


## ARTICLE OPEN



# GLP-1 receptor agonist exenatide uncouples food intake from hedonic and anticipatory regulation in non-human primates: insights from an operant meal schedule paradigm

Balázs Knakker<sup>1,7</sup>, Judit Inkeller<sup>1,7</sup>, Péter Kovács<sup>2,6</sup>, Balázs Lendvai<sup>2,3</sup> and István Hernádi<sup>1,4,5</sup> 

© The Author(s) 2024

Glucagon-like peptide 1 (GLP-1), a neuroendocrine signal of energy balance and satiety, has a major role in regulating food intake behaviour. Here we investigated the effects of the GLP-1 agonist exenatide on palatability-driven feeding regulation in adult male rhesus macaques ( $n = 5$ ) using a novel operant food intake paradigm with four meal schedule conditions where two types of pellets with different palatability values were offered as meal in all combinations in two consecutive daily feeding sessions (S1 and S2). In control conditions, a strong, palatability-driven anticipatory effect was found in S1, followed by a complementary positive contrast effect in S2. After acute subcutaneous treatment with 1 µg/kg dose of exenatide 1 h before S1, food intake decreased to the same very low level in all meal schedule conditions in S1, completely erasing the previously observed anticipatory effect. Conversely, exenatide induced hypoglycaemia in an anticipatory meal schedule dependent pattern. Interestingly, the previously observed positive contrast effect was spared in S2, with a weaker residual effect specifically on the consumption of the more palatable pellet type. To conclude, the food intake reducing effects of exenatide may temporally evolve from strong anorectic to weak anhedonic modulations, where hedonic experience and anticipation during the early anorectic phase is conserved but uncoupled from food intake behaviour.

*Neuropsychopharmacology* (2025) 50:410–418; <https://doi.org/10.1038/s41386-024-01981-5>

## INTRODUCTION

In general, food intake (what, when, and how much food is consumed) is controlled by the interaction between biological and environmental factors and is modulated by multiple neuronal pathways controlling homeostasis, reward processing and decision making. In addition, to best serve survival, energy homeostasis is directly regulated by overlapping neuronal and hormonal signalling that alters appetite, food craving and foraging behaviour to balance short-term fluctuations in energy intake. High-palatability food can enhance its own intake even beyond homeostatic levels via hedonic sensitisation [1], thus leading to regular overeating and hence to obesity [2].

Specific gut hormones, such as glucagon-like peptide 1 (GLP-1) are released in response to ingestion and regulate food intake both in animals and humans by influencing specific appetite-regulating circuits in the brain. GLP-1 plays a role in both the homeostatic and hedonic control of feeding behaviour [3]. GLP-1 is a member of the incretin neuropeptide family, secreted by intestinal enteroendocrine L-cells of the distal ileum and large intestine [4, 5] and also in the nucleus of the solitary tract (NTS) in response to nutrient intake [6, 7]. Under normal physiological conditions, GLP-1 receptors (GLP-1R) are expressed on the dendritic terminals of vagal afferents innervating the

gastrointestinal tract [8], in diencephalic brain regions involved in homeostatic and metabolic control of feeding such as the lateral hypothalamus, and the nucleus arcuatus, and also in other brain regions that specifically regulate reward processing and motivated behaviour such as the ventral tegmental area (VTA) and the nucleus accumbens (NAc) [9]. Among its various actions, GLP-1 stimulates glucose-dependent insulin secretion, inhibits glucagon secretion, delays gastric emptying, enhances feelings of satiety, and consequently reduces food intake [10–12]. In addition, GLP-1 has also been demonstrated to play a major role in maintaining glucose homeostasis by lowering blood glucose levels specifically when the concentration of blood glucose exceeds the normal fasting levels [13].

Exenatide (Ex), a 39-amino-acid GLP-1 analogue peptide, is a potent agonist with high affinity for GLP-1Rs and has a significantly longer biological half-life than the exogenously administered GLP-1, which makes it an efficient treatment option in noninsulin-dependent diabetes mellitus [14–17]. Exenatide reduces postprandial and fasting plasma glucose levels via an insulinotropic effect and via the suppression of plasma glucagon levels in the fasted state [18]. In addition, Ex also penetrates the blood-brain barrier and acts on central GLP-1Rs [19]. In accordance with the behavioural consequences of the ensuing widespread

<sup>1</sup>Grastyán E. Translational Research Centre, University of Pécs, Pécs, Hungary. <sup>2</sup>Department of Pharmacology and Drug Safety Research, Gedeon Richter Plc., Budapest, Hungary.

<sup>3</sup>Richter Department, Semmelweis University, Budapest, Hungary. <sup>4</sup>Department of Neurobiology, Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary.

<sup>5</sup>Institute of Physiology, Medical School, University of Pécs, Pécs, Hungary. <sup>6</sup>Present address: VRG Therapeutics, Fűvészkeret utca 3., Budapest 1083, Hungary. <sup>7</sup>These authors contributed equally: Balázs Knakker, Judit Inkeller. ✉email: [hernadi@gamma.ttk.pte.hu](mailto:hernadi@gamma.ttk.pte.hu)

Received: 21 February 2024 Revised: 15 August 2024 Accepted: 26 August 2024

Published online: 4 September 2024

central and peripheral effects of GLP1-R activation, Ex strongly reduces appetite and food intake in rodents, non-human primates, and humans likewise [3, 20–22].

Data from animal studies further suggest that the appetite suppressant (anorectic) effects of GLP-1R agonists are also manifested through mesolimbic GLP-1R activation, that in turn alters the rewarding value of food [23–29]. These effects are especially important with respect to treatment of hedonic overeating in obesity, a more recent indication of GLP1-R agonists [30, 31]. The hedonic value of a food is determined by its palatability (flavour, texture, etc.), the environment in which the food interacts with the subject, and ultimately the homeostatic and motivational state of the subject. Motivational states are modulated by specific anticipatory and decision-making cognitive domains, which have a fundamental role in the regulation of the intake of foods that are desired or craved, especially in non-human primates and humans. Despite the success of GLP-1Rs in targeting hedonic overeating in obesity, the way homeostatic, hedonic, cognitive and contextual factors are entwined in the regulation of eating in humans remains unclear [32]. For example, human diets are organised around specific habitual and planned meals scheduled around a day, so our food consumption is also generally regulated by anticipation of a possible later availability of a food item, and also the hedonic value of the available and anticipated food. While relevant research in rodents have successfully encapsulated similar phenomena in clever experimental designs [33], in this domain the differences between rodents and humans are arguably larger than in the case of homeostatic regulation serving basic survival instincts. To increase the translational potential of the available preclinical models, it has become necessary to devise humanised meal-schedule tasks in non-human primates and test the effects GLP1-R modulation on primate scheduled food intake, especially as only a limited number of studies are currently available to support the existing clinical findings and foster further research in the field.

To address this gap, we previously developed a two-session operant food intake paradigm with four meal schedule conditions assigned on a weekly basis. A meal schedule was defined by two types of pellets with different palatability values offered as meal in all combinations with two daily feeding sessions (S1 and S2) [34]. In this paradigm, the total daily food consumption of lean and healthy adult animals remained stable over several weeks on a level that clearly avoided overconsumption and covered their homeostatic needs [34]. Within these homeostatic limits, animals consumed more of the more palatable pellet offered in S2 after an S1 with the less palatable meal – a marked successive positive palatability contrast effect. More surprisingly, after the current weekly meal schedule had been revealed to the animals on Mondays (diet naïve state), the food consumption pattern of the animals changed in a remarkable way on Tuesdays and remained so for the rest of the week (diet-aware state). Specifically, the animals displayed a strong, palatability-driven negative anticipatory effect in S1: food consumption strongly decreased in anticipation of the more palatable S2 meal, compared to when the less palatable meal was offered in S2.

In the present study, utilising the above-described novel, highly translatable two-session operant food intake paradigm, rhesus macaques were administered GLP-1R agonist Ex to elucidate how GLP-1R agonism differentially modulates homeostatic feeding regulation, hedonic valuation and palatability-driven anticipation and decision making across the two daily feeding sessions.

## MATERIALS AND METHODS

### Animals and housing

Five 4-year-old, pair-housed, pre-trained, healthy male rhesus macaques (*Macaca mulatta*) were used in the experiments. Their body weight was  $4.4 \pm 0.3$  kg and  $5.5 \pm 0.5$  kg (mean  $\pm$  SEM) at the beginning and at the end

of the study, respectively. The animals were kept in home cages that were uniformly sized  $200 \times 100 \times 200$  cm (length  $\times$  width  $\times$  depth) and were equipped with wooden rest areas. Besides natural light from windows, illumination from full-spectrum artificial light was provided for 12 h starting at 7:00 AM each day. Temperature ( $24 \pm 1^\circ\text{C}$ ) and relative humidity ( $55 \pm 10\%$ ) of the animal house were kept constant, with continuous airflow.

On experimental days (weekdays, Mondays to Fridays), daily food allowance was only offered during the food intake sessions (see section 'Two-session operant food intake paradigm' below). On weekend days (Saturdays and Sundays), animals received usual dry lab chow (Altromin Spezialfutter GmbH & Co, Lage, DE; nutritionally complete, 3.315 kcal/g) with a variety of vegetable and fruit supplements. The calorie intake from the pellets consumed during the experimental days and calorie intake on weekend days were similar, and fully complied with the recommended daily energy requirements of the animals (see section 'Energy requirements' in Supplementary Methods). Water was *ad libitum* available on every day of the week.

### Ethical compliance

All procedures were conducted in the Grastyán Translational Research Centre of the University of Pécs. The study was approved by the Animal Welfare Committee of the University of Pécs, and the Hungarian National Scientific Ethical Committee on Animal Experimentation. Ethical permission was issued by the Department of Animal Health and Food Control of the County Government Offices of the Ministry of Agriculture (permission number: BA02/2000-13/2015). Measures were taken to minimise pain and discomfort of the animals in accordance with the Directive 40/2013 (II.14): "On animal experiments" issued by the Government of Hungary, and the Directive 2010/63/EU "On the protection of animals used for scientific purposes" issued by the European Parliament and the European Council.

### Two-session operant food intake paradigm

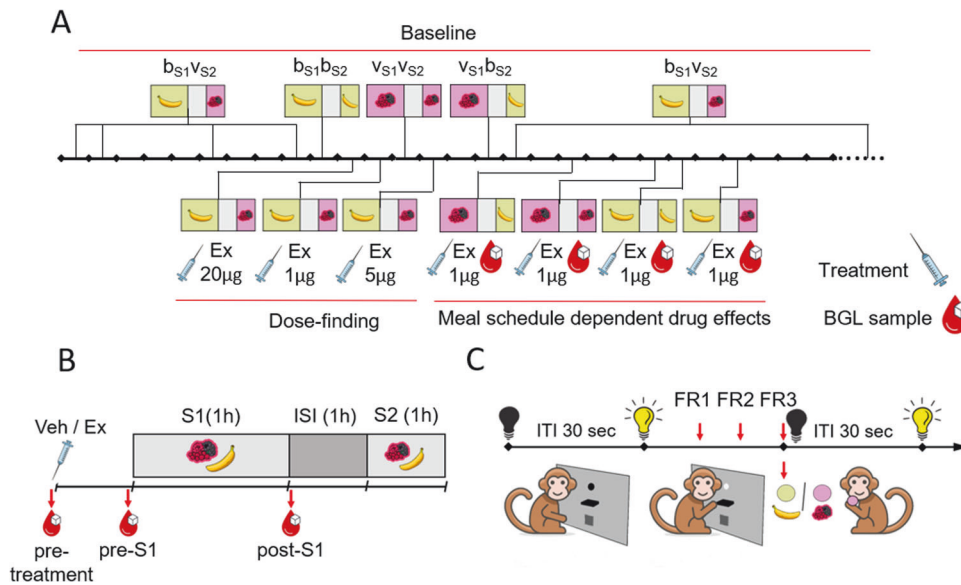
The experiments were performed using a two-session operant food intake behavioural paradigm that was previously developed in our laboratory [34]. All the data analysed in this study were collected on the experimental weeks when all five animals were involved in the two-session operant food intake paradigm [34] on each weekday, tested simultaneously in five separate operant chambers (see also Supplementary Methods, 'Operant chambers'). Sessions consisted of repeated individual trials where the animals received a single one-gram food pellet upon three consecutive lever presses (fixed ratio 3 reinforcement schedule, FR3) per trial (see Fig. 1C). Lever presses during the 30-s ITI were neither rewarded with food pellets nor punished with extra time-out. Two experimental sessions were run per day: Session 1 (S1) started at approx. 9:30 AM and lasted 2 h (or until max. 200 pellets were earned), and Session 2 (S2) started at approx. 12:30 PM and lasted 1 h (or until max. 100 pellets were earned). There was a one-hour inter-session interval (ISI) between S1 and S2 when the subjects were returned to their home cages (see Fig. 1B).

The session durations and the number of available pellets were previously determined so as not to limit the animals' food consumption: There were only 3 out of 175 occasions for S1 and 9 out of 175 occasions for S2 when the sessions were 1–5 min shorter because of reaching the maximal pellet consumption.

During a session, food pellets of only one type were available: either the standard, low-palatability banana flavoured pellets (denoted 'b', Dustless Precision Pellets®, Bio-Serv, Inc., Frenchtown, New Jersey, US; nutritionally complete, 3.35 kcal/g) and the more palatable 'very berry' (forest fruit) flavoured pellets (denoted 'v', Supreme Mini-Treats, Bio-Serv, Inc., Frenchtown, New Jersey, US; nutritionally complete, 3.46 kcal/g). Food pellet types were selected based on food preference test, as described in our previous study [34]. All possible combinations of the two types of pellets assigned to the two daily sessions were tested, resulting in 4 different meal schedule conditions: S1 banana / S2 banana ( $b_{S1}b_{S2}$ ), S1 banana / S2 very berry ( $b_{S1}v_{S2}$ ), S1 very berry / S2 banana ( $v_{S1}b_{S2}$ ), S1 very berry / S2 very berry ( $v_{S1}v_{S2}$ ). Each different meal schedule was consistently offered for 5 consecutive experimental days, from Mondays to Fridays.

### Procedures and drug administration

On Baseline weeks we recorded the animals' feeding behaviour in the two-session operant paradigm without pharmacological treatments. Two experiments were conducted, a *Dose-finding experiment* involving 3 treatment weeks with the  $b_{S1}v_{S2}$  meal schedule, and the *Meal schedule*



**Fig. 1 Timeline and schematic experimental design.** **A** Timeline of the experimental weeks. On the timeline, pictograms and acronyms indicate the weekly meal schedule for all experimental weeks. Experimental weeks without pharmacological treatments are called Baseline weeks as indicated above the timeline. Below the timeline, the treatment weeks of the *Dose-finding* and *Meal schedule* experiments are shown, labelled with treatment types (syringe pictogram), administered doses, and blood sampling (blood drop pictogram). Treatment weeks involved both Vehicle and Exenatide treatment days (see **B** and main text). **B** Daily schedule of the food intake task. On each weekday the animals performed the food intake task for 2 experimental sessions (S1 and S2) separated by a one-hour intersession interval (ISI). Within a session, only one type of food pellet was offered: either less palatable (banana flavoured) pellets or more palatable ('very berry' flavoured) pellets, as also indicated by pictograms. Timing of treatment administration and blood sampling are indicated by pictograms. **C** Trial structure of the food intake task. At the beginning of each trial, a cue light switched on, indicating that food items were available upon three consecutive lever presses (red arrows, fixed ratio 3 reinforcement schedule, FR3). After pellet delivery, the cue light was switched off for a 30-sec intertrial interval (ITI).

experiment involving 4 treatment weeks, one for each of the 4 meal schedule conditions. On treatment weeks, Vehicle treatment was administered on Tuesdays, and drug treatment was administered on Wednesdays. The timeline of all the experimental weeks – treatment and Baseline weeks – is shown on Fig. 1A.

We investigated the effects of peripheral administration the GLP-1 agonist exenatide (Ex, Byetta 10 µg injection pen; synthetic exendin-4; AstraZeneca AB., Stockholm Sweden; batch number: 15H029). In our experiments, animals received acute, subcutaneous (under the skin of the thigh) injection of Ex on Wednesdays, 1 h prior to S1. Since pharmacokinetic data of the short-acting formulation of Ex applied in the present study is only available for rodents [35] and humans [36] (but not rhesus macaques), the timing of the drug administration and the two sessions was determined based on the behavioural findings of Scott and Moran [22], where the dose-dependent anorectic effects of Ex in rhesus macaque operant food consumption was seen up to 6 h post-administration. Exenatide was extracted from the injection pen and was diluted with a vehicle solution (pH = 4.5, acetate buffer with mannitol) containing the main components of the original vehicle from the product to achieve a larger dosing volume (0.1 mL/kg) set based on individual body weight. Exenatide or vehicle solutions were administered via single subcutaneous injections. The daily dosing formulations were prepared freshly (max. 2 h) prior to administration. Vehicle (sham) treatments were administered on Tuesdays with the same timing as the genuine treatments. At least 6 days of recovery time was introduced between pharmacological treatments to eliminate possible residual effects from the previous treatment and to enable monitoring food intake on subsequent days (Thursdays and Fridays).

In the *Dose-finding* experiment, we determined the effective and side effect-free dosing by administering Ex at doses of 1, 5, or 20 µg/kg in meal schedule b<sub>S1</sub>V<sub>S2</sub> (see Fig. 1A). All subjects were observed and assessed for side effects such as changes in alertness and general activity, the presence of excessive salivation, gagging, and regurgitation following each injection until the end of the experimental day (4 pm). Following the *Dose-finding* experiment, we conducted a *Meal schedule* experiment, where we investigated the acute effects of 1 µg/kg Ex in all four meal schedules. The dosing formulation protocol, the route of administration, dose volumes, and the timing of dosing were the same in both of experiments.

### Blood glucose level sampling

For the measurement of blood glucose levels (BGL) in the *Meal schedule* experiment, blood samples were taken from the monkeys seated in a standard primate chair, which was otherwise used for the transport of animals and for experimental sessions from their home cages. Monkeys had been previously accustomed to the procedure of taking blood samples and trained to voluntarily offer their hind limb for sampling to minimise stress. After puncturing the skin with a needle tip on the large toe of the hind limb, a drop of blood was applied to the test strip. Blood glucose levels were measured with EasyTouch blood glucose monitor (Wellmed EasyTouch® GCU, ET-301 Multi-Function Monitoring System, Biopetik Technology Inc., Jhunan Township, TW) in all four meal schedule conditions according to the following timetable: immediately before administration of drug or vehicle (pre-treatment); just before S1 (pre-S1) and after S1 (post-S1).

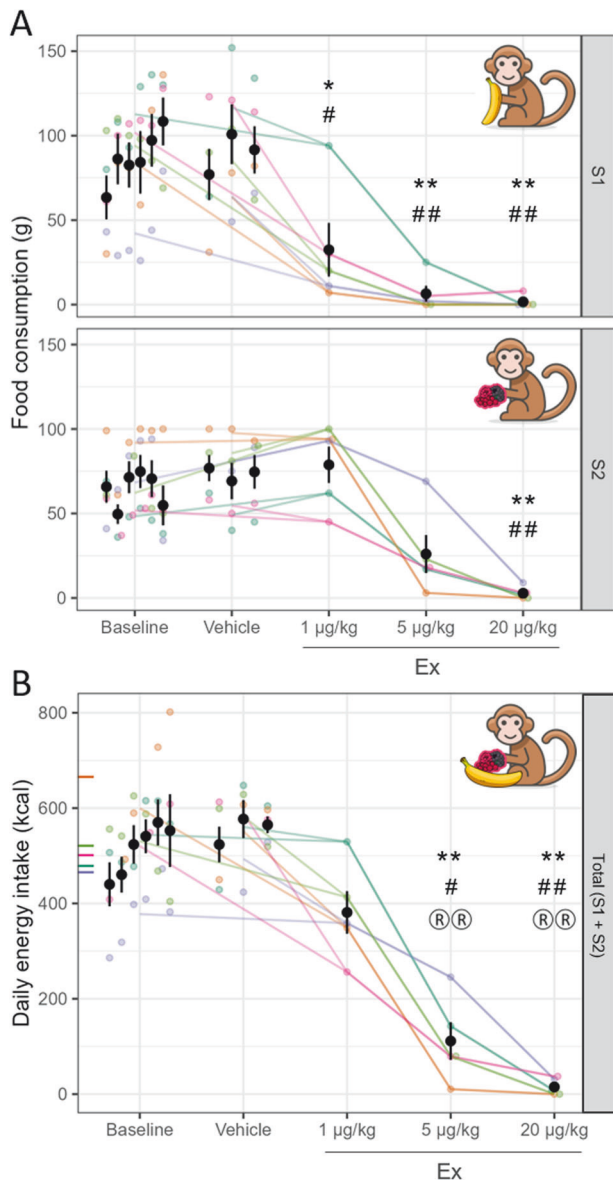
### Statistical analysis

Data loading and preparation was partly done in MATLAB R2021a (The Mathworks, Natick, MA, USA); analyses and graphics were made in R 4.3.2. Where applicable, statistical tests are two-sided. Degrees of freedom for F-tests were corrected for nonsphericity using the Greenhouse-Geisser method.

**Food consumption analyses.** We investigated the total food consumption (g) for each session. The number of pellets delivered was registered by the operant testing apparatus. Leftover pellets were collected from the operant chambers at the end of the sessions and their number was registered manually. Food consumption for each session was calculated by subtracting the number of leftover pellets from the number of pellets delivered.

For both experiments, two different control conditions were used: Baseline and Vehicle control. Using Vehicle control is standard practice, since it controls for the potential effects of treatment administration itself. However, from our previous study [34] we clearly saw that to detect the feeding regulation effects that we described, using only Vehicle control data in the present study would not necessarily provide enough statistical power in the small sample size setting necessitated by the use of non-





**Fig. 2 Dose-finding experiment in the food intake paradigm.** Dose-dependent effects of Ex on food consumption (A) and on total daily energy intake (B). Effects of 1, 5 or 20 µg/kg Ex (x axis) on food consumption (y axis on A) in S1 (upper panel) and S2 (lower panel) and daily energy intake (y axis on B) of the two-session food intake paradigm compared to Vehicle control days (Vehicle, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ ) and no-treatment Baseline days (Baseline, #:  $p < 0.05$ , ##:  $p < 0.01$ ). P-values are Dunnett-corrected for the 3 comparisons within each analysis. Colours indicate individual animals, points correspond to data from one session on A and one day on B. Black points and whiskers are condition averages and s.e.m. across animals, with the 6 Baseline and 3 Vehicle days shown separately. On the y axis of panel B inward pointing ticks show the daily energy intake recommendation for each animal (colour-coded) based on their current weight. On B, \* markers denote significant differences from nutritional recommendation values (\*:  $p < 0.05$ , \*\*:  $p < 0.01$ ).

human primates. Thus, here we also used Baseline control data where no pharmacological or vehicle treatment was administered to compare drug effects with the normal, habitual feeding behaviour in the task with higher statistical power. Specifically, for the *Dose-finding experiment*, the Vehicle control was data averaged from the 3 Vehicle sessions from the days (Tuesdays) preceding the drug administration days. Baseline control for the *Dose-finding experiment* was the average from 6 sessions from the same days of the Baseline weeks as the drug administration days (Wednesdays).

Vehicle controls for the *Meal schedule experiment* were not averaged, one Vehicle day from the same week was used for control in each (weekly) meal schedule condition. Baseline control for the analysis of the *Meal schedule experiment* was calculated using days from Tuesday to Friday averaged, from Baseline weeks with the corresponding meal schedule conditions. The Baseline control weeks of the *Meal schedule experiment* were analysed in detail in our previous paper where we showed that the animals displayed a clear anticipation effect from Tuesdays to Fridays [34]. Since our present aim was to investigate the pharmacological modulation of this effect, we deliberately included Tuesdays to Fridays (diet-aware days) in the experimental design and analysis (see also the Introduction). For detailed control analyses of the Baseline and Vehicle conditions, see 'Control analyses of Vehicle and Baseline data' section of the Supplementary Results.

Data analyses for S1 and S2 were conducted separately as, due to their differing duration, food consumption levels and corresponding variances were different between the two sessions. Thus, for both experiments, four repeated measures ANOVAs were conducted on food consumption data, separate ones for the two control conditions used and for S1 and S2 (S1 with Vehicle control, S1 with Baseline control, S2 with Vehicle control, S2 with Baseline control).

The ANOVAs for the *Dose-finding experiment* involved only the Treatment factor (4 levels: Control – Vehicle or Baseline, Ex 1 µg/kg, Ex 5 µg/kg, Ex 20 µg/kg). The ANOVAs for the analysis of the *Meal schedule experiment* involved the following factors: S1 meal type ( $b_{S1}$  or  $v_{S1}$ ), S2 meal type ( $b_{S2}$  or  $v_{S2}$ ) and Treatment (2 levels: either Vehicle or Baseline control, Ex 1 µg/kg).

**Daily energy intake analysis.** The daily energy intake was calculated based on the food consumption values described above and the metabolisable energy content of the pellets as supplied by the manufacturer. Repeated measures ANOVAs identical to those performed on S1 and S2 were run on daily energy intake data, except that the models were parametrised so that each animal's recommended daily energy intake (see 'Energy requirements' in Supplementary Methods) was the intercept. This way we could also quantify and statistically test the difference of each Treatment condition (including the Baseline and Vehicle controls) from the daily nutritional recommendation. Since the main focus of this analysis was relating the recommended daily intake, further results were considered redundant with the detailed S1 and S2 analyses and are not reported here.

**Blood glucose level analysis.** We analysed the BGL data from samples taken during the *Meal schedule experiment*. Please refer to the section 'Blood glucose level analysis' in the Supplementary Results for details.

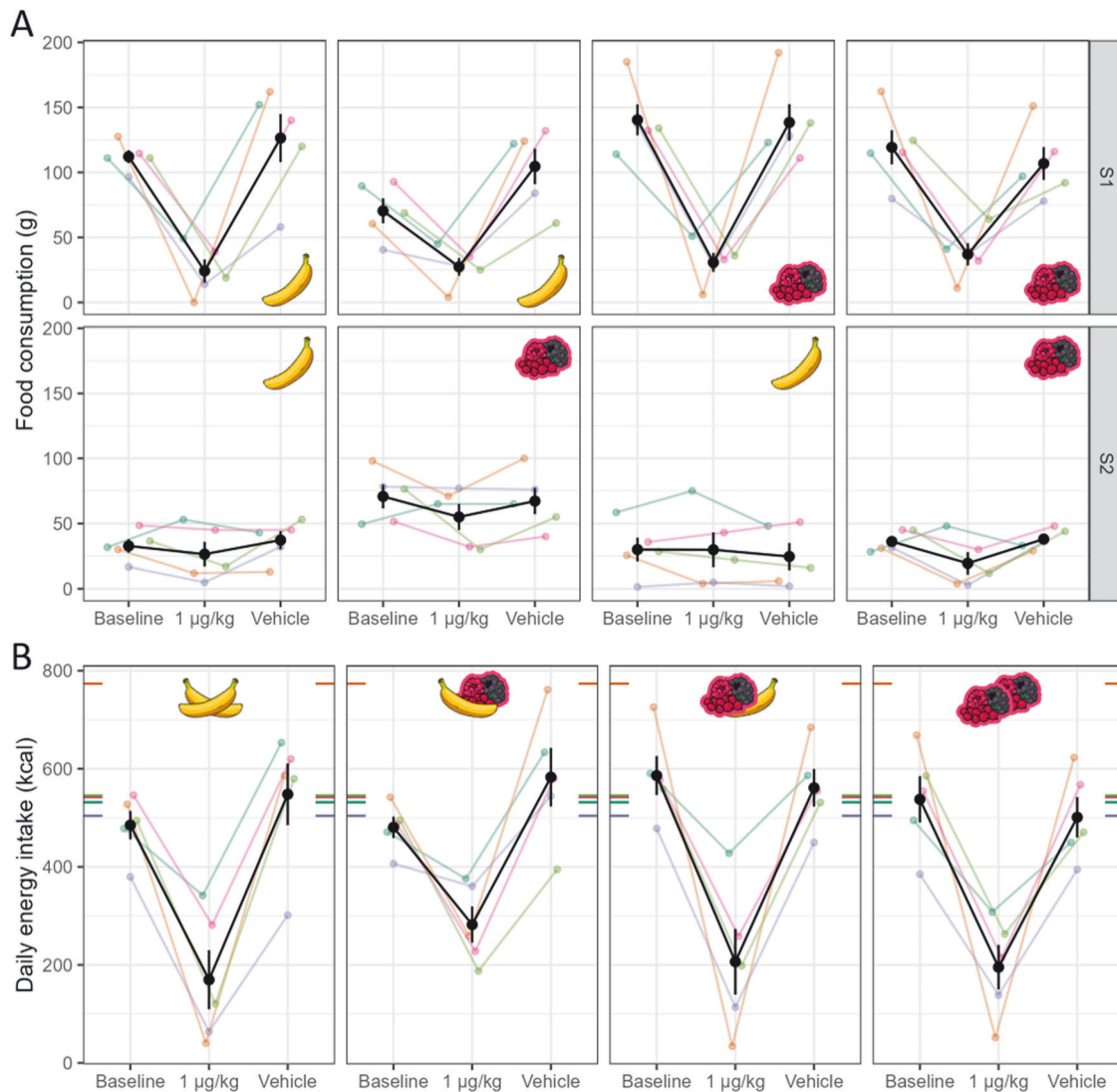
## RESULTS

We first conducted a *Dose-finding experiment* using meal schedule  $b_{S1}v_{S2}$  to probe the effects of Ex on food intake in the two-session operant paradigm. Based on the results, we selected the optimal dose of Ex which reduced food intake without observable side effects and went on to investigate the hypothesised meal schedule dependent effects of Ex (see Fig. 1).

### Dose-finding experiment

In the *Dose-finding experiment* the total daily energy intake significantly and dose-dependently decreased under Ex treatment compared to control conditions ( $F_{2,0.8,1} = 60.21$ ,  $p = 1.3 \times 10^{-5}$  using Vehicle control;  $F_{1,8,7,2} = 38.24$ ,  $p = 0.0002$  using Baseline control; see Fig. 2B). The Vehicle and Baseline control energy intake did not differ significantly from nutritional recommendations based on age and body weight, but the daily energy intake was significantly lower than Vehicle, Baseline, and also the recommended nutritional value for the 5 and 20 µg/kg doses of Ex, while it only marginally decreased for the 1 µg/kg dose (see Supplementary Table 1 for details).

In S1, Ex significantly decreased food consumption in all three doses (against Vehicle:  $F_{1,6,5} = 26.36$ ,  $p = 0.001$ ; against Baseline:  $F_{1,8,7,2} = 22.82$ ,  $p = 0.0009$ ; see Fig. 2A, top; Supplementary Table 2, top), with close to zero consumption in the two higher dose levels (5 and 20 µg/kg). In S2 overall, Ex still caused strong, significant decrements in food consumption (against Vehicle:  $F_{1,7,6,8} = 22.86$ ,



**Fig. 3 Meal schedule experiment in the food intake paradigm.** The effects of 1 µg/kg Ex across meal schedule (panels from left to right) and treatment conditions (x axis) on food consumption (y axis, **A**) across sessions (lower and upper part of **A**), and on daily total energy intake (y axis, **B**). Pictograms represent meal schedule conditions. Colours indicate individual animals; coloured points correspond to data from one session on **A** and one day on **B**. Black points and whiskers are condition averages and s.e.m. across animals. Coloured tick marks on the edges of sub-panels on **B** show recommended energy intake of each animal calculated based on weight measured during the experiment.

$p = 0.001$ ; against Baseline:  $F_{1,9,7,7} = 20.01$ ,  $p = 0.001$ ; see Fig. 2A, bottom; Supplementary Table 2, bottom), however, the 1 µg/kg dose of Ex was not effective, and close-to-zero floor consumption levels were only reached for the highest dose of Ex (20 µg/kg).

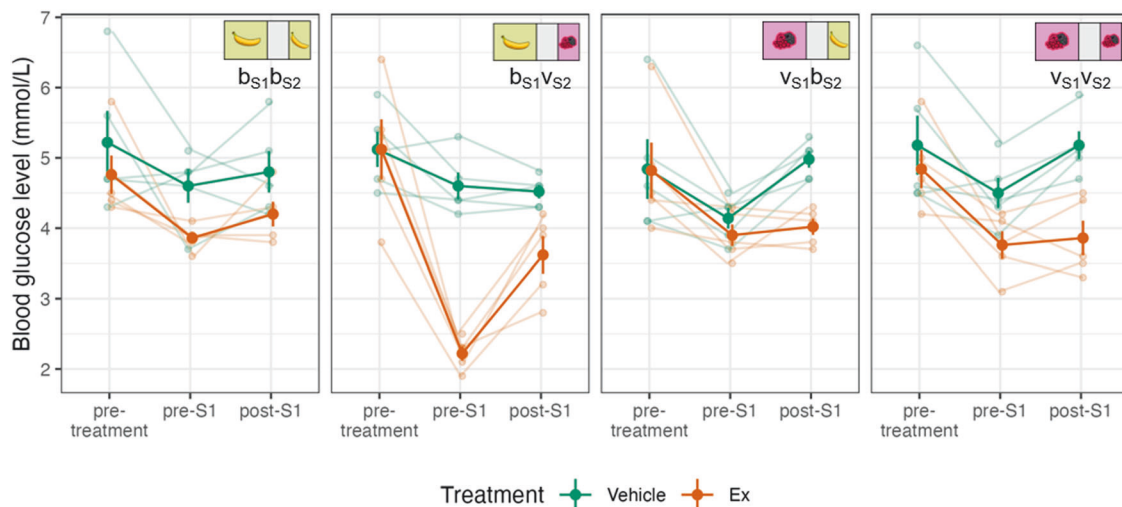
After the 20 µg/kg dose of Ex, all the animals showed transient hypoactivity, coordination problems and gastrointestinal symptoms, while after the 5 µg/kg dose, side effects were observed only in one animal. For the 1 µg/kg dose, all animals showed normal behaviour and general activity.

We verified that food consumption did not differ significantly between the Baseline and the Vehicle control conditions, with no significant temporal trends in either (see ‘Control analyses of Vehicle and Baseline data’ section of the Supplementary Results).

#### Meal schedule dependent experiment

Based on the results, we selected the 1 µg/kg dose of Ex which reduced food intake without observable side effects, and went on to investigate the hypothesised meal schedule dependent effects of Ex in all four possible meal schedule conditions (see Fig. 3).

In our previous study [34] validating and describing this paradigm, we found a palatability-driven anticipation effect: S1 food consumption in the two-session paradigm strongly decreased in the  $b_{S1}v_{S2}$  condition in anticipation of the more palatable S2 meal type, compared to when the less palatable meal was offered in S2 (the  $b_{S1}b_{S2}$  condition). Importantly, this palatability-driven anticipation effect was replicated in the present study (Vehicle – control condition in the present study, S2 meal type:  $F_{1,4} = 8.48$ ,  $p = 0.044$ , Baseline – previous study data, S2 meal type:  $F_{1,4} = 13.80$ ,  $p = 0.021$ ; Control type  $\times$  S2 meal type:  $F_{1,4} = 0.13$ ,  $p = 0.73$ , see also Fig. 3). To facilitate comparison between the two studies, we include the data from the previous study as the no-treatment Baseline control condition (see also Methods) in addition to the placebo-treatment Vehicle control condition. In the Supplementary Results (‘Control analyses of Vehicle and Baseline data’) we show that the new Vehicle dataset is similar to the previously analysed ‘Baseline’ condition in the present study, except for a substantially weakened S1 meal type effect – an effect that is orthogonal to the focal anticipation effect described above.



**Fig. 4** Blood glucose levels in the Meal schedule experiment. Blood glucose levels (y axis) from the three sampling times (pre-treatment, pre-S1, post-S1, on x axis) in the four meal schedule conditions (panels left to right) under Vehicle and Ex treatment (green and orange, respectively).

Total daily energy intake significantly decreased under 1  $\mu\text{g/kg}$  dose of Ex treatment relative to nutritional recommendations ( $d = -366 \pm 84$  kcal,  $t_4 = -4.35$ ,  $p = 0.012$ ), Vehicle ( $d = -335 \pm 62$  kcal,  $t_4 = -5.45$ ,  $p = 0.0055$ ) and Baseline controls ( $d = -309 \pm 62$  kcal,  $t_4 = -4.99$ ,  $p = 0.0075$ ; see Fig. 3B and Supplementary Table 2). In contrast, energy intake in the Vehicle and Baseline conditions matched the nutritional recommendations (Vehicle relative to recommended:  $d = -31 \pm 32$  kcal,  $t_4 = -0.97$ ,  $p = 0.38$ ; Baseline relative to recommended:  $d = -57 \pm 30$  kcal,  $t_4 = -1.91$ ,  $p = 0.13$ ).

Under Ex treatment, food intake in S1 strongly decreased (main effect of Treatment against Vehicle:  $F_{1,4} = 29.28$ ,  $p = 0.0056$ ; against Baseline:  $F_{1,4} = 41.96$ ,  $p = 0.003$ ) to the same low level (from  $119.0 \pm 11.8$  g in Vehicle to  $29.9 \pm 6.9$  g in Ex) regardless of meal schedule condition (under Ex treatment, S1 meal type:  $F_{1,4} = 2.01$ ,  $p = 0.23$ ; S2 meal type:  $F_{1,4} = 1.14$ ,  $p = 0.35$ ; S1 meal type  $\times$  S2 meal type:  $F_{1,4} = 0.35$ ,  $p = 0.58$ ), as also implied by the Treatment  $\times$  S2 meal type interactions (against Vehicle control:  $F_{1,4} = 6.97$ ,  $p = 0.058$ , against Baseline control:  $F_{1,4} = 9.72$ ,  $p = 0.036$ ). Thus, the palatability-driven anticipatory effect in S1 was completely erased by the 1  $\mu\text{g/kg}$  dose of Ex.

In S2, the overall effect of Ex on food consumption was weak, also in accordance with the Dose-finding results (Treatment main effect against Vehicle:  $F_{1,4} = 2.37$ ,  $p = 0.20$ , against Baseline:  $F_{1,4} = 1.52$ ,  $p = 0.29$ ). However, Ex had a slightly stronger effect on the consumption of the more palatable food (mean  $\pm$  SE average Treatment effect:  $-15.4 \pm 6.5$  g against Vehicle and  $-16.2 \pm 9.5$  g against Baseline) compared to the effect for the less palatable food ( $-2.8 \pm 5.9$  g against Vehicle and  $-3.2 \pm 6.7$  g against Baseline) as supported by a Treatment  $\times$  S2 meal type interaction (using Vehicle days as control:  $F_{1,4} = 12.37$ ,  $p = 0.025$ ; using Baseline days as control:  $F_{1,4} = 8.46$ ,  $p = 0.044$ ).

The characteristic pattern in S2 was the successive positive palatability contrast effect – a dramatic increase in food intake in the  $b_{S1}v_{S2}$  meal schedule condition compared to all the remaining three meal schedule conditions [34]. Importantly, this pattern was fully preserved under Ex treatment (S1 meal type  $\times$  S2 meal type under Ex:  $F_{1,4} = 8.94$ ,  $p = 0.040$ ), and was not moderated by Treatment (Treatment  $\times$  S1 meal type  $\times$  S2 meal type, Vehicle control:  $F_{1,4} = 2.50$ ,  $p = 0.19$ ; Treatment  $\times$  S1 meal type  $\times$  S2 meal type, Baseline control:  $F_{1,4} = 0.60$ ,  $p = 0.48$ ; S1 meal type  $\times$  S2 meal type, Vehicle control:  $F_{1,4} = 4.94$ ,  $p = 0.09$ ; Baseline control:  $F_{1,4} = 9.53$ ,  $p = 0.037$ ).

Blood glucose levels (BGL) were measured during the four meal schedules both on the Vehicle and the Ex treatment days (see Fig. 4). BGL in general substantially dropped from pre-treatment to pre-S1 ( $F_{1,4} = 8.79$ ,  $p = 0.041$ ), change-score analysis – further analyses are baseline-adjusted models or raw values. (For more detailed statistics, see Supplementary Results.) During Vehicle treatment in S1 with very berry flavoured pellets offered, BGL increased (from pre-S1 to post-S1) by  $0.76 \pm 0.13$  mmol/L, a significantly (Time  $\times$  S1 meal type:  $t_{50,0} = 4.30$ ,  $p = 8 \times 10^{-5}$ ) larger increase compared to sessions when banana flavoured pellets were offered, where it stagnated ( $+0.06 \pm 0.13$  mmol/L). This effect was erased by Ex treatment (post-S1 minus pre-S1, very berry consumed, Ex treatment:  $+0.11 \pm 0.18$  mmol/L), which is not surprising given the similar low consumption rates across conditions under the effect of the drug.

Exenatide strongly decreased pre-S1 BGL specifically in the  $b_{S1}v_{S2}$  meal schedule condition ( $d = -2.38 \pm 0.25$  mmol/L,  $t_{6,8} = -9.7$ ,  $p = 3 \times 10^{-5}$ , uncorrected contrast), and only to a smaller extent in the other conditions ( $d \geq -0.73$ ,  $t \geq -3.5$ ,  $p \geq 0.006$ ; pre-S1, Treatment:  $F_{1,4,2} = 41.50$ ,  $p = 0.0025$ ; Treatment  $\times$  S1 meal type  $\times$  S2 meal type:  $t_{50,4} = 3.50$ ,  $p = 0.001$ ). This effect substantially weakened in the post-S1 blood sample (Treatment  $\times$  Time  $\times$  S1 meal type  $\times$  S2 meal type interaction in the main model:  $t_{50,0} = -2.61$ ,  $p = 0.012$ ), though BGL was still significantly lower under Ex compared to Vehicle control treatment (post-S1, Treatment:  $F_{1,4,1} = 24.23$ ,  $p = 0.0073$ ).

## DISCUSSION

In the present study, we investigated the potential effects of Ex on palatability-based food preference and anticipation in rhesus macaques, besides confirming its known effects on homeostatic food intake regulation. In the Meal schedule experiment, we found that Ex treatment in a dose of 1  $\mu\text{g/kg}$  strongly decreased food consumption in S1 (1 to 3 h after treatment) and erased the previously observed palatability-driven negative anticipatory effect. Interestingly, no marked food intake reduction effect of Ex was observed in S2 (4 to 5 h after treatment), and, surprisingly, the previously observed successive positive contrast in food intake in S2 also persisted under Ex treatment. However, Ex treatment was shown to mildly reduce intake of the more palatable, but not the less palatable food in S2. Additionally, Ex decreased both fasting and postprandial blood glucose levels (BGL) measured



before and after S1, and the Ex effect on fasting BGL depended on the meal schedule.

Extending our previous results, here we confirmed that in control conditions, homeostatic regulation of food consumption was captured by the two-session feeding paradigm as the daily energy intake in the operant task matched nutritional recommendations to maintain growth and energy homeostasis. Ex, in line with its established effect on food intake [34], dose-dependently decreased food consumption in our paradigm so that the energy intake fell below both control and recommended nutritional values.

Though no studies have analysed the anorectic effects of Ex in non-human primates so far in a paradigm with repeated, scheduled feeding sessions, the previous results of Scott and Moran on rhesus macaques are still broadly compatible with our present findings [22]. In their study [22], animals were tested in a single six-hour operant food intake session starting 15 min after intramuscular administration of Ex. Despite important procedural differences from our present study, a closer look at their findings also reveals a strong initial anorectic effect of Ex in the first 1 to 3 h, and the food consumption reductions in the time period of 4 to 5 h post-administration were of comparable size to our present findings in S2. We extended those earlier results by investigating the effect of Ex under different meal schedules, showing that the late effect of Ex is not only smaller but depends on the palatability of the offered food, implying that it is also more context-dependent compared to the strong early effect of Ex.

In the Vehicle and Baseline control conditions, when the more palatable food was available in S2, the animals reduced their consumption in S1 anticipating the more valued meal, so that the consumption decrement in S1 matched the excess amount eaten in S2 – we referred to this phenomenon as the palatability-driven negative anticipatory effect [34]. In the *Meal schedule experiment*, after the acute administration of the 1 µg/kg dose of Ex food consumption decreased to the same very low level in all four meal schedule conditions, that is, the palatability-driven negative anticipatory effect was erased. Ex is known to decrease the homeostatic drive, i.e., hunger, to a level so low that is known to weaken or eradicate hedonic contrast effects [33, 37, 38]. Based on this, in our study 1 µg/kg Ex erased the palatability-driven anticipatory effect via generally inhibiting hedonic discrimination mechanisms or uncoupling them from the regulation of feeding behaviour.

In S2, we did not observe a strong, meal schedule independent reduction in feeding that would have been expected given a sustained pharmacological effect, but neither did we find compensatory consumption after the reduced S1 feeding rates that would have been expected due to declining drug effects. Food consumption in each condition was approximately in the same range as that of the respective control (placebo-treatment Vehicle or no-treatment Baseline) conditions with the same meal schedule. Importantly, the characteristic successive positive palatability contrast effect, that is, the excess S2 consumption on the  $b_{S1V_{S2}}$  meal schedule condition relative to all other conditions, was also conserved. This positive contrast effect requires awareness of the hedonic values of both the presently experienced and the previously consumed food types. Based on this, we hypothesise that even after radically lower food consumption in S1 following the administration of Ex, before entering S2, the hedonic state (or sensory-specific satiation [39]) attained during S1 and carried over to S2 was similar to that of the control conditions.

While Ex spared the positive palatability contrast effect, it specifically decreased the consumption of the more palatable, but not the less palatable food in S2. This is in line with previous studies in rodents showing that systemically administered Ex diminished the previously established palatability-based place preference and general motivation measured by the progressive

ratio operant test. Exenatide also decreased preference of sucrose solution against water and more palatable against less palatable food, which are interpreted as anhedonic effects. [24, 29, 40–43]. Exenatide also had strong anhedonic effects in the same paradigms when injected directly into reward-related brain areas such as the ventral tegmental area (VTA), the nucleus accumbens (NAc), and even in the nucleus tractus solitarius (NTS), a structure previously thought to regulate homeostatic feeding only [25–29]. Results from a blood-oxygen dependent functional MRI study [44] also suggest that mesolimbic reward-related areas may also mediate the anorectic effects of Ex in humans, with a hypothesised mechanism of decreasing responsiveness to contextual food cues (incentive salience, ‘wanting’) and increasing activity that correlates with consummatory reward value (‘liking’) [45]. Thus, human and rodent studies show that GLP-1R activation in reward-related mesolimbic and brainstem nuclei reduces the motivational incentive of the most rewarding stimulus/food available. GLP-1R modulation on homologous neural circuits in the rhesus macaque is a viable candidate mechanism for the observed anorectic effects of Ex in the current study.

The BGL values measured in the pre-treatment samples and on Vehicle control days were consistent with the BGL under laboratory conditions during everyday procedures reported in the literature [46]. In line with clinical and experimental data [47, 48], Ex strongly decreased BGL relative to the Vehicle condition both before and after S1. In the case of Vehicle treatment, higher values of postprandial BGL were obtained after S1 when the animals consumed a slightly higher amount of food because the more palatable meal was available in S1. This discrepancy disappeared due to Ex treatment which can be explained by the similar low food consumption in all four meal schedule conditions. Under Vehicle treatment, fasting BGL values consistently decreased during the hour preceding the start of S1. This is in accordance with previous human studies showing that food expectancy and delaying meals lead to decreasing blood glucose levels [47, 48]. During food expectation – that is, in the cephalic phase – even thinking about the food can initiate several physiological processes like increased insulin secretion, increased ghrelin levels, increased digestive juice secretion, and also decreasing blood glucose levels [47, 49, 50]. These processes constitute the early activation of the processes of postprandial metabolism, to prepare the organism for the digestion and absorption of the forthcoming food and nutrients [49]. Exenatide treatment caused a further drop in the fasting BGL in all four meal schedule conditions. Importantly, it was the  $b_{S1V_{S2}}$  meal schedule condition where the BGL decreasing effect of Ex was exceptionally strong, even pushing BGL into the hypoglycaemic range which was not reached under any other conditions. A possible explanation for this strong hypoglycaemic effect is that the palatability-related anticipation effect that we have shown in our previous study [34] is the strongest in the  $b_{S1V_{S2}}$  meal schedule condition, and the cephalic phase effects of this anticipatory process were even amplified by Ex. This is a striking observation, especially if we consider the fact that the negative anticipatory effect was completely erased from the S1 food consumption behaviour by Ex.

Several factors have limited the investigation of motivation and food reward sensitivity in animals in previous food intake studies. Frequently, animals were on an *ad libitum* feeding regime outside the scope of the experiments, where postprandial processes were left uncontrolled. Our novel two-session operant food intake paradigm addresses these limitations using a well-controlled feeding regime that permits the investigation of reward-related food intake behaviour without the confounding influence of food available outside the context of the experiment. Our paradigm also did not require food deprivation: in contrast to several previous laboratory-based experiments (e.g., [51, 52]), the animals were allowed to eat in an unrestrained and practically *ad libitum*

manner (see Methods). Thus, the behavioural pattern of food consumption in this paradigm can be considered endogenously controlled, so it is better suited to investigate close-to-natural feeding regulation of the animals under laboratory circumstances.

It is important to note that we used lean, healthy young adult animals in our studies. Since the regulation of food intake through these complex processes of dynamic interactions between the homeostatic and hedonic control systems and the higher-order regulatory adjustments functioned normally in the young animals used in our studies, which limits the direct applicability of these results to maladaptive eating behaviour or disorders in the regulation of feeding. However, we believe that this paradigm could be used well in the future to study multiple aspects of feeding regulation in obesity models with dysregulated feeding behaviour (i.e., in animals that overeat during *ad libitum* feeding).

To sum up, here we report that in our novel two-session operant food intake paradigm, GLP-1 agonism differentially modulated homeostatic, hedonic and anticipatory aspects of palatability-driven feeding behaviour. Exenatide had a strong early (1 to 3 h post-administration, S1) homeostatic anorectic effect, and the previously observed palatability-driven negative anticipatory effect in S1 disappeared completely under Ex treatment. Food consumption levels recovered by S2 (4 to 5 h post-administration), and in this late phase a weak, palatability-dependent food intake reduction was observed – suggesting an anhedonic effect. The positive palatability contrast effects in S2 were largely conserved, suggesting that even though it did not influence food consumption behaviour, hedonic experience in S1 was conserved under the early Ex effect. Intriguingly, by amplifying preprandial blood glucose level decreases, Ex induced hypoglycaemia specifically in the focal condition of the palatability-driven negative anticipatory effect that was eradicated by it on the level of food consumption behaviour.

To conclude, hedonic behavioural patterns following S1 and anticipatory blood sugar level effects preceding S1 together suggest that during S1 hedonic and anticipatory regulatory mechanisms were present under the early Ex effect, but were uncoupled from ongoing feeding behaviour. Understanding the underlying biological pathways and their complex interactions in disease-models will open new avenues in designing more targeted therapies and predicting potential therapeutic outcomes in humans.

## DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## REFERENCES

- Zheng H, Lenard NR, Shin AC, Berthoud H-R. Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. *Int J Obes*. 2009;33:58–513.
- Lutter M, Nestler EJ. Homeostatic and hedonic signals interact in the regulation of food intake. *J Nutr*. 2009;139:629–32.
- Hayes MR, Skibicka KP, Grill HJ. Caudal brainstem processing is sufficient for behavioral, sympathetic, and parasympathetic responses driven by peripheral and hindbrain glucagon-like-peptide-1 receptor stimulation. *Endocrinology*. 2008;149:4059–68.
- Brubaker PL, Drucker DJ. Minireview: glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. *Endocrinology*. 2004;145:2653–9.
- Mojsov S, Kopczynski MG, Habener JF. Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and the pancreas. *J Biol Chem*. 1990;265:8001–8.
- Vrang N, Larsen PJ. Preproglucagon derived peptides GLP-1, GLP-2 and oxyntomodulin in the CNS: role of peripherally secreted and centrally produced peptides. *Prog Neurobiol*. 2010;92:442–62.
- McGrath R, Glastras S, Hocking S, Tjoeng I, Krause M, Fulcher GR. Central Functions of Glucagon-like Peptide-1: Roles in Energy Regulation and Neuroprotection. *J Steroids Horm Sci*. 2015;6:152.
- Hayes MR, Mietlicki-Baase EG, Kanoski SE, De Jonghe BC. Incretins and amylin: neuroendocrine communication between the gut, pancreas, and brain in control of food intake and blood glucose. *Annu Rev Nutr*. 2014;34:237–60.
- Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol*. 1999;403:261–80.
- Holst JJ. Glucagon-like peptide-1, a gastrointestinal hormone with a pharmaceutical potential. *Curr Med Chem*. 1999;6:1005–17.
- Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest*. 1998;101:515–20.
- Drucker DJ. The biology of incretin hormones. *Cell Metab*. 2006;3:153–65.
- Nadkarni P, Chepurly OG, Holz GG. Regulation of Glucose Homeostasis by GLP-1. In: Tao Y-X, editor. *Glucose Homeostasis and the Pathogenesis of Diabetes Mellitus*. vol. 121, Academic Press; 2014. p. 23–65.
- Parkes D, Jodka C, Smith P, Nayak S, Rinehart L, Gingerich R, et al. Pharmacokinetic actions of exendin-4 in the rat: Comparison with glucagon-like peptide-1. *Drug Dev Res*. 2001;53:260–7.
- Gautier J-F, Choukem S-P, Girard J. Physiology of incretins (GIP and GLP-1) and abnormalities in type 2 diabetes. *Diabetes Metab*. 2008;34:S65–S72.
- Heppner KM, Perez-Tilve D. GLP-1 based therapeutics: simultaneously combating T2DM and obesity. *Front Neurosci*. 2015;9:92.
- Hinnen D. Glucagon-like peptide 1 receptor agonists for type 2 diabetes. *Diabetes Spectr*. 2017;30:202–10.
- Edwards CMB, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, et al. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am J Physiol Endocrinol Metab*. 2001;281:E155–E161.
- Kanoski SE, Fortin SM, Arnold M, Grill HJ, Hayes MR. Peripheral and central GLP-1 receptor populations mediate the anorectic effects of peripherally administered GLP-1 receptor agonists, liraglutide and exendin-4. *Endocrinology*. 2011;152:3103–12.
- Mack CM, Moore CX, Jodka CM, Bhavsar S, Wilson JK, Hoyt JA, et al. Antiobesity action of peripheral exenatide (exendin-4) in rodents: effects on food intake, body weight, metabolic status and side-effect measures. *Int J Obes*. 2006;30:1332–40.
- Williams DL, Baskin DG, Schwartz MW. Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety. *Endocrinology*. 2009;150:1680–7.
- Scott KA, Moran TH. The GLP-1 agonist exendin-4 reduces food intake in non-human primates through changes in meal size. *Am J Physiol Regul Integr Comp Physiol*. 2007;293:R983–R987.
- van Bloemendaal L, Uzzerman RG, ten Kulve JS, Barkhof F, Konrad RJ, Drent ML, et al. GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. *Diabetes*. 2014;63:4186–96.
- Dickson SL, Shirazi RH, Hansson C, Bergquist F, Nissbrandt H, Skibicka KP. 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*. 2012;32:4812–20.
- Alhadeff AL, Rupprecht LE, Hayes MR. 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*. 2012;153:647–58.
- Mietlicki-Baase EG, Ortinski PI, Rupprecht LE, Olivos DR, Alhadeff AL, Pierce RC, et al. The food intake-suppressive effects of glucagon-like peptide-1 receptor signaling in the ventral tegmental area are mediated by AMPA/kainate receptors. *Am J Physiol Endocrinol Metab*. 2013;305:E1367–E1374.
- Mietlicki-Baase EG, Ortinski PI, Reiner DJ, Sinon CG, McCutcheon JE, Pierce RC, et al. Glucagon-like peptide-1 receptor activation in the nucleus accumbens core suppresses feeding by increasing glutamatergic AMPA/kainate signaling. *J Neurosci*. 2014;34:6985–92.
- Alhadeff AL, Grill HJ. Hindbrain nucleus tractus solitarius glucagon-like peptide-1 receptor signaling reduces appetitive and motivational aspects of feeding. *Am J Physiol Regul Integr Comp Physiol*. 2014;307:R465–R470.
- Richard JE, Anderberg RH, Götteson A, Gribble FM, Reimann F, Skibicka KP. Activation of the GLP-1 receptors in the nucleus of the solitary tract reduces food reward behavior and targets the mesolimbic system. *PLoS ONE*. 2015;10:e0119034.
- Stenlid R, Cerenius SY, Wen Q, Aydin BK, Manell H, Chowdhury A, et al. Adolescents with obesity treated with exenatide maintain endogenous GLP-1, reduce DPP-4, and improve glycemic control. *Front Endocrinol*. 2023;14:1293093.
- Fox CK, Clark JM, Rudser KD, Ryder JR, Gross AC, Nathan BM, et al. Exenatide for weight-loss maintenance in adolescents with severe obesity: a randomized, placebo-controlled trial. *Obesity*. 2022;30:1105–15.
- Lee PC, Dixon JB. Food for thought: reward mechanisms and hedonic overeating in obesity. *Curr Obes Rep*. 2017;6:353–61.
- Flaherty CF. *Incentive relativity*. New York, NY, US: Cambridge University Press; 1996.
- Inkeller J, Knakker B, Kovács P, Lendvai B, Hernádi I. Intrinsic anticipatory motives in non-human primate food consumption behaviour. *iScience*. 2024;27:109459.



35. U.S. Food and Drug Administration. *Clinical Pharmacology and Biopharmaceutics Review(s)*. 2009; NDA 21-919. Retrieved from [https://www.accessdata.fda.gov/drugsatfda\\_docs/nda/2009/021919s000pharmr.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/021919s000pharmr.pdf)
36. Bray GM. Exenatide. *Am J Health-Syst Pharm*. 2006;63:411–8.
37. Gleitman H, Steinman F. Depression effect as a function of retention interval before and after shift in reward magnitude. *J Comp Physiol Psychol*. 1964;57:158–60.
38. Gonzalez RC, Fernhoff D, David FG. Contrast, resistance to extinction, and forgetting in rats. *J Comp Physiol Psychol*. 1973;84:562–71.
39. Hetherington MM, Caton S, Nekitsing C. Sensory-Specific Satiation and Satiety. *Time-Dependent Measures of Perception in Sensory Evaluation*, Wiley; 2017. p. 48–66.
40. Zhang X, Wang Y, Long Y, Wang L, Li Y, Gao F. et al. Alteration of sweet taste in high-fat diet induced obese rats after 4 weeks treatment with exenatide. *Pep-tides*. 2013;47:115–23.
41. Williams DL, Hyvarinen N, Lilly N, Kay K, Dossat A, Parise E, et al. Maintenance on a high-fat diet impairs the anorexic response to glucagon-like-peptide-1 receptor activation. *Physiol Behav*. 2011;103:557–64.
42. Mella R, Schmidt CB, Romagnoli P, Teske JA, Perez-Leighton C. The food environment, preference, and experience modulate the effects of exendin-4 on food intake and reward. *Obesity*. 2017;25:1844–51.
43. Sandoval D, Barrera JG, Stefater MA, Sisley S, Woods SC, D'Alessio DD, et al. The Anorectic Effect of GLP-1 in Rats Is Nutrient Dependent. *PLoS ONE*. 2012;7:e51870.
44. van Bloemendaal L, Veltman DJ, ten Kulve JS, Groot PFC, Ruhé HG, Barkhof F, et al. Brain reward-system activation in response to anticipation and consumption of palatable food is altered by glucagon-like peptide-1 receptor activation in humans. *Diabetes Obes Metab*. 2015;17:878–86.
45. Berridge KC. Wanting and liking: observations from the neuroscience and psychology laboratory. *Inquiry*. 2009;52:378
46. Lapin BA, Gvozdiak TE, Klotz IN. Blood glucose levels in rhesus monkeys (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) under moderate stress and after recovery. *Bull Exp Biol Med*. 2013;154:497–500.
47. Campfield LA, Smith FJ. Blood glucose dynamics and control of meal initiation: a pattern detection and recognition theory. *Physiol Rev*. 2003;83:25–58.
48. Ott V, Friedrich M, Prilop S, Lehnert H, Jauch-Chara K, Born J, et al. Food anticipation and subsequent food withdrawal increase serum cortisol in healthy men. *Physiol Behav*. 2011;103:594–9.
49. Power ML, Schulkin J. Anticipatory physiological regulation in feeding biology: Cephalic phase responses. *Appetite*. 2008;50:194–206.
50. Feldman M, Richardson CT. Role of thought, sight, smell, and taste of food in the cephalic phase of gastric acid secretion in humans. *Gastroenterology*. 1986;90:428–33.
51. Vaughan KL, Kaiser T, Peadar R, Anson RM, de Cabo R, Mattison JA. Caloric restriction study design limitations in rodent and nonhuman primate studies. *J Gerontol Ser A*. 2018;73:48–53.
52. Rowland NE. Food or fluid restriction in common laboratory animals: balancing welfare considerations with scientific inquiry. *Comp Med*. 2007;57:149–60.

## ACKNOWLEDGEMENTS

We would like to thank Zoltán Gödri for valuable technical contribution and for their assistance in animal care. We would like to thank Dr. Attila Trunk for technical help with experiment setup and initial data processing.

## AUTHOR CONTRIBUTIONS

Conceptualisation, BK, JI, PK and IH; methodology, BK, JI, PK and IH; Investigation, JI; software, BK; formal analysis, BK; data curation, BK, JI; writing – original draft, BK, JI and IH; writing – review & editing, BK, JI, PK, BL and IH; visualisation, BK, JI; resources, BL and IH; project administration, BL and IH; funding acquisition, BL and IH; supervision, IH.

## FUNDING

This publication was supported by the National Laboratory of Translational Neuroscience, RRF-2.3.1-21-2022-00011. This research was supported by the project No. TKP2021-EGA-16, implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme. Open access funding provided by University of Pécs.

## COMPETING INTERESTS

BL is employee of Gedeon Richter Plc. PK was an employee of Gedeon Richter Plc. during the planning and experimental phase of the study. These do not alter our adherence to journal policies on sharing data and materials. The remaining authors (JI, BK, IH) declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## ADDITIONAL INFORMATION

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41386-024-01981-5>.

**Correspondence** and requests for materials should be addressed to István Hernádi.

**Reprints and permission information** is available at <http://www.nature.com/reprints>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024