mode, Newport) and signaled to the console. The Doric studio software controlled the photometry system (version 5.4.1.1) and recorded the photo- and behavioral inputs with 227 samples/s (decimation of 50). We analyzed the photometry data with custom-made Python scripts (version 3.7.4). First, we removed the autofluorescence and filtered (butter low pass filter, 6 Hz) and smoothened (50 samples) the light signals. Then the ΔF/F signal was calculated around the cue and reward collection for each trial. We included only reward trials in which the reward was collected. We averaged the ΔF/F signal during the cue and around reward collection (onset + 5 s) for both channels. As there were no group differences in the isobestic channel, we only plotted the GCaMP channel.

2.7. Histological verification

After the behavioral experiments, the mice were perfused and the brains were stained to determine fiber location and virus expression. We anesthetized the mice with pentobarbital (0.1 ml i.p. of 200 mg/ml, Euthanimal 20%, Alfasan), after which they were perfused with PBS and 4% paraformaldehyde (PFA) in PBS. We dissected the brains and let them post-fixate overnight, followed by incubation in 30% sucrose and freezer storage (−20 °C). The brains were sectioned (20 μm) and captured on a microscope slide (SuperFrost Plus, VWR). The slices were stained for GFP (component of GCaMP construct), tyrosine hydroxylase (TH, a marker of dopaminergic neurons) and DAPI (a nuclear counterstaining) using a standardized protocol. The staining included 1-h blocking, overnight primary antibody incubation (1:500 rabbit-αTH, AB152, Millipore; 1:500 chicken-αGFP, GFP-1020, Aves), 2 h secondary antibody incubation (1:500 goat anti-rabbit 568, ab175471 and goat anti-chicken 488, ab150169, Abcam) and 20 min of DAPI (0.5 mg/ml). We imaged the sections under the EPI fluorescent microscope (Axio imager m2, Zeiss).

2.8. Statistical analysis

The statistical tests were performed in SPSS (version 27) and GraphPad Prism (version 9.5.0) with the individual mouse as the experimental unit. Behavioral and photometry data were analyzed with a paired *t*-test or a Wilcoxon signed-rank test, dependent on the distribution of the data. The data within a session were analyzed with a two-way repeated measures ANOVA. The responder analysis was analyzed with a mixed model ANOVA. In all Figs, the statistical outcome is as follows *p < 0.05, **p < 0.01, ***p < 0.001.

3. Results

3.1. Measuring VTA dopamine activity during the sucrose conditioning task in vivo

We expressed a cre-dependent GCaMP6s virus in the VTA of pitx3-cre mice to indirectly measure the activity of the dopamine cells (Fig. 1A) and verified properly targeted expression with staining (Fig. 1B and C). A fiber was implanted above the injection site, and all fiber tips were located in or above the VTA (Fig. 1D). When the mice recovered from surgery, they were trained on the task where a 5 s tone and light cue predicted the availability of a 20% sucrose reward (Fig. 1E). Trained mice lick mainly when a reward is available (Fig. 1F) and show increased signal (ΔF/F) from the 465 nm (GCaMP) channel during food cue exposure and reward retrieval (Fig. 1G).

3.2. Semaglutide increased VTA dopaminergic activity during reward collection

Next, we studied if 1 mg/kg semaglutide influenced VTA dopamine signaling and food reward seeking. In the first 20 trials, we measured baseline task performance without intervention to assess responses over weeks. The average lick and photometry responses after cue and reward were not different between the weeks (Fig. 1H–J), as well as the number of rewards collected and latency at the food cup (data not shown). After the baseline measurement, we i.p. injected semaglutide (1 mg/kg) or vehicle and continued the sucrose conditioning task 15 min after administration (Fig. 2A). When semaglutide was injected, the mice decreased the number of obtained rewards (Fig. 2B) [$Z = -2.327$, $p = 0.020$], although the latency to enter the food port during reward collection was similar between conditions (Fig. 2C) [$t_{(15)} = -1.588$, $p = 0.133$]. Semaglutide lowered the total number of licks (Fig. 2D and E) [$t_{(15)} = 2.868$, $p = 0.012$], during the food predictive cue (Fig. 2F) [$t_{(15)} = 2.969$, $p = 0.001$], during reward collection (Fig. 2G) [$Z = 2.275$, $p = 0.023$], and in between trials (Fig. 2I) [$t_{(15)} = 2.143$, $p = 0.049$]. The number of licks in the first bout during reward collection (a measure of liking (Johnson, 2018)) was not altered by the semaglutide injection (Fig. 2H) [$t_{(15)} = -0.650$, $p = 0.525$]. Taken together, semaglutide reduced the total number of rewards that were collected and the number of licks during and in between reward collection was reduced.

Mice that received the semaglutide treatment had comparable VTA dopamine neuron activity during the food predictive cue as after a vehicle injection (Fig. 2J and K) [**$t(15) = -0.377$, $p=0.712$**]. However, semaglutide increased the dopamine activity during reward collection from an average ΔF/F of 0.595 ± 1.960 to 1.158 ± 2.037 (Fig. 2L and M) [average $t_{(15)} = 2.794$, $p = 0.014$; peak **$Z = -2.947$, $p = 0.003$**]. After the task, chow intake was monitored in the home cage. After semaglutide, the mice decreased their chow intake (Fig. 2N) [$t_{(15)} = 6.45$, $p < 0.001$].

Thus, 1 mg/kg of peripheral semaglutide reduced sucrose licking and chow intake. Interestingly, the dopamine activity during sucrose collection was increased after a semaglutide injection.

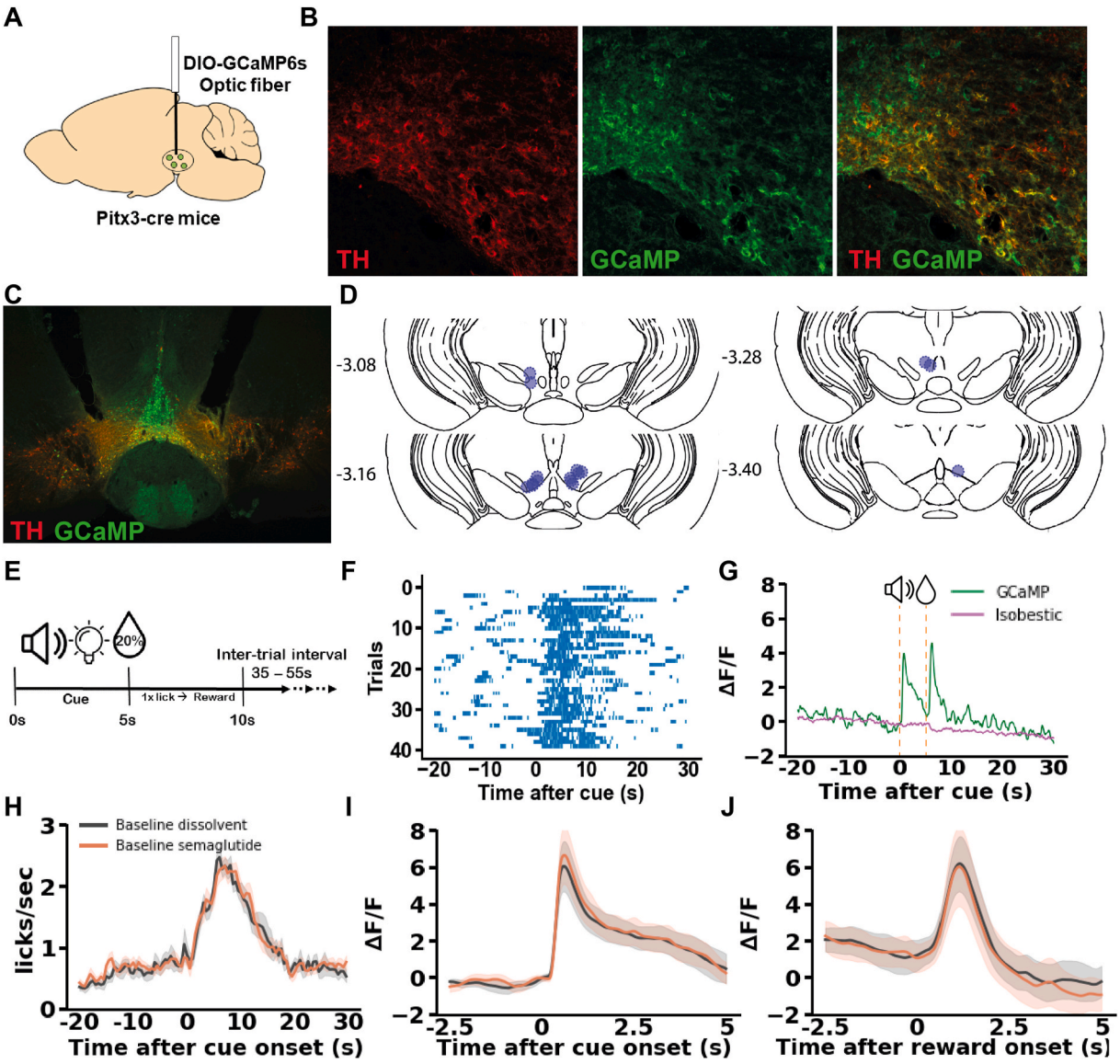

Fig. 1. Measuring VTA dopamine activity during the sucrose conditioning task. A schematic diagram of the targeted VTA dopamine neurons (green dots) with implanted fiber (**A**). An illustration of histological verification of co-localization of the GCaMP on the dopamine neurons (TH staining) (**B**). In all recorded animals, the fiber placement and virus expression (**C**) was determined. All recorded fiber tip locations of the photometry experiments are illustrated in (**D**). in the experimental task (**E**) the mice learned to lick after the offset of the cue (5 s) to obtain a sucrose reward. The licking pattern over trials is illustrated in (**F**). With simultaneous photometry recordings (**G** typical example of the average signal of a session) we observed a response to a food predictive cue (0–5 s) and sucrose reward delivery (±5–7 s). Repeating the task without interventions (baseline before semaglutide or control treatment) gave similar lick and photometry responses during cue and reward (**H-J**). The lines represent the mean in line graphs, and the adjacent transparent color shows the SEM. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Responses in the sucrose conditioning task within a session

As semaglutide is known to enhance satiation and reduce meal size, we reasoned that it impacted later trials more than early trials. Therefore in a secondary analysis, we studied the evolution of behavioral and photometry responses over the session. We calculated averages of participated trials every 20 trials, thus studying three blocks.

During a session, the number of rewards collected decreased over trials, most strongly in the semaglutide treated group (Fig. 3A) [trials $F_{(2,30)} = 4.574$, $p = 0.018$; trials x treatment: $F_{(2,30)} = 7.644$, $p = 0.002$]. The reduction in rewards obtained was most prominent in the last 20 trials [40–60 trial $p_{corrected} = 0.025$].The latencies to pick up the rewards increased during the session (Fig. 3B), although without significant differences after semaglutide [trials $F_{(1.350,18.903)} = 18.226$, $p < 0.001$; trials x treatment $F_{(2,28)} = 3.176$ $p = 0.057$].

The general photometry demodulated signal (a measure of bleaching), decreased over trials but was not affected by semaglutide (Fig. 3C) [trials $F_{(1.028,\ 15.422)} = 91.135$, $p < 0.001$; trials x treatment $F_{(1.151,\ 17.272)} = 0.339$, $p = 0.599$]. In the photometry signal during the cue, we observed a decreased signal over trials which was not affected by semaglutide treatment (Fig. 3D) [trials $F_{(1.350,20.250)} = 13.024$, $p < 0.001$; treatment $F_{(1,15)} = 0.108$, $p = 0.747$; trials x treatment $F_{(2,30)} = 0.821$, $p = 0.450$]. In contrast, the photometry signal during reward collection increased after a semaglutide injection compared to vehicle without change over trials (Fig. 3E) [trials $F_{(1.343,17.463)} = 0.579$, $p = 0.505$; treatment $F_{(1,13)} = 5.609$, $p = 0.034$; trials x treatment $F_{(1.431,\ 18.605)} = 1.676$, $p = 0.215$]. Additionally, the photometry signal during reward collection was also averaged per 20 rewards collected (Fig. 3F). Here, again a main effect of the semaglutide treatment was found, that was not affected by previous rewards [trials $F_{(2,16)} = 0.961$, $p = 0.403$; trials x

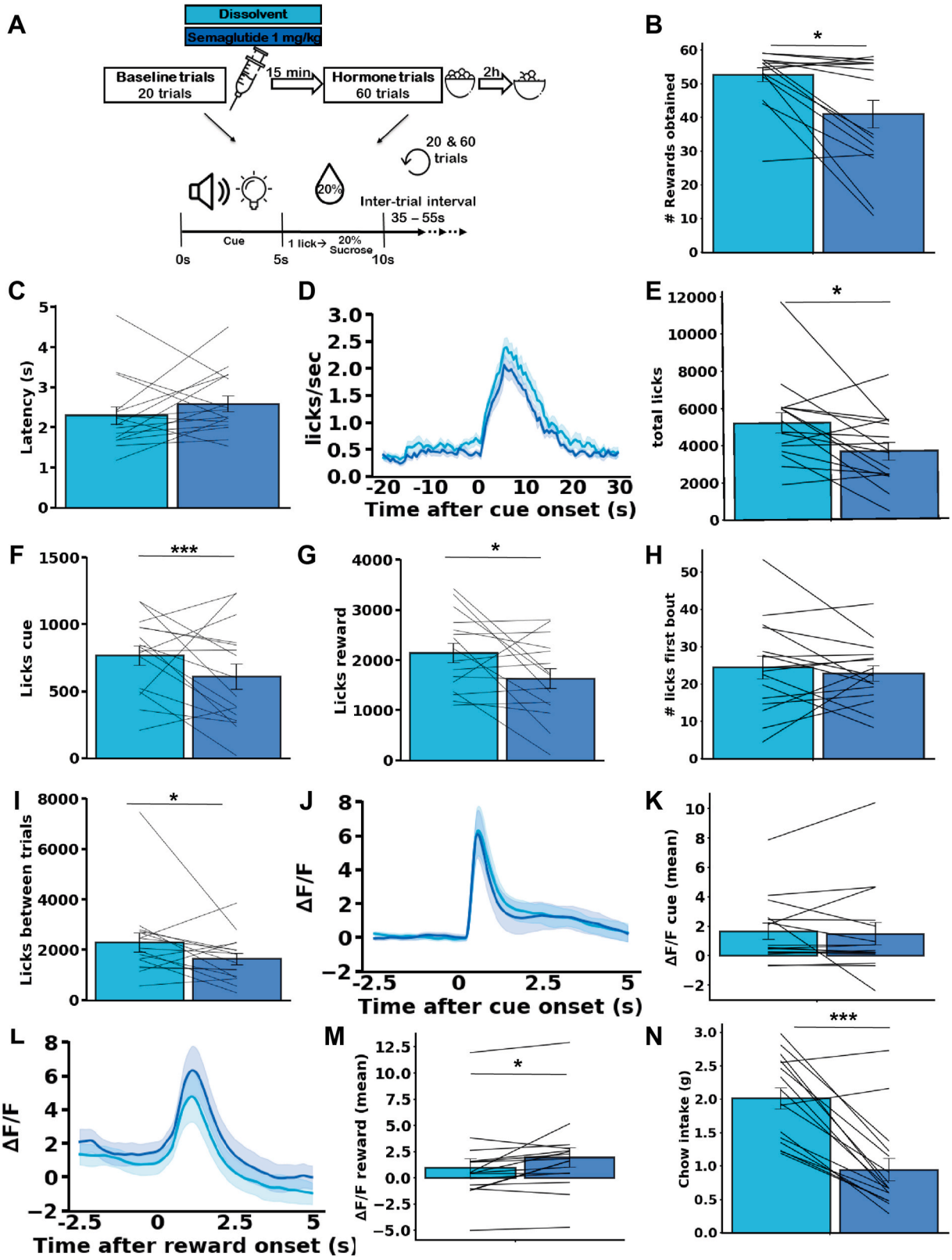

Fig. 2. 1 mg/kg semaglutide decreased sucrose and chow intake, while VTA dopamine response was increased during reward consumption.
An illustration of the experiment (**A**) in which we assessed baseline responses to the sucrose conditioning task, whereafter we injected 1 mg/kg semaglutide or vehicle and monitored their behavior and photometry responses during the sucrose conditioning task. The task performance included the number of sucrose rewards collected (**B**), the average latency of food cup approach during the cue (**C**) and the total number of licks during the task (**E**). The average licks of the mice per condition over time (**D**) and quantified during the cue (**F**), reward (**G**), in the first bout of reward collection (**H**) and in between trials (**I**). The average photometry signals during cue and reward are plotted over time (**J**, **L**) and quantified (**K, M**). After the task, we monitored the first 2 h of home cage chow intake (N). In bar graphs, bars represent means, and lines indicate individual values. The lines represent the mean in line graphs, and the adjacent transparent color shows the SEM. *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

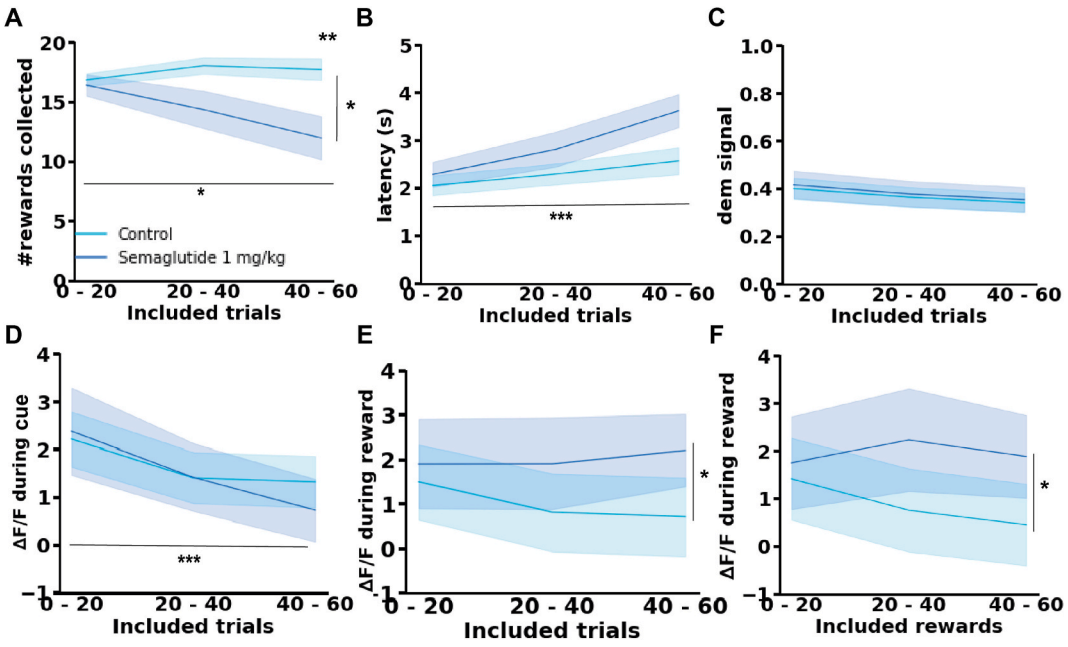

Fig. 3. 1 mg/kg semaglutide reduced motivation for sucrose after 20 trials, while the dopamine activity during reward collection was increased during the whole session.

We calculated a 20 trials average for participated trials in the session (**A**) and the latencies to retrieve the rewards (**B**). The average photometry signal, demodulated (dem) signal, was plotted per 20 trials (**C**). The 20-trial average dopamine activity during the cues (**D**) was plotted. The photometry signal during reward collection was averaged per 20 trials (**E**) and per 20 rewards collected (**F**). The lines represent the mean and the adjacent transparent color shows the SEM. *p < 0.05, **p < 0.01, ***p < 0.001. *'s on the right mean main effect of condition. *'s below means main effect of trial block, *'s above the lines mean differences between conditions at the specific trial block. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

treatment $F_{(2,16)} = 1.482$, $p = 0.257$; treatment $F_{(1,8)} = 6.874$, $p = 0.031$].

Taken together, semaglutide decreased motivation for sucrose after 40 trials, while it enhanced dopamine neuronal activity during reward collection which remained high throughout the session.

3.4. Responder vs. non-responders

A subset of the mice reacted behaviorally stronger to the semaglutide treatment, most evident in the number of rewards obtained during the task (Fig. 2B). As food seeking behaviour and VTA activity are causally related (Chang et al., 2016; Steinberg et al., 2013; Van Zessen et al., 2021), it could be that strong responsive animals drove the semaglutide-induced differences in licks and reward photometry signal. A mouse was allocated to the non-responder group when semaglutide reduced the number of collected rewards by less than 5 rewards. A mouse was allocated to the responder group when semaglutide reduced the number of reward collected by more than 5. Most mice in the responder group collected at least 20 rewards less after semaglutide injection.

Statistical analysis confirmed differences in the number of rewards collected between the responders and non-responders (Fig. 4A) [treatment x responder: $F_{(1, 14)} = 34.540$, $p < 0.001$]. Further analysis that focused on the interaction between (non-)responders and treatment did not reveal differences in the latency at the food cup (Fig. 4B) [$F_{(1, 14)} = 2.152$, $p = 0.165$]. The licks during cue and reward were specifically reduced in the responder group after semaglutide treatment (Fig. 4D and E) [licks cue $F_{(1, 14)} = 11.270$, $p = 0.005$; licks reward $F_{(1, 14)} = 7.688$, $p = 0.015$]. The licks outside the cue and reward period were not differently affected between the responder groups (Fig. 4F) [$F_{(1, 14)} = 0.875$, $p = 0.365$], nor the total licks and licks in first collection bout (Fig. 4C, G) [total licks $F_{(1, 14)} = 3.673$, $p = 0.076$; bout $F_{(1, 14)} = 0.076$, $p = 0.787$].

The GCaMP activity during reward increased after semaglutide [$F_{(1, 14)} = 7.291$, $p = 0.017$], independent of the responder group (Fig. 4H) [$F_{(1, 14)} = 0.012$, $p = 0.914$]. Most mice reduced their home cage chow intake after semaglutide, also in the non-responder group. On average, there was less chow eaten in the responder group [chow responder vs non-responder group $F_{(1, 14)} = 6.735$, $p = 0.021$] without significant interaction with treatment (Fig. 4I) [$F_{(1, 14)} = 1.836$, $p = 0.197$].

We conclude that semaglutide-induced reduction in obtaining rewards also affected cue and reward-related licking, but not latency, dopamine activity during reward collection and home cage chow intake. Thus, the enhanced dopamine response during reward collection was independent of behavioral response, whereas the dopamine activity during cue was.

3.5. Lower semaglutide doses did not impact dopamine responses

We repeated the experiment by injecting lower doses of semaglutide (0.1 and 0.3 mg/kg). Here we did not observe alterations in obtained rewards (Fig. 5A and B) [0.1 mg/kg $t_{(6)} = -0.97$, $p = 0.368$; 0.3 mg/kg $t_{(5)} = -0.05$, $p = 0.964$], reward dopamine activity (Fig. 5C) [0.1 mg/kg $Z = 0.06$, $p = 0.949$; 0.3 mg/kg $t_{(5)} = 1.28$, $p = 0.256$] nor other parameters in the task (data not shown). However, the home cage chow intake was again reduced after the task (Fig. 5D) [0.1 mg/kg $t_{(6)} = 2.46$, $p = 0.049$; 0.3 mg/kg $t_{(6)} = 18.66$, $p < 0.001$]. A separate food intake experiment ensured pharmacological responsiveness also in the first hour after injection (Fig. 5E and F) [$F_{(3,39)} = 34.658$, $p < 0.001$, post hocs 0.1, 0.3 and 1 mg/kg all $p_{corrected} < 0.001$]. Lower doses of semaglutide (0.1 and 0.3 mg/kg) did not alter performance in the sucrose conditioning task nor VTA dopamine activity, while chow intake was reduced.

4. Discussion

We find that the peripheral administration of 1 mg/kg GLP-1 agonist semaglutide impacts on food reward-seeking and VTA dopamine signaling during a Pavlovian sucrose conditioning paradigm.

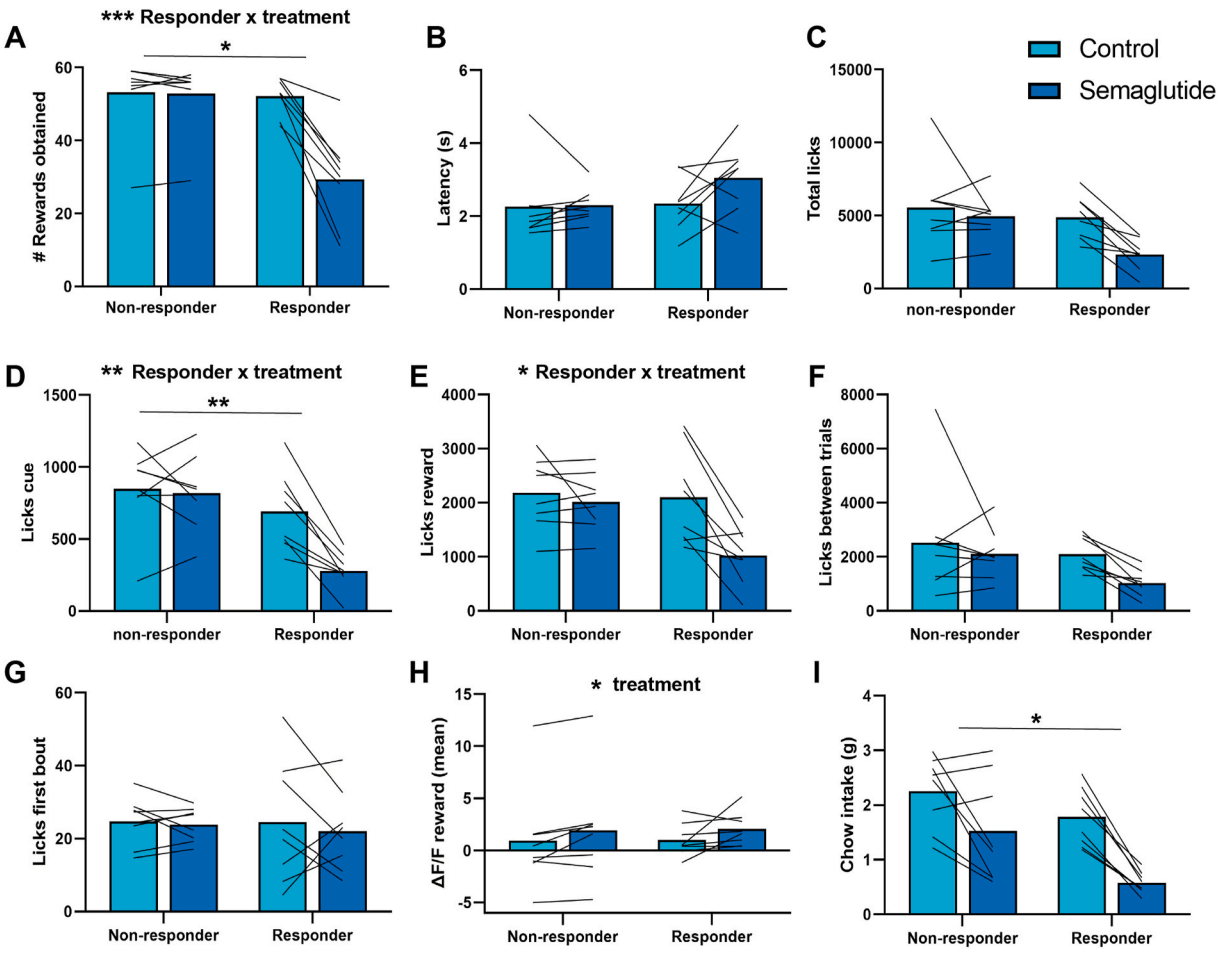

Fig. 4. Semaglutide-induced increased dopamine activity during sucrose was not affected by an altered behavioral response.
The mice were divided into responders and non-responders based on the difference in the number of rewards collected after semaglutide or control treatment. The outcomes of the sucrose conditioning task are plotted here for the responder and non-responder groups, including the number of rewards obtained (**A**), latency at the food cup (**B**), licks (**C-G**), and chow intake (**I**). Also, the photometry signal during cue reward (**H**) is plotted for the responder and non-responder groups. Bars represent means, and lines indicate individual values. *p < 0.05, **p < 0.01, ***p < 0.001. *'s responder x treatment means there was a significant interaction effect.

Semaglutide reduced the number of sucrose rewards and licks in the task. At the same time, semaglutide increased the VTA dopamine neuron activity during sucrose collection, independent of task performance as mice without drug-induced reduction in reward-seeking showed increased neuronal activity during reward consumption. In contrast, the VTA dopamine neuron activity during cue was not altered by semaglutide. We conclude that semaglutide reduced appetite and reward-seeking while it increased VTA dopamine neuron activity during reward consumption.

By investigating semaglutides' effect on a second-to-second time scale, we found it to increase VTA dopamine activity during reward collection but not during cue. This contrasts with a previous study that found decreased VTA dopamine activity during a reward-predictive cue after exendin-4 application in the ventricle (Konanur et al., 2020). Also from other studies using other GLP-1 analogs, we expected rather decreased than increased dopamine levels after semaglutide (Egecioglu et al., 2013a, 2013b Fortin and Roitman, 2017; Reddy et al., 2016; Sørensen et al., 2015; Vallöf et al., 2016, 2019). The direct administration of Exendin-4 to the brain, compared to intraperitoneal administration may explain the difference in cue-associated dopaminergic activity. Studies on GLP-1R binding sites and c-fos activation after peripheral semaglutide did not find evidence for direct access to the midbrain (Gabery et al., 2020; Hansen et al., 2021), while exendin-4 does (Hernandez et al., 2018). One interpretation for the different impact of exendin-4 compared to semaglutide on dopamine signaling is that semaglutide indirectly activates dopamine neurons via nuclei that are close to the brain's circumventricular organs, such as the NTS, arcuate nucleus of the hypothalamus (ARC) or lateral septum.

We found that semaglutide decreased chow intake while increasing VTA dopamine activity during reward collection but not during the cue. Dopamine is known to impact on different aspects of feeding and food-seeking. Dopamine stimulants such as amphetamine and methylphenidate exert anorexigenic effects (Efron et al., 1997; Sanghvi et al., 1975), while decreased dopamine levels are associated with increased food intake (Cordeira et al., 2010; Verhagen et al., 2009). Additionally, dopamine-deficient mice stop eating, which could be reversed after injecting L-DOPA (Zhou and Palmiter, 1995). A different pattern arises when studying food-seeking, as temporarily inhibiting VTA dopamine neurons reduced food-seeking (Chang et al., 2016; Van Zessen et al., 2021), while excitation of these neurons increased food-seeking behaviors and prevented extinction (Pan et al., 2021; Steinberg et al., 2013). In dopamine-transporter knockouts, which have higher baseline dopamine levels, motivation to obtain food reward is increased without impacting on Pavlovian conditioning (Cagniard et al., 2006). In our Pavlovian experiment, semaglutide increased dopamine activity during reward, decreased food intake and reduced reward seeking. This challenges the current hypothesis on the causality between dopamine activity and food-seeking behaviors. Processes underlying reward cues and consumption are independent processes and it remains to be determined how they impact on food-seeking.

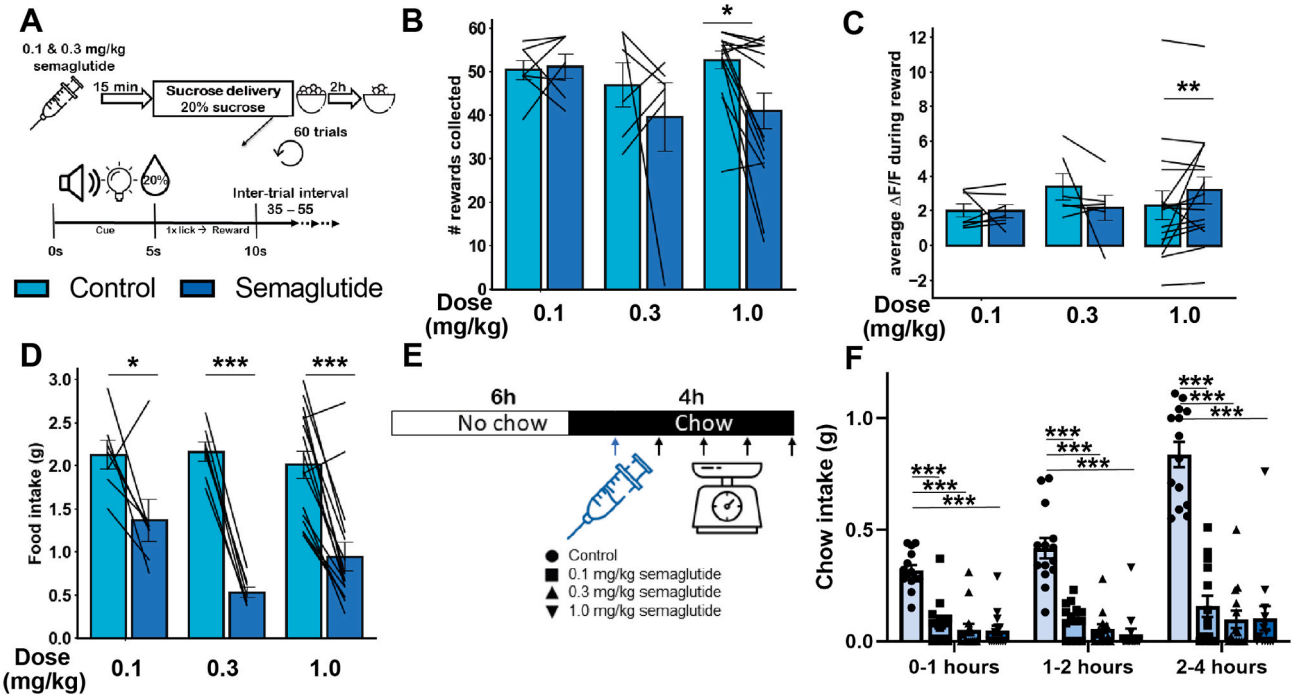

Fig. 5. Lower doses of semaglutide did not alter rewards obtained and dopamine activity for sucrose, while chow intake remains reduced.
We repeated the experiment while injecting lower doses of semaglutide, whereafter the Pavlovian sucrose conditioning paradigm was performed (**A**). The results of 1 mg/kg serve as a reference and are from Fig. 2. The obtained rewards (**B**), average dopamine activity during reward collection (**C**) during the task and home cage chow intake in the first 2 h after the task (**D**) are plotted. To ensure pharmacological efficacy during the sucrose conditioning task, we monitored chow intake in the first 4 h after injection (**E, F**). The bars represent group means with individual values displayed in lines and symbols. Error bars represent SEM. *p < 0.05, **p < 0.01, ***p < 0.001.

Additionally, dopamine is also associated with liking and saliency of rewards. In our study, the 'liking' of sucrose, as measured by the number of licks in the first bout of reward collection (Johnson, 2018), remained unchanged after semaglutide administration. This suggests that semaglutide does not alter 'liking,' even though the motivation to acquire the reward is reduced, and dopamine activity is increased. One theory is that the VTA DA activity during reward collection is related to salience (Schultz, 2016), making the reward more noticeable after semaglutide.

Earlier studies suggest semaglutide cannot directly access the midbrain but can assess brain areas such as the NTS, ARC or lateral septum (Gabery et al., 2020; Hansen et al., 2021; Jensen et al., 2018). These areas are previously related to influence dopamine signaling and reward-related behaviors. GLP-1R agonists applied in the NTS affected dopamine signaling and diminished reward-seeking behaviors (Alhadeff et al., 2012; Richard et al., 2015; Vallöf et al., 2019). Semaglutide was found to reduce the activity of the ARC and diminish chow intake (Ghidewon et al., 2022). The lateral septum projects to the VTA and is involved in valence and goal-directed behaviors (Wirtshafter and Wilson, 2021). GLP-1 agonists in the lateral septum suppress motivation for food and alcohol reinforcement (Terrill et al., 2019). Thus, there are several pathways via which peripherally administered semaglutide could impact VTA dopamine responsivity. Further studies should reveal via which pathway the VTA dopamine activity is altered.

Semaglutide decreased total sucrose intake during the session. This is consistent with previous literature showing that semaglutide and other GLP-1R agonists reduced palatable food intake, such as chocolate (Gabery et al., 2020). In addition, studies on participants with obesity have shown that semaglutide diminished the preference for energy-dense foods (Blundell et al., 2017). The reduction in sucrose intake was only present after injection of the 1 mg/kg dose, while chow intake was reduced already with a 0.1 mg/kg dose, possibly due to larger quantity of chow provided, compared to the limited amount of sucrose (+-10 kcal in chow vs. 0.4 kcal in the sucrose) that did not satiate.

This study has several limitations. Firstly, high doses of GLP-1 agonists, including semaglutide, can induce nausea and malaise-like symptoms (Kanoski et al., 2012; O'Neil et al., 2018). Although we did not find any malaise signs, we cannot exclude that it may have influenced our findings. Additionally, we conducted our experiments with food-restricted, lean mice to ensure high motivation for sucrose. It would be interesting to repeat the experiments in an ad libitum-fed state and/or overweight mice to determine whether this mechanism also plays a role in a satiated or obesogenic state. Thirdly, we provided little portions of sucrose (8 μl) to keep the mice motivated throughout the task. These quantities are unlikely to be satiating and may explain why lower doses of semaglutide we tested did not reduce task performance while suppressing food intake after the Pavlovian session. Lastly, we determined the effect of semaglutide starting 15 min following intraperitoneal injection, which is rather fast. Although we cannot exclude that semaglutide effects develop over later periods, we believe that an intraperitoneal injection results in rapid distribution, which is confirmed by its effects on motivation and cue-induced dopamine signaling.

In conclusion, a high dose of semaglutide decreased motivation for sucrose, while dopamine activity during reward collection increased. Future research should investigate the underlying mechanisms that mediate these effects. Overall, our study contributes to understanding the GLP-1R agonist semaglutide on reward-related behaviors and neuronal signaling in mice.

Contributors

KK: study design, data collection, data analysis, data interpretation, literature search, generation of figures, writing of the manuscript; DK: data collection, data interpretation; EE: data collection, study design; ML: data collection; LD: data collection, FD: data interpretation, writing of the manuscript; RA: study design, data interpretation, literature

search, writing of the manuscript.

Role of funding source

This work was supported by the ERANET-NEURON 2018 (#MIGBAN FKZ: 01EW1906A) grant and NWO ALW open (OCENW.KLEIN.071).

Declaration of competing interest

The authors have no conflicts of interest.

Acknowledgments

The authors thank Frank Meye for the valuable discussions, Bregje Titulaer for piloting previous related experiments. Semaglutide was kindly provided by Novo Nordisk via the compound-sharing program.

References

Ahmann, A.J., Capehorn, M., Charpentier, G., Dotta, F., Henkel, E., Lingvay, I., Holst, A. G., Annett, M.P., Aroda, V.R., 2018. Efficacy and safety of once-Weekly semaglutide versus exenatide ER in subjects with type 2 diabetes (SUSTAIN 3): a 56-Week, open-Label, randomized clinical trial. Diabetes Care 41, 258–266. https://doi.org/10.2337/dc17-0417.

Alhadeff, A.L., Rupprecht, L.E., Hayes, M.R., 2012. GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. Endocrinology 153, 647–658. https://doi.org/10.1210/en.2011-1443.

Alvarez, E., Roncero, I., Chowen, J.A., Thorens, B., Blázquez, E., 1996. Expression of the glucagon-like peptide-1 receptor gene in rat brain. J. Neurochem. 66, 920–927. https://doi.org/10.1046/j.1471-4159.1996.66030920.x.

Aranäs, C., Edvardsson, C.E., Shevchouk, O.T., Zhang, Q., Witley, S., Blid Sköldheden, S., Zentveld, L., Vallöf, D., Tufvesson-Alm, M., Jerlhag, E., 2023. Semaglutide reduces alcohol intake and relapse-like drinking in male and female rats. EBioMedicine 93, 104642. https://doi.org/10.1016/j.ebiom.2023.104642.

Blundell, J., Finlayson, G., Axelsen, M., Flint, A., Gibbons, C., Kvist, T., Hjerpsted, J.B., 2017. Effects of Once-weekly Semaglutide on Appetite Energy Intake Control of Eating, Food Preference and Body Weight in Subjects with Obesity.

Cagniard, B., Balsam, P.D., Brunner, D., Zhuang, X., 2006. Mice with chronically elevated dopamine exhibit enhanced motivation, but not learning, for a food reward. Neuropsychopharmacology 31, 1362–1370. https://doi.org/10.1038/sj.npp.1300966.

Chang, C.Y., Esber, G.R., Marrero-Garcia, Y., Yau, H.J., Bonci, A., Schoenbaum, G., 2016. Brief optogenetic inhibition of dopamine neurons mimics endogenous negative reward prediction errors. Nat. Neurosci. 19, 111–116. https://doi.org/10.1038/nn.4191.

Cordeira, J.W., Frank, L., Sena-Esteves, M., Pothos, E.N., Rios, M., 2010. Brain-derived neurotrophic factor regulates hedonic feeding by acting on the mesolimbic dopamine system. J. Neurosci. 30, 2533–2541.

Dickson, S.L., Shirazi, R.H., Hansson, C., Bergquist, F., Nissbrandt, H., Skibicka, K.P., 2012. The glucagon-like peptide 1 (GLP-1) analogue, exendin-4, decreases the rewarding value of food: a new role for mesolimbic GLP-1 receptors. J. Neurosci. 32, 4812–4820. https://doi.org/10.1523/JNEUROSCI.6326-11.2012.

Efron, D., Jarman, F., Barker, M., Dip, G., Psych, E., 1997. Attention deficit hyperactivity disorder: a double-blind , crossover trial. Pediatrics 100.

Egecioglu, E., Engel, J.A., Jerlhag, E., 2013a. The glucagon-like peptide 1 analogue exendin-4 attenuates the nicotine-induced locomotor stimulation, accumbal dopamine release, conditioned place preference as well as the expression of locomotor sensitization in mice. PLoS One 8, 1–7. https://doi.org/10.1371/journal.pone.0077284.

Egecioglu, E., Steensland, P., Fredriksson, I., Feltmann, K., Engel, J.A., Jerlhag, E., 2013b. The glucagon-like peptide 1 analogue Exendin-4 attenuates alcohol mediated behaviors in rodents. Psychoneuroendocrinology 38, 1259–1270. https://doi.org/10.1016/j.psyneuen.2012.11.009.

Fortin, S.M., Roitman, M.F., 2017. Central GLP-1 receptor activation modulates cocaine-evoked phasic dopamine signaling in the nucleus accumbens core. Physiol. Behav. 176, 17–25. https://doi.org/10.1016/j.physbeh.2017.03.019.

Gabery, S., Salinas, C.G., Paulsen, S.J., Ahnfelt-Rønne, J., Alanentalo, T., Baquero, A.F., Buckley, S.T., Farkas, E., Fekete, C., Frederiksen, K.S., Helms, H.C.C., Jeppesen, J.F., John, L.M., Pyke, C., Nøhr, J., Lu, T.T., Polex-Wolf, J., Prevot, V., Raun, K., Simonsen, L., Sun, G., Szilvásy-Szabó, A., Willenbrock, H., Secher, A., Knudsen, L.B., 2020. Semaglutide lowers body weight in rodents via distributed neural pathways. JCI Insight 5. https://doi.org/10.1172/jci.insight.133429.

Ghidewon, M., Wald, H.S., McKnight, A.D., De Jonghe, B.C., Breen, D.M., Alhadeff, A.L., Borner, T., Grill, H.J., 2022. Growth differentiation factor 15 (GDF15) and semaglutide inhibit food intake and body weight through largely distinct, additive mechanisms. Diabetes Obes. Metabol. 24, 1010–1020. https://doi.org/10.1111/dom.14663.

Gutniak, M., Ørskov, C., Holst, J., Ahrén, B., Efendić, S., 1992. Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subject and patients with diabetes mellitus. N. Engl. J. Med. 326, 1316–1322.

Hansen, H.H., Perens, J., Roostalu, U., Skytte, J.L., Salinas, C.G., Barkholt, P., Thorbek, D.D., Rigbolt, K.T.G., Vrang, N., Jelsing, J., Hecksher-Sørensen, J., 2021. Whole-brain activation signatures of weight-lowering drugs. Mol. Metabol. 47, 101171 https://doi.org/10.1016/j.molmet.2021.101171.

Hayes, M.R., Bradley, L., Grill, H.J., 2009. Endogenous hindbrain glucagon-like peptide-1 receptor activation contributes to the control of food intake by mediating gastric satiation signaling. Endocrinology 150, 2654–2659. https://doi.org/10.1210/en.2008-1479.

Hayes, M.R., Mietlicki-Baase, E.G., Kanoski, S.E., De Jonghe, B.C., 2014. Incretins and amylin: neuroendocrine communication between the gut, pancreas, and brain in control of food intake and blood glucose. Annu. Rev. Nutr. 34, 237–260. https://doi.org/10.1146/annurev-nutr-071812-161201.

Hernandez, N.S., Ige, K.Y., Mietlicki-Baase, E.G., Molina-Castro, G.C., Turner, C.A., Hayes, M.R., Schmidt, H.D., 2018. Glucagon-like peptide-1 receptor activation in the ventral tegmental area attenuates cocaine seeking in rats. Neuropsychopharmacology 43, 2000–2008. https://doi.org/10.1038/s41386-018-0010-3.

Holst, J.J., 2007. The physiology of glucagon-like peptide 1. Physiol. Rev. 87, 1409–1439. https://doi.org/10.1152/physrev.00034.2006.

Jensen, C.B., Pyke, C., Rasch, M.G., Dahl, A.B., Knudsen, L.B., Secher, A., 2018. Characterization of the glucagonlike peptide-1 receptor in male mouse brain using a novel antibody and in situ hybridization. Endocrinology 159, 665–675. https://doi.org/10.1210/en.2017-00812.

Johnson, A.W., 2018. Characterizing ingestive behavior through licking microstructure: underlying neurobiology and its use in the study of obesity in animal models. Int. J. Dev. Neurosci. 64, 38–47. https://doi.org/10.1016/j.ijdevneu.2017.06.012.

Kanoski, S.E., Rupprecht, L.E., Fortin, S.M., De Jonghe, B.C., Hayes, M.R., 2012. The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide. Neuropharmacology 62, 1916–1927. https://doi.org/10.1016/j.neuropharm.2011.12.022.

Konanur, V.R., Hsu, T.M., Kanoski, S.E., Hayes, M.R., Roitman, M.F., 2020. Phasic dopamine responses to a food-predictive cue are suppressed by the glucagon-like peptide-1 receptor agonist Exendin-4. Physiol. Behav. 215, 112771 https://doi.org/10.1016/j.physbeh.2019.112771.

Lau, J., Bloch, P., Schäffer, L., Pettersson, I., Spetzler, J., Kofoed, J., Madsen, K., Knudsen, L.B., McGuire, J., Steensgaard, D.B., Strauss, H.M., Gram, D.X., Knudsen, S. M., Nielsen, F.S., Thygesen, P., Reedtz-Runge, S., Kruse, T., 2015. Discovery of the once-weekly glucagon-like peptide-1 (GLP-1) analogue semaglutide. J. Med. Chem. 58, 7370–7380. https://doi.org/10.1021/acs.jmedchem.5b00726.

López-Ferreras, L., Richard, J.E., Noble, E.E., Eerola, K., Anderberg, R.H., Olandersson, K., Taing, L., Kanoski, S.E., Hayes, M.R., Skibicka, K.P., 2018. Lateral hypothalamic GLP-1 receptors are critical for the control of food reinforcement, ingestive behavior and body weight. Mol. Psychiatr. 23, 1157–1168. https://doi.org/10.1038/mp.2017.187.

Masaki, T., Ozeki, Y., Yoshida, Y., Okamoto, M., Miyamoto, S., Gotoh, K., Shibata, H., 2022. Glucagon-like peptide-1 receptor agonist semaglutide improves eating behavior and glycemic control in Japanese obese type 2 diabetic patients. Metabolites 12. https://doi.org/10.3390/metabo12020147.

Matsuyama, T., Komatsu, R., Namba, M., Watanabe, N., Itoh, H., Tarui, S., 1988. Glucagon-like peptide-1 (7-36 amide): a potent glucagonostatic and insulinotropic hormone. Diabetes Res. Clin. Pract. 5, 281–284. https://doi.org/10.1016/S0168-8227(88)80063-9.

McMahon, L.R., Wellman, P.J., 1998. PVN infusion of GLP-1-(7-36) amide suppresses feeding but does not induce aversion or alter locomotion in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 274, 23–29. https://doi.org/10.1152/ajpregu.1998.274.1.r23.

Morales, M., Margolis, E.B., 2017. Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat. Rev. Neurosci. 18, 73–85. https://doi.org/10.1038/nrn.2016.165.

O'Neil, P.M., Birkenfeld, A.L., McGowan, B., Mosenzon, O., Pedersen, S.D., Wharton, S., Carson, C.G., Jepsen, C.H., Kabisch, M., Wilding, J.P.H., 2018. Efficacy and safety of semaglutide compared with liraglutide and placebo for weight loss in patients with obesity: a randomised, double-blind, placebo and active controlled, dose-ranging, phase 2 trial. Lancet 392, 637–649. https://doi.org/10.1016/S0140-6736(18)31773-2.

Pan, W.X., Coddington, L.T., Dudman, J.T., 2021. Dissociable contributions of phasic dopamine activity to reward and prediction. Cell Rep. 36, 109684 https://doi.org/10.1016/j.celrep.2021.109684.

Pratley, R.E., Aroda, V.R., Lingvay, I., Lüdemann, J., Andreassen, C., Navarria, A., Viljoen, A., 2018. Semaglutide versus dulaglutide once weekly in patients with type 2 diabetes (SUSTAIN 7): a randomised, open-label, phase 3b trial. Lancet Diabetes Endocrinol. 6, 275–286. https://doi.org/10.1016/S2213-8587(18)30024-X.

Reddy, I.A., Pino, J.A., Weikop, P., Osses, N., Sørensen, G., Bering, T., Valle, C., Bluett, R. J., Erreger, K., Wortwein, G., Reyes, J.G., Graham, D., Stanwood, G.D., Hackett, T.A., Patel, S., Fink-Jensen, A., Torres, G.E., Galli, A., 2016. Glucagon-like peptide 1 receptor activation regulates cocaine actions and dopamine homeostasis in the lateral septum by decreasing arachidonic acid levels. Transl. Psychiatry 6, e809. https://doi.org/10.1038/tp.2016.86.

Richard, J.E., Anderberg, R.H., Göteson, A., Gribble, F.M., Reimann, F., Skibicka, K.P., 2015. Activation of the GLP-1 receptors in the nucleus of the solitary tract reduces food reward behavior and targets the mesolimbic system. PLoS One 10, 1–21. https://doi.org/10.1371/journal.pone.0119034.

Salamone, J.D., Correa, M., Yohn, S., Lopez Cruz, L., San Miguel, N., Alatorre, L., 2016. The pharmacology of effort-related choice behavior: dopamine, depression, and individual differences. Behav. Process. 127, 3–17. https://doi.org/10.1016/j.beproc.2016.02.008.

Sanghvi, I.S., Singer, G., Friedman, E., Gershon, S., 1975. Anorexigenic effects of d-amphetamine and 1-dopa in the rat. Pharmacol. Biochem. Behav. 3, 81–86. https://doi.org/10.1016/0091-3057(75)90084-2.

Schirra, J., Kuwert, P., Wank, U., Leicht, P., Arnold, R., Goke, B., Katschinski, M., 1997. Differential effects of subcutaneous GLP-1 on gastric emptying, antroduodenal motility, and pancreatic function in men. Proc. Assoc. Am. Phys. 109, 84–97.

Schultz, W., 2016. Dopamine reward prediction-error signalling: a two-component response. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn.2015.26.

Schultz, W., Dayan, P., Montague, P.R., 1997. A neural substrate of prediction and reward. Science 275, 1593–1599. https://doi.org/10.1126/science.275.5306.1593.

Smidt, M.P., von Oerthel, L., Hoekstra, E.J., Schellevis, R.D., Hoekman, M.F.M., 2012. Spatial and temporal lineage analysis of a Pitx3-driven cre-recombinase knock-in mouse model. PLoS One 7, 1–6. https://doi.org/10.1371/journal.pone.0042641.

Sørensen, G., Reddy, I.A., Weikop, P., Graham, D.L., Stanwood, G.D., Wortwein, G., Galli, A., Fink-Jensen, A., 2015. The glucagon-like peptide 1 (GLP-1) receptor agonist exendin-4 reduces cocaine self-administration in mice. Physiol. Behav. 149, 262–268. https://doi.org/10.1016/j.physbeh.2015.06.013.

Steinberg, E.E., Keiflin, R., Boivin, J.R., Witten, I.B., Deisseroth, K., Janak, P.H., 2013. A causal link between prediction errors, dopamine neurons and learning. Nat. Neurosci. 16, 966–973. https://doi.org/10.1038/nn.3413.

Terrill, S.J., Holt, M.K., Maske, C.B., Abrams, N., Reimann, F., Trapp, S., Williams, D.L., 2019. Endogenous GLP-1 in lateral septum promotes satiety and suppresses motivation for food in mice. Physiol. Behav. 206, 191–199. https://doi.org/10.1016/j.physbeh.2019.04.008.

Vallöf, D., Kalafateli, A.L., Jerlhag, E., 2019. Brain region specific glucagon-like peptide-1 receptors regulate alcohol-induced behaviors in rodents. Psychoneuroendocrinology 103, 284–295. https://doi.org/10.1016/j.psyneuen.2019.02.006.

Vallöf, D., MacCioni, P., Colombo, G., Mandrapa, M., Jörnulf, J.W., Egecioglu, E., Engel, J.A., Jerlhag, E., 2016. The glucagon-like peptide 1 receptor agonist liraglutide attenuates the reinforcing properties of alcohol in rodents. Addiction Biol. 21, 422–437. https://doi.org/10.1111/adb.12295.

Van Zessen, R., Flores-Dourojeanni, J.P., Eekel, T., van den Reijen, S., Lodder, B., Omrani, A., Smidt, M.P., Ramakers, G.M.J., van der Plasse, G., Stuber, G.D., Adan, R.A.H., 2021. Cue and reward evoked dopamine activity is necessary for maintaining learned pavlovian associations. J. Neurosci. 41, 5004–5014. https://doi.org/10.1523/JNEUROSCI.2744-20.2021.

Verhagen, L.A.W., Luijendijk, M.C.M., Hillebrand, J.J.G., Adan, R.A.H., 2009. Dopamine antagonism inhibits anorectic behavior in an animal model for anorexia nervosa. Eur. Neuropsychopharmacol 19, 153–160. https://doi.org/10.1016/j.euroneuro.2008.09.005.

Wirtshafter, H.S., Wilson, M.A., 2021. Lateral septum as a nexus for mood, motivation, and movement. Neurosci. Biobehav. Rev. 126, 544–559. https://doi.org/10.1016/j.neubiorev.2021.03.029.

Zhou, Q.Y., Palmiter, R.D., 1995. Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 83, 1197–1209. https://doi.org/10.1016/0092-8674(95)90145-0.