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| Supplementary Table 1. Preclinical studies on GLP-1 and palatable food intake  |
| ***Name of First Author/ Publication Year*** | ***Mouse/ Rat Line & Age or weight*** | ***Definition of the Model/Application of GLP-1 agonist (dose and method)*** | ***Experimental Groups*** | ***Assessments*** | ***Main Findings*** |
| **1. Preclinical** |
| **1.a Rats** |
| (Asarian et al., 1998) | Male Sprague–Dawley rats | Rats received sham feeding for 2 weeks to stabilize 45 min of sham intake/GLP-1 (3, 10, or 30 μg) i.c.v. | 1. aCSF2. GLP-1 (3 μg)3. GLP-1 (10 μg) | Feeding, grooming, exploratory behavior consisting of sniffing, locomotion, rearing, resting, and anomalous behaviors 5 min after injectionSucrose sham-fed intakes during 0, 15, 30, 45 min intervals 5 min after injectionSucrose intake (numbers of sucrose licks, bursts, clusters, and interlick intervals) at 15 min start of each test | Sham feeding of 0.8 mol/L sucrose was inhibited (almost %50) by each GLP-1 doses at all times, but GLP-1 did not terminate sham feedingThe frequency of sham feeding behavior (initial rate of licking, licks/minute) was reduced by GLP-1(3 and 10 μg)3 μg of GLP-1 decreased feeding during 45-min of behavioral testsGLP-1 did not alter other behavioral categories measuredThe frequency of resting behavior did not change by GLP-1 GLP-1 (3 and 10 μg) reduced significantly burst size and cluster size |
| (Edwards et al., 2000) | Adult male Wistar rats (250–300 g)  | ICV injected GLP-1 (3 nmol) or Agrp (83–132) in combination with GLP-1  | 1. ICV injected saline or a-melanocyte-stimulating hormone (a-MSH) at doses of 0.3, 1, 3 or 10 nmol2. ICV injected saline or a-MSH at a dose of 1 nmol3. ICV injected saline or CART 0.2 nmol, Agrp 1 nmol or CART and Agrp 4. ICV injected saline or GLP-1 3 nmol, CRF 0.3 nmol, Agrp 1 nmol, Agrp in combination with GLP-1  | Food intake was measured at 1, 2, 4, 8, and 24 h | 1 nmol a-MSH significantly reduced food intake at 1 h and 2 h and 1 nmol Agrp inhibited this anorectic effect CART reduced 1, but not 2 h food intake and Agrp did not affect this resultGLP-1 significantly reduced 1, 2, 4, and 8 h food intake, CRF significantly reduced 1, 2 and 4 h food intake and Agrp did not affect these results at each time pointCART, GLP-1, and CRF, unlike leptin, were not mainly dependent on MC4-R or MC3-R or any other postulated Agrp sensitive pathway for their function |
| (Dossat et al., 2011) | Naive male Wistar rats (325 g) | Intra-Lateral ventricle (LV) 0, 0.033, 0.15, 0.3, 1, or 3g of GLP-1Intra-NAc core 0.1, 0.025 μg of GLP-1/ 0.1 μg of GLP-1/ 1, 2, 3 μg of GLP-1 Intra-NAc shell 0.025 or 0.1 μg of GLP-1  | Exp1: Lateral ventricle (LV) injections of1. 0, 0.033, 0.15, 0.3, 1, or 3g of GLP-12. 0, 2.5, 5, 10, or 20 g of Ex9Exp2: NAc core injections of1. 0.025 μg of GLP-12. 0.1 μg of GLP-1 3. 1, 2, 3 μg of GLP-1 4. 0.5 μl saline Exp3:1. Intra-NAc core saline2. 0.1 μg of GLP-13. 1 ml of 0.6 M LiCl 4. 0.02% saccharin | Body weightFood intake measured 1, 2, and 24 h after injectionConditioned taste aversion (CTA)/Retrograde tracing into the NAc coreIHC for GLP-1 levelsc-Fos | Retrograde injections into the NAc core labeled caudal level NTS neurons and these NTS neurons were all GLP-1 positive; therefore, these results identified a strong GLP-1 projection to this forebrain region1 and 3 μg intra-LV GLP-1 injections reduced food intake at 1 and 2 h (but not 24 h); lower doses had no effect20 μg Ex-9 increased food intake at 1, 2, and 24 h; lower doses did not affect chow intake0.025 and 0.1 μg intra-NAc core GLP-1 injections reduced food intake at 1, 2, and 24 h, so even subthreshold intra-NAc core infusions resulted in decreased food intakeThe core of NAc was more relevant to the GLP-1 action then the shell because of the intra-NAc shell injection of the same doses of GLP-1 had no effect Intra-NAc core 3 μg Ex-9 increased food intake at 2 h and there was a trend of increased food intake at 24 h, meaning GLP-1 release from neurons at this site played a limiting role in the consumption of foodThere was no effect on body weight in both GLP-1 and Ex-9 injectionsIntra-NAc core GLP-1 (0.1 μg) injections increased the number of c-Fos-positive nuclei in the NAc core regionNAc core was a second site for GLP-1’s food consumption effects without induction of CTA, so anorexia observed after NAc GLP-1 injections was not due to viscerosensory stress, but due to increased satiety |
| (Dailey et al., 2012) | Sprague Dawley rats (287.5 ± 12.5 g) | High fat (6 h) and chocolate (2 h) entrainment on separate groups | 1. Standard chow meal entrained2. Standard chow ad libidum3. High-fat diet (HFD) meal entrained4. High-fat diet (HFD) ad libidum5. Chocolate entrained6. No chocolate meal given | Food anticipatory activity (FAA) 4 h before mealtime/Blood glucose levelsPlasma ghrelin and insulin levelsPlasma GLP-1 levels Plasma collected from rats killed at 90, 60, 30 min prior to mealtime | The chocolate-entrained group did not show the same anticipatory increases in ghrelin and GLP-1 seen in the chow- or high-fat-entrained animalsIn all entrainment groups, an increase in glucose and a decrease in insulin occurred before mealtimeElevations in ghrelin and GLP-1 plasma levels were not necessary for the FAA to occur; thus, no correlation between these systems could be deducedAnticipatory changes in peptides might be due to food restriction or decreased body weight compared with the ad libitum control animals and not by different meal entrainmentsGLP-1 increased before meal feeding in ratsThe amount of food eaten or the energy status of the animal might be more important than the feeding pattern for the preprandial rhythm in ghrelin and GLP-1Earlier increase in glucose in the groups that were expecting a greater caloric load might be correlated with the earlier onset and longer-lasting FAA response |
| (Mathes et al., 2012) | Male Sprague-Dawley rats | RYGB surgeryAll groups were tested in three conditions: water restricted, fasted, nondeprivedChow fed/Ex-9 (30 ug/kg) IPEx-4 (1 ug/kg) IP | Exp12: 1. Sham + Vehicle2. RYGB + Vehicle3. Sham + Ex-94. RYGB + Ex-95. Sham + Ex-46. RYGB + Ex-4Exp3:1. Ex-9 2. Ex-4 3. Ex-4 + Ex-9  | Short-term brief-access licking test (while fasted pre-surgically and post-surgically and while nondeprived post-surgically and 5 h after injections/ another group while post-surgically or 15 min after injections both fasted and non-deprived)Body weight  | Overall, sucrose lick scores of non-deprived rats were higher with Ex-9 than vehicle-injected rats/ however, this effect was small (15 min after injection)0.3 M sucrose intake was decreased significantly with Ex-4 injection in fasted rats (30 min and 1 h after trial initiation)When Ex-9 and Ex-4 injected together, this effect was decreased with Ex-9 |
| (Alhadeff et al., 2012) | Male Sprague-Dawley rats | Ex-4 (0.025 μg and 0.05 μg, unilateral) into VTA, NAc core and shell 1 h after the onset of light cycleEx-9 (10 μg) directed to either the VTA, NAc core or NAc shellimmediately before the onset of the dark cycle | 1. aCSF2. Ex-4 (0.025μg)3. Ex-4 (0.05μg) | Cumulative sucrose intake (sucrose presented immediately after injections, assessed at 2 h, 5 h, and 24 h after injection)Modified chow or high-fat food intake (assessed at 1, 3, 6, and 24 h after injection)Pica response (14 h after injection)Body weight (24 h after injection)/Double immunohistochemistry for Fluorogold (monosynaptic retrograde tracer) and GLP-1 Immunoreactivity for PPG levels in VTA, NAc core, NAc shell | Neurons that express PPG in the NTS projected directly to the VTA, NAc core, and NAc shellSucrose intake was reduced by intra-VTA and intra-NAc core (but not shell) Ex-4 (both subthreshold and above threshold doses) at 20, 30, 40, 50, and 60 min after injection Ex-4 at both doses resulted in reduced body weightBoth doses of Ex-4 into the VTA decreased HF diet intake at 6 and 24 h and increased chow intake at 3 h, along with 24 h reduced body weight gain, which indicated a shift in preference of highly-palatable foods0.05 μg Ex-4 into NAc core decreased HF diet intake at 3, 6, and 24 h, while 0.025 μg only decreased HF diet intake at 24 h, along with 24 h body weight decrease0.05 μg Ex-4 into NAc shell decreased HF diet intake at 6, and 24 hEx-4 into NAc shell increased chow intake at 3 h24 h of chow intake and body weight did not change by Ex-4 NAc (both core and shell)Intake of HF food was increased by intra-VTA (3, 6 h) and intra-NAc core (1, 3 h) Ex-9/ intra-NAc shell Ex-9 did not affect the intake of HF foodIntra-VTA, intra-NAc core, and shell Ex-4 did not induce a pica response or decrease chow intake |
| (Dickson et al., 2012) | Male Sprague Dawley rats (250 g) | Partially food restricted/ lower levels of endogenous GLP-1/Ex-4 (0.3 μg/kg, 2.4 μg/kg) IP Third ICV Ex-4 (0.03 μg, 0.1 μg, 0.3 μg, 1.5 g, 3.0 μg)Lateral ICV Ex-4 (0.2 μg) and Ex-3 (20 μg)Intra-VTA and -NAc Ex-4 (0.03 or 0.1 μg)Intra-VTA Ex-4 (0.01 μg) | 1. Vehicle2. Ex-43. Third ICV vehicle4. Third ICV Ex-45. Lateral ICV vehicle 6. Lateral ICV Ex-4 + vehicle 7. Lateral ICV Ex-4 + Ex-38. VTA Ex-49. NAc Ex-4  | PR operant conditioning at 10 min post-injection at 10, 30, 60, 120 min time pointsCPP at 10 min post-injectionMotor activity (locomotor and rearing) for 1 hPica responseBody weight for 1 and 24 hFood intake for 1 and 24 h | Ex-4 IP. decreased lever pressing in the operant conditioning test and abolished preference in the CPP testICV Ex-4 decreased the number of sugar rewards earned at most doses and at all time pointsEx-3 reversed the Ex-4 induced decrease in food-motivated behaviorAt a dose subthreshold ICV, intra-VTA Ex-4 (0.03 and 0.1 μg) reduced food-motivated behavior, chow food intake, and body weight without affecting locomotor activity or rearingNAc Ex-4 (only at 0.1 μg) decreased operant responding and food intake less effectively than VTA and did not affect body weightNAc Ex-4 also reduced locomotor activity and rearing for a short amount of timeVTA Ex-4 decreased operant responding for sucrose and food intake in satiated rats alsoSatiated rats that were more willing to work for sucrose at baseline (high responders) were more affected by VTA Ex-4 compared to less motivated rats (low responders)Neither VTA nor NAc Ex-4 treatments caused pica response |
| (Labouesse et al., 2012) | Male Sprague–Dawley rats (160-180 g) | SDA surgery: transection of the left dorsal vagal rootlets and dorsal esophageal vagal trunk resulting in complete vagal deafferentation below the diaphragmFood deprivation for 4 h before injections and 6 h before c-Fos/Ex-4 (1.0 and 0.1 μg/kg) IP | Exp1:1. SDA surgery2. Sham operationExp2:1. Ex-42. Bolus (1 ml/kg BW) | Cumulative food intake (0.5, 1, 2, 4 and 20 h)Meal patterns (first and second meal size and duration, inter-meal interval)Two-bottle choice paradigm (flavor avoidance learning)/IHC for c-Fos in NTS, DMX, AP, caudal VLM, PBN, DR, PVN, Arc, CeA, NAc | High dose (1.0 μg/kg) Ex-4 reduced 0.5 and 1 h food intake in both sham and SDA ratsLow dose (0.1 μg/kg) Ex-4 decreased 0.5 and 1 h food intake in sham, but not SDA-operated ratsBoth Ex-4 doses decreased food intake in other time points regardless of surgerySDA rats tended to have decreased food intake regardless of Ex-4Low dose Ex-4 decreased first meal size only in sham-operated ratsFirst meal duration, second meal size or duration, inter-meal interval, and number of meals in the dark phase did not depend on the surgeryEx-4 induced a conditioned flavor avoidance in the SDA group but not in the sham-operated group/ indicating vagal afferents protected against Ex-4 mediated CFAEx-4 caused activity in the AP, NTS, PBNle, CVLM, CeA, NAc and DMX regardless of surgeryEx-4 did not activate neurons in the PBN, DR, ArcEx-4 caused activity in the PVN of sham-operated rats but not SDA rats |
| (Liang et al., 2013) | Adult, male Sprague Dawley rats (250-275 g at arrival and 638 g during the drug injection tests)  | Ex-4 (1, 3.2, 10 µg/kg) IP | 1. Naltrexone (NTX) (0, 0.32, 1 and 3.2 µg/kg)2. Ex-4 (0, 1, 3.2, 10 µg/kg)3. NTX + Ex-4 | Short-term food intakeConditioned taste aversion (CTA) learning | NTX and Ex-4 decreased food intake dose-dependentlyNTX and Ex-4 combination decreased food intake and the amount of decrease was higher, which indicated an additive effectIn the CTA test, NTX did not change support acquisitionBoth Ex-4 doses (1 or 3.2 µg/kg) and combinations of NTX and Ex-4 changed acquisition of CTA rapidly and robustly |
| (Dossat et al., 2013) | Naive male Wistar rats | Exp1: Sweetened condensed milk (SCM)Exp2: 0.25M sucroseExp3: 0.10 sucroseExp4: 0.1% saccharinExp5: 3 ml IG 40% sucrose or saline/Intra-NAc core Ex-9 (3 μg on experiments 1-4 and 2 μg on experiment 5) 15 minutes prior to testing | Exp1-4:1. Ex-92.SalineExp5:1. Vehicle + intragastric saline2. Vehicle + intragastric sucrose3. Ex-9 + intragastric saline4. Ex-9 + intragastric sucrose | Body weightFood intakeLicking microstructural variables | Ex-9 increased first meal size and decreased the number of meals of sweetened condensed milk, without affecting total licksEx-9 increased 1st and 3rd meal size and total licks of 0.25M sucrose, without affecting the number/schedule of meals, satiety ratio, or within-burst interlick intervalFor 0.25M sucrose 1st meal, Ex-9 increased meal duration and initial lick rate, without affecting burst variablesFor 0.25M sucrose 2nd meal, Ex-9 increased burst size and duration and decreased the number of burstsFor 0.25M sucrose 3rd meal, Ex-9 increased burst size and duration without affecting the number of burstsEx-9 increased 2nd meal size and total licks of 0.10 sucrose, without affecting number/schedule of meals, satiety ratio, meal duration, number of bursts, or within-burst interlick intervalFor 0.25M sucrose 1st meal, Ex-9 increased the initial number of licks, burst size, and duration in the first segment of the mealFor 0.25M sucrose 2nd meal, Ex-9 increased burst size and durationEx-9 did not affect any variable for 0.1% nonnutritive saccharin, indicating the necessity of nutrients in the gut for the action of NAc GLP-1REx-9 reversed IG sucrose-induced decrease in size of first meal and burst size and duration in the first and third segmentIG sucrose decreased the number of licking bursts and first meal duration regardless of Ex-9IG sucrose or Ex-9 did not affect the number of licks in the first minute or within-burst interlick interval |
| (Mietlicki-Baase et al., 2013) | Adult male Sprague-Dawley non-obese (381.7 g, 11-14 weeks of age) rats | HFD (60% kcal from fat) during and for 2 weeks prior to testing/Intra-VTA Ex-9 (10 μg) unilateralIntra-VTA Ex-4 (0.05 μg) unilateralEx-4 (3 g/kg) IP | Exp1:1. VTA Ex-9 + Ex-4 or saline2. VTA saline + Ex-4 or salineExp2:1. VTA CNQX (AMPA/kainate receptor antagonist) + VTA Ex-4 or saline2. VTA MK-801 (NMDA receptor antagonist) + VTA Ex-4 or saline3. VTA saline + VTA Ex-4 or saline | HFD food intake for 24 hBody weight at 24 hMeal patterns (meal size, number of meals) for 24 h/Immublotting in the VTA for TH at 15 min post-injection/Patch-clamp electrophysiological recordings in the VTA before and during Ex-4 application (1 μM) | Intra-VTA Ex-9 attenuated the IP Ex-4-induced decrease in HFD food intake and body weightIntra-VTA Ex-4 was found to reduce HFD food intake (from 3 to 24 h) by decreasing meal size and these reductions were suppressed by AMPA/kainate receptor antagonist CNQXNMDA-R’s were not involved in the food intake and meal size suppressive effects of intra-VTA Ex-4Intra-VTA Ex-4 increased TH levels in the VTA compared to salineEx-4 increased sEPSC frequency in VTA dopamine, without changing sEPSC decay, time, peak amplitude, and charge transfer |
| (Alhadeff and Grill, 2014) | Adult male Sprague-Dawley rats (250 –300 g) | HFD (45%, 60% kcal fat)/100-nl unilateral Ex-4 (0.025 or 0.05 μg)100-nl unilateral mNTS injection of 0.025 μg of Ex-4 (0.025 or 0.05 μg) | 1. Vehicle2. Ex-4 (0.025 μg) | HFD intake at 1, 3, 6, and 24 h post-injectionBody weight at 24 h post-injectionPR operant responding 3 h after injectionCPP for palatable food 3 h after injection | mNTS-GLP-1R was found to decrease HFD intake and reduced PR responding for sucrose mNTS-GLP-1R caused a reduction of CPP for palatable foodmNTS-GLP-1R was observed to reduce chow intake and body weight, while not inducing pica response |
| (Alhadeff et al., 2014) | Adult male Sprague–Dawley rats (250–300 g) | HFD (45% kcal fat) for 5 days/Unilateral lateral parabrachial nucleus (lPBN) Ex-4 (0.025 or 0.05 μg depending on assessment)Ex-9 (10 or 20 μg) | Pica, chocolate pellets experiments: 1. Control 2. 0.05 μg Ex-4HFD, activity:1. Control 2. 0.025 μg Ex-4 3. 0.05 μg Ex-44. 20 μg Ex-9 | Body weight at 24 hHFD food (at 1, 3, 6, and 24 h), chow, and water intake (at 24 h)Pica responseOperant lever respondingActivity test/lPBN Fluorogold tracing IHC for GLP-1 in NTS | 71.3% of GLP-1 producing neurons of the NTS monosynaptically projected to the IPBN ipsilaterallyCerebral aqueduct delivery of neither dose of Ex-4 nor Ex-9 affected HFD intake at all time points compared to vehicleIntra-lPBN Ex-4 decreased cumulative (6 and 24 h) and noncumulative (3-6 h) chow and water intake without causing pica, but it did not significantly reduce body weightIntra-lPBN Ex-4 decreased cumulative (6 and 24 h) and noncumulative (3-6 h) HFD and water intake, and body weightIntra-lPBN Ex-9 increased cumulative chow (6 and 24 h) and HFD (6 h) intake without affecting body weightIntra-lPBN Ex-4 decreased number of chocolate pellets earned and active lever pressesNeither intra-lPBN Ex-4 nor Ex-9 significantly affected total distance traveled or total active time |
| (Yang et al., 2014) | Adult male Sprague Dawley rats (250 –275 g) | HFD or chow for 6 weeksPair-fed groups were included (control for food intake and body weight)/Ex-4 (3.2 μg/kg) IP, twice daily for 9 consecutive days | 1. HFD +Ex-42. HFD +Vehicle 3. HFD + Pair-def4. Standard chow (SC) + Ex-45. SC + Vehicle 6. SC + Pair-fed | Food intake daily (1, 2, 4, 8, and 24 h of intake)Body weight daily/Blood glucose, insulin, and leptin levelsAgouti gene-related protein (Agrp), neuropeptide Y (NPY), proopiomelanocortin (POMC) expressions in the hypothalamic arcuate nucleus (ARC) TH expression in the VTADopamine receptor (D1, D2) expressions in the NAc | Ex-4 decreased body weight and food intake in both SC and HFD/ this effect was seen earlier in the HF groupLeptin and insulin levels were increased in HFD group/ long-term Ex-4 treatment successfully decreased these effectsHFD decreased POMC but did not affect the expression of NPY or Agrp in the ARCEx-4 increased POMC expression while decreasing NPY, and this effect was independent of the reduction of food intake and body weight Mesolimbic TH and D1R gene expression were significantly decreased in chronic HFD rats/ Ex-4 and food restriction reduced these decreased expressions (only D1R changes reached significance) |
| (Mietlicki-Baase et al., 2014) | Adult male Sprague Dawley rats (375– 425 g) | HFD (60% kcal from fat) during and for 1 week prior to testing/Intra-NAc core injection of Ex-4 (0.05 µg)Bath application of 1µM Ex-4 for electrophysiological recordings | 1. NAc core AMPA/kainate receptor antagonist, CNQX (0.3 µg) or NAc core NMDA antagonist, AP-5 (1 µg) + vehicle2. CNQX or AP-5 + Ex-43. Vehicle4. Vehicle + Ex-4 | Body weight at 24 hCumulative HFD food intake (1, 3, 6, and 24 h)/Fast-scan cyclic voltammetry for dopamine release measurements/Whole-cell voltage-clamp recordingsPaired-pulse ratio (PPR) experiments | Ex-4 did not alter dopamine release in NAc core slicesEx-4 increased the frequency of NAc core MSN mEPSCs but did not affect kinetics or amplitude, indicating a presynaptic effect of GLP-1R activationEx-4 bath application decreased the PPR of evoked EPSCs, further supporting a presynaptic effect by increasing the probability of glutamate releaseEx-4 moderately suppressed the frequency of MSNs AP firing and slightly decreased (but significantly) resting membrane potentialIntra-NAc Ex-4 decreased HFD intake and CNQX pretreatment reduced this effectIntra-NAc core Ex-4 reduced 24h body weight gain and CNQX reversed this effectAP-5 did not alter the anorexigenic effects of Ex-4 |
| (Anderberg et al., 2014) | Male Sprague−Dawley rats (250 g at the beginning) | Rats were food-restricted (10 g of chow) overnight/Ex-4 (0.2 μg/) i.c.v. | Exp1:1. Food2. No foodExp2:1. Vehicle2. SKF, D1 receptor agonist (2 μg)3. SCH, D1 receptor antagonist (1 or 5 μg)4. QNP, D2 receptor agonist (10 μg)5. ETC, D2/D3 receptor antagonist (10 μg)6. Ex-4Exp3:1. Vehicle + vehicle2. Vehicle + Ex-4 (0.3 μg)3. ETC (3.3 μg) + Ex-44. ETC + vehicle | Chow intake for 20 min at 1, 2, and 24 hLocomotor activity at 20 min after injectionPR operant conditioning for sucrose at 20 min after injection/Amygdala dopamine turnover 30 min after injections or food intakeDOPAC and HVA tissues concentrations 30 min after injections or food intake | Chow food intake and Ex-4 increased DOPAC and HVA levels along with amygdala dopamine turnoverOperant responding for sucrose, chow intake, or locomotor activity was not changed by D1 receptor activation or D1 receptor blockadeFood intake was decreased along with food-motivated behavior by D2 receptor activation and increased by D2 receptor blockade in the amygdalaFood intake at all time points was decreased by central Ex-4 and D2/D3 receptor blockade reversed this effect Ex-4 reduced operant responding for sucrose, but D2/D3 receptor blockade did not attenuate this effect |
| (Hansen et al., 2014) | Male Sprague-Dawley rats (8 weeks of age) for acute drug treatmentMale Sprague-Dawley rats (6 weeks of age) for DIO study | Two-choice diet: rats fed standard rodent chow or HFHS diet for 17 weeks (diet-induced obese rats, DIO)/GLP-1 (0.4 mg/kg s.c.)Linagliptin (1.5 mg/kg) perorally or (0.5 mg/kg) s.c.Liraglutide (0.2 mg/kg) s.c.Acute or chronic treatment (bi-daily, 2 and 10 h into the light phase, for 28 days total) | Exp1: Acute treatment1. Vehicle2. Linagliptin 0.1 mg/kg3. Linagliptin 0.5 mg/kg4. GLP-1 0.2 mg/kg5. GLP-1 0.4 mg/kg6. Liraglutide Exp2: Chronic treatment (DIO rats)1. Vehicle2. Linagliptin 0.5 mg/kg3. Linagliptin 1.5mg/kg4. GLP-1 0.4 mg/kg5. Linagliptin 0.5 mg/kg + GLP-1 0.4 mg/kg6. Linagliptin 1.5mg/kg + GLP-1 0.4 mg/kg7. Liraglutide  | Whole-body composition and abdominal fat analysis in DIO ratsCumulative food intake 6-20 h for acute treatmentChow, high-fat palatable diet (HPFC) and cumulative daily caloric intake for chronic treatment/Active and total plasma GLP-1 levels and DPP-IV enzyme activity of DIO ratsISH for CART, NPY, μ-opioid receptor, prepro-enkephalin, prepro-dynorphin, TH, dopamine transporter in the forebrain, midbrain, and hypothalamus of DIO rats | Linagliptin alone did not affect acute food intake at any time point and GLP-1 alone slightly and short-lastingly reduced food intake at 4 h after treatmentA significant reduction in food intake was observed in combined linagliptin and GLP-1 in a dose-dependent manner until 6 h post-treatmentLiraglutide alone significantly decreased food intake from 4 h post-treatment until the end of the 20th hourBoth routes of linagliptin used in combined treatment affected the body weight loss equallyThe body weight was also reduced by liraglutide aloneA marked increase in chow preference and decrease in HFHS diet intake was observed in both combined linagliptin and GLP-1, and liraglutide alone; however, this increase was more pronounced in the combined treatmentActive and total plasma GLP-1 levels after 4 weeks of treatment were significantly higher compared to vehicle control and also GLP-1 alone in DIO ratsPrepro-dynorphin mRNA levels in the caudate-putamen but not in the NAc were increased in linagliptin (s.c.) and GLP-1 combined in DIO rats, but this effect was not observed with liraglutide aloneNo significant differences were seen in other transcripts and regions between treatment groups |
| (Wright and Rodgers, 2014) | 10 adult male Lister hooded rats(beginning weights: Exp1 202.5 g, Exp2 215.4 g) | Exp1. Ex-4 (0.025, 0.25 and 2.5 μg/kg) Exp2. Ex-4 (0.025 μg/kg and 0.25 μg/kg) | Exp1:1. Vehicle2. Ex-4 Exp2:1. Vehicle2. Ex-4 0.025 μg/kg + saline3. Ex-4 0.25 μg/kg + 0.1 mg/kg NTX 15 min between injections | Latency for food location and eating for palatable mash 15 min after injectionsFrequency and duration of food intake, drinking, grooming, scratching, sniffing, and locomotion behaviors for palatable mash 15 min after injectionsBehavioral satiety sequence for 1 h of palatable mash 15 min after injectionsWeight gain post-treatment | Ex-4 significantly reduced food intake in a dose-dependent manner The lowest dose of Ex-4 (0.025 μg/kg) failed to reduce food consumption and change eating behaviorAt 0.25 and 2.5 μg/kg doses, food intake, frequency, and consumption rate was decreased and these effects were more significant at 2.5 μg/kgDuration of eating bouts was increased, but there was no effect on the duration of eating at 0.025 μg/kg, but the total eating rate was observed to be declined dose-dependentlyDuration and frequency of sniffing, grooming and other locomotion behaviors and also resting periods were increased with Ex-4 dose-dependentlyHighest doses of both Ex-4 and NTX alone reduced eating behaviors considerably and produced an augmented dose-dependent anorectic effectNTX and Ex-4 combination failed to cause a more substantial decrease in food intake behaviors and degree of consumption relative to responses to Ex-4 and NTX alone |
| (Richard et al., 2015) | Adult, male Sprague-Dawley rats (180–250 g at arrival and 400 g during the drug injection tests) | Intra-NTS microinjection of Ex-4 (0.05 μg and 0.2 μg) | For CPP:1. Ex-4 (0.05µg) injected rats2. aCSF injected ratsFor RNA isolation and mRNA expression:1. 0.2 µg Ex4 injected rats2. aCSF injected rats  | Palatable food-choice test (at 1,3, 6 h after injection)Pica test (at 1,3,6, and 24h after injection)Food induced operant conditioning Conditioned place preference (CPP) (30 min after injection)/GLP-1 fiber detectionTH, Drd1a, Drd2, Drd3, Drd5, Slc6a3, Gad1, Creb1, FosB mRNA expression | Intra NTS Ex-4 injection suppressed palatable food intake contrary to vehicle-treated ratsChow intake was notably decreased with the chow introduction to rats alone after Ex-4 injection in a dose-dependent mannerEx4 injection decreased body weight at 22h No significant augmentation was seen in kaolin intake and there was no pica responseIntra-NTS Ex-4 administration reduced the number of sucrose rewards gained, which implicated a decline in food-motivated behavior whereas there was no change in locomotor activityTH positive neurons and PPG positive neurons were colocalized at area postrema TH encoding mRNA was fourfold, D2R in the VTA was twofold enhanced after intra-NTS injection; however, there was no alteration in FosB, Creb1, Gad1, D1aR, D3R, D5R expression in the VTAFosB, Creb1, Gad1, D1aR, D2R, D3R, D5R, and DAT did not significantly change in the NAc after Ex-4 injection |
| (Hsu et al., 2015) | Adult male Sprague-Dawley rats (320 - 450 g) | Exp1d. Western diet (41% kcal fat)Exp2a. 45 mg pellet (fat + sucrose)/Bilateral HPFv injections of Ex-4 (0.015, or 0.03 μg)Ex-9 (0, 1.25, 2.5, or 5, 10 μg) | Exp1a. HPFv or lateral ventricle (LV) Ex-4 (0.015, or 0.03 μg) or vehicleExp1b. HPFv Ex-4 (0.03 μg) or vehicleExp1c. HPFv Ex-(9-39) (1.25, 2.5, or 5 μg) or vehicleExp1d. HPFv Ex-4 (0.03, or 0.06 μg) or vehicleExp2a. HPFv Ex-4 (0.03 μg) or vehicleExp2b. HPFv Ex-4 (0.03 μg) or vehicleExp3. HPFv Ex-4 (0.03 μg) or vehicle, or 0.15 M LiCl or saline | Chow intake at 1, 3, 6, and 24 h after injectionsBody weight at 24 h post-injectionMeal pattern immediately after injectionOperant responding for food, PR reinforcement schedule 3 h post-injectionCPP at 3 h post-injectionConditioned flavor avoidance (CFA) at 3 h post-injection/IHC for GLP-1 in the hindbrainGLP-1 levels in blood, CSF, and HPFv | Ex-4 delivered to HPFv, but not LV reduced cumulative food intake at 3, 6, and 24 h and body weight at 24 hHPFv Ex-4 decreased chow intake, meal size but did not affect meal frequency 10 μg HPFv Ex-9 increased food intake only at 6hHPFv Ex-4 increased preference for chow diet over the Western dietHPFv Ex-4 decreased the total number of active lever presses, the number of pellets earned, and operant response to foodHPFv Ex-4 did not affect CPP or CFAGLP-1R axons were not detected in ventricular or dorsal HPFGLP-1R axons were found closely opposed to ventricular ependyma, including PVN, BNST, and subfornical organFG injected into LV was found in some GLP-1R neurons in the NTSActive GLP-1 was found from highest to lowest in the serum, CSF and HPFv lysates |
| (Richard et al., 2016) | Female and male Sprague-Dawley rats (160–200 g) | Food-restricted (50% of normal intake) or non-deprived state/Ex-4 into left ventricle (LV) (0.1 or 0.3 μg/μL)ICV Ex-4 (0.3 μg/μL) | Exp1: Non-deprived or food-restricted1. Vehicle2. Ex-4 into LVExp2: Food-restricted1. Estrogen receptor antagonist, ICI (10 μg/μL) into LV + vehicle2. Vehicle 3. ICI (10 μg/μL) into LV + Ex-44. Ex-4Exp3: Food-restricted1. Estrogen receptor-α (ERα) antagonist, MPP2. Vehicle3. MPP4. Ex-4 | PR operant conditioning at 20 min after injectionChow (for 24 h) and palatable (peanut butter, for 1 h) food intake | Central GLP-1R activation in non-deprived animals was observed to decrease food-motivated behavior significantly more in females relative to malesNot only desire for sucrose and peanut butter but also desire for chow intake decreased after 24 h in both sexes after Ex-4In food-restricted animals, Ex-4 again decreased motivated eating behavior, but this time, sex was statistically insignificantICI was observed to attenuate effects of Ex-4 on food behavior, in both sexesEx-4 alone or in combination with ICI reduced peanut butter consumption in females, but not in malesEx-4 alone or in combination with ICI reduced chow consumption in males; this reduced consumption was only seen in Ex-4 in femalesMPP attenuated effects of Ex-4 on food behavior in both sexes |
| (Vogel et al., 2016) | Adult male Sprague-Dawley rats (200-250 g) | 2 μg GLP-1-estrogen (1.87 μg GLP-1, and 0.13 μg estrogen) s.c.0.125 μg GLP-1-estrogen (0.117 μg GLP-1, and 0.008 μg estrogen) ICV0.075 μg GLP-1-estrogen (0.07 μg GLP-1, and 0.005 μg estrogen into VTA, LH, NTS, and supramammillary nucleus (SUM) | 1. GLP-1 2. GLP-1-estrogen3. Estrogen4. Vehicle | Sucrose driven PR response in operant conditioning after 90 (s.c.) or 30 (central) min injectionsChow intake for 1 and 24 hBody weight for 1 and 24 hPica responseLocomotor activity in the activity chamber at 30 min post-injection/SPECT-imaging of regional cerebral blood flow (rCBF) | Peripheral (s.c.) GLP-1-estrogen reduced food intake and body weight more than sole GLP-1 or estrogen (GLP-1, estrogen or vehicle alone did not reach significance)Peripheral conjugate injection reduced the number of sucrose pellets earned and active lever presses more efficiently than GLP-1 or estrogen aloneAfter peripheral injections, pica responses were similar in all groups ICV GLP-1-estrogen application had the same effect and did not cause malaise or locomotor impairmentThe SUM was found to be the target site of GLP-1-estrogen as increased blood flow was observed mostly in this areaGLP-1 alone successfully reduced food reward in the VTA/ however, the conjugate was not effective Intra-SUM GLP-1-estrogen reduced food reward and food intake more than GLP-1 or estrogen aloneIntra-LH GLP-1-estrogen reduced food intake and body weight more than GLP-1 or estrogen-onlyIntra-NTS GLP-1-estrogen reduced food intake and caused a trend toward decreased body weight more than GLP-1 or estrogen |
| (Terrill et al., 2016) | Naive male Wistar rats | HFD (%60 fat)/Ex-4 (0.2 μg) i.c.v. Unilateral, intra-LS Fluor 647-labeled Ex-4 (0.025 μg) Intra-LV Ex-4 (0.025, 0.05, or 0.1 μg) 30 min before darkIntra-LS Ex-4 (0.025 μg) 30 min before darkIntra-LS Ex-9 (10 μg) 30 min before dark | Exp1-2:1. Veh2. Intra-LS Ex- 4 (0.01 or 0.025 μg)3. Intra-LS Ex-9 (5 or 10 μg)Exp3:1. Veh2. Ex-4Exp4: 1. Veh2. Ex-9Exp5-8:1. Veh2. Ex-93. Ex-4 | Chow intake at 1, 2, 4, and 20 h after injectionChow intake at 24 h after injection in other behavioral testsHFD (sucrose and corn oil) intake every 30 min for 2 hPica response (kaolin intake) for 20 hMeal pattern analysisElevated plus maze (EPM)PR operant responding for 2 h/Fluorescent immunostaining and fluorescently labeled Ex-4 in LS | Chow intake was reduced with doses of 0.05 and 0.1 μg at 20 h; however, 0.025 μg of Ex-4 did not differ from saline Body weight was reduced by intra-LS Ex-4 as Ex-4 decreased overnight chow and HFD intakeChow intake was increased by intra-LS Ex-9Average dark-phase meal size and average light-phase meal size were decreased after intra-LS Ex-4 Intra-LS Ex-4 did not induce pica responseSucrose intake and corn-oil emulsion intake were increased with intra-LS Ex-9 injection, but chow intake was not changedBreakpoint and total active lever presses and overnight chow intake were increased by intra-LS Ex-9Total arm entries or entries into open arms were not affected by Ex-4, but Ex-4 significantly decreased the number of entries into closed arms in EPMEnsure preload significantly reduced PR respondingIn both the non-preload and Ensure conditions, intra-LS Ex-9 showed no effect on the number of sucrose reinforcers earned or total active lever presses Chow intake was reduced by Ensure compared control no-preload conditionIntra-LS Ex-9 increased 24-h total kilocalorie intake after Ensure load |
| (Alhadeff et al., 2017) | Adult male Sprague-Dawley rats (250-265 g) | Novel adeno-associated virus (AAV-GLP-1R) that has RNA sequences to knock down GLP-1R to NTS | 1. AAV-Control2. AAV-GLP-1R | Food intake dailyBody weight dailyMeal patterns by feedometer 3 days for 2 weeks post-injectionOperant responding for 45 mg sucrose pellets/Real-time PCR for GLP-1R mRNA levels | AAV transfected tissue showed 66.5% reduction in expression of GLP-1R compared to the control group in NTS tissuesAAV-GLP-1R resulted in increased chow intake, dark cycle cumulative food intake, and meal size, but it did not increase the body weight and dark cycle meal numberNo difference in light cycle cumulative food intake or meal number was observedNTS AAV-GLP-1R increased the motivation to take the palatable food after training to receive 45 mg sucrose intakeNTS GLP-1R mRNA expression negatively correlated with lever presses and reinforcers earned in the PR responding task |
| (Ong et al., 2017) | Adult male Sprague Dawley rats (250 - 265 g) | Chow or high-fat diet (HFD/ 45% kcal/fat)/Intra-PVT Ex-4 (12.5 ng, 25 ng, 50 ng) Ex-9 (5 and 10 μg) | 1. HFD2. Chow3. HFD + Ex-44. Chow + Ex-4 | Chow and HFD intake at 1, 2, 4, 6, and 24 h post-injectionMeal patternsCPP for HFD 4 h after injectionsCue-induced reinstatement of sucrose-seeking behavior 4 h after injectionsPR responding for sucrose reward 4 h after injectionsPica response/IHC for c-Fos, GLP-1, FLEx, and FG-labeled cells in the caudal NTS and PVT/Whole-cell patch clamp recordings for PVT-NAc projecting neurons | Intra-PVT Ex-4 decreased chow and HFD intake while intra-PVT Ex-9 increased it, independent of malaiseIntra-PVT Ex-4 attenuated CPP for HFD and lever pressing for sucroseFood intake activated NTS PPG neurons, PVT projecting NTS neurons, and NTS PPG neurons that monosynaptically project to PVTPVT GLP-1 axons were found closely opposed to NaC projecting fluorescein Ex-4 labeled cellsEx-4 suppressed AP firing on PVT-to-NAc projecting neurons partly through synaptic mechanisms/ these inhibitory effects were blocked by Ex-9 (dose-dependently)Ex-4 induced suppression of AP firing was smaller and delayed when given with synaptic blockers (CNQX for glutamate receptors, PTX for GABA receptors)Ex-4 resulted in hyperpolarization of PVT-to-NAc neurons in the presence of synaptic blockersEx-4 decreased frequency, but not amplitude, of spontaneous and miniature EPSCs in PVT-to-NAc (both core and shell) projecting cells |
| (Hsu et al., 2018) | Male Sprague–Dawley rats (320-450 g) | 1. Implanted bilaterally with vHP and mPFC-targeted cannulae, unilateral mPFC injection of AP-5 or vehicle, then vHP exendin-4 delivery 2. Short hairpin RNA targeting GLP-1R mRNA was cloned, packaged into an adeno-associated virus (AAV1), delivered bilaterally to the vHP (vHP GLP-1R KD group)/Intra-ventral hippocampus (vHP) injection of Ex-4 (0.03 μg)200 nl AAV-GLP-1R shRNA (vHP GLP-1R KD group) | 1. mPFC AP-5 + vHP Ex-42. mPFC CNQX (0.3 μg) + vHP Ex-4Bilateral vCA1 administration of: 1. Control (aCSF)2. Control (AAV1-GFP)3. AAV1-GLP-1R shRNA | Body weightFood intakeOperant conditioningDifferential reinforcement of low rates of responding (DRL)/FISH for GLP-1R in ventral hippocampal (vCA1)Immunohistochemistry (IHC) for PHAL, pNR2B, Anterograde neural pathway tracing from the vHP field CA1 and AAV1-GFP | All FG back-labeled vCA1 cell bodies co-expressed GLP-1R mRNAvHP GLP-1R to mPFC NMDA-R neural pathway regulated impulsive responding for palatable food, but not AMPA/kainate receptor signalingmPFC pretreatment with vehicle followed by vHP Ex-4 exhibited a significant decrease in food intake and body weightvHP GLP-1R KD rats tended to choose greater meal size with a compensatory decrease in meal frequency, as well as a significant increase in inter meal interval compared to controlsvHP GLP-1R KD rats earned a significantly greater number of pellets across a 5-day training period in comparison to control AAV and aCSF controlEx-4 also significantly increased efficacy (pellets earned/ total number of levers pressed) in an inhibitory control task  |
| (Maske et al., 2018) | Naive male (25-300 g at arrival)Female (175-200 g at arrival) Wistar rats | Intragastric (IG) nutrient infusion for sucrose (15 kcal) and Ensure (9.3 kcal)/Ex-9 (100 μg/kg IP) | Exp1&2:1. Saline 2. IG infusion of 10 ml 40% sucrose solution Exp 3:1. Saline 2. IG infusion of 10 ml Original Chocolate Ensure Exp4: 1. 0.9 % saline 2. 1.46 % saline 3. 3.415% salineExp5: 1. Vehicle 2. Ex-9 (100 μg/kg) 3. DVZ (0.5 mg/kg) 4. 10 ml of 0.9% saline 5. IG infusion of 10 ml Original Chocolate Ensure  | Progressive ratio (PR) schedule of reinforcementBody weight Overnight chow intakeOvernight water intake/Vaginal cytology to define the estrous cycle in female rats | IG infusion reduced active lever presses, breakpoint, and number of reinforcers earned, which can partly be mediated by a decrease in motivation to get food in both male and female ratsWhen released in response to IG infusion, GLP-1 might play a role in this effect in malesIG infusion of saline was not sufficient to reduce motivation for food, so it is the caloric content of the infusion that decreased the motivation for foodFood deprivation did not alter the effect of IG nutrients to reduce motivation for food The gastrointestinal capacity did not play a role in the suppression of PR responding or breakpoint IG infusion of Ensure decreased active lever presses, breakpoint, and number of reinforcers, meaning reinforcer, and the IG nutrient didn't have to be the sameThe suppressive effect of Ensure was blocked by the Ex-9 treatment in males but not females, measured by active lever presses and reinforces earned, without affecting the chow intakeDevazepide, a CCKa-R antagonist, treatment failed to influence the reducing effect of IG infusion on food motivation in both males and females |
| (López-Ferreras et al., 2018) | Male and female Sprague-Dawley rats (5 weeks old) | GLP-1R KO in LHEx-4 (0.05 μg and 0.15 μg)Ex-9 (10 μg)  | Exp1:1. Ex- 4 (0.05 μg)2. Ex-4 (0.15 μg)3. aCSFExp2:1. Ex-9 (10 μg)2. aCSFExp3: 1 h per day for one week, every other day1. Fat diet 2. Chow diet 3. Sucrose diet | Progressive ratio operant conditioning for sucrose at 20 min after injectionFood seeking with head-pokes into the chamberFood intake at 1 and 24 h after operant conditioning testFood intake and body weight daily (GLP-1 KD mice)Intraperitoneal glucose tolerance test/Fasting blood glucose analysis qPCR for IL6, IL1, melanin-concentrating hormone (MCH), neurotensin, orexin expressionsViral tract tracingIn situ hybridization (ISH) | Intra-LH Ex-4 (0.05 and 0.15 μg) significantly decreased food-motivated behavior (both reduced number of sucrose rewards earned and number of active lever presses) in male ratsIntra-LH Ex-4 (0.05 and 0.15 μg) decreased food intake at both 1 and 24 h in malesOnly 0.15 μg intra-LH Ex-4 injection resulted in decreased sucrose rewards earned and food intake (at 24 h) in femalesIntra-LH Ex-4 decreased food reinforcement only in the estrus phase and not in metestrus/diestrus and reduced food intake at 1 and 24 h was more robust in the estrus phase as wellBilateral LH Ex-9 injections increased food reward earned, active lever presses, and food-seeking in males and not in femalesBilateral LH Ex-9 did not affect food intake at 1 and 24 hGLP-1R KO increased operant responding for food reward and food intake in non-restricted male rats but not in femalesLH-GLP-1R activation reduced orexin expression but only in female rats during the estrus phaseIL1, IL6 expressions significantly increased in male rats after LH-GLP-1 Ex-4 injections, and this increase in IL6 was only seen in consumption of sucrose but not chow or lardExpression of MCH (an orexigenic LH neuropeptide) was reduced by LH-GLP-1R activation in both males and females (during all female cycles), which could be associated with both the reinforcement and adiposity effects of LH-GLP-1R activationFemale brains more readily compensated for the decreased LH-GLP-1R signalILs and MCH were affected in both sexes, where reduction in orexin and neurotensin detectable only in females, specifically in the estrus phase |
| (Howell et al., 2019) | Male Sprague-Dawley rats | Ad libitum access to standard rodent chow and water were given to mice/Ex-4 (IP, 0.1–1.0 μg/kg/ VTA, 0.01–0.1 μg) | 1. VTA Ex-4 + 300 pmol ghrelin2. VTA Ex-4 (0.01–0.1 μg) + saline vehicle | Operant responding for food reward with banana-flavored sucrose pellet reinforcers | The operant responding for food reward was increased by ghrelin administration into the VTA, and the peripheral injection of Ex-4 decreased this effectBoth IP and VTA Ex-4 injection caused a significant decrease in operant responding for food reward that was constructed by ghrelin administration |
| (Jones et al., 2019) | Male and female Sprague-Dawley rats  | Standard low-fat chow and water ad libitum for 2 weeksSome rats received a Western diet (high-fat (42%) and high-carbohydrate (39%) WD)/Liraglutide (10 μg/kg) IP | 1. Chow + saline2. Chow + liraglutide3. Western diet (WD) + saline4. Western diet (WD) + liraglutide | Body composition and body weight at terminal training (before diet and drug)Transfer cue training: 12 daily, 4 min trials with a 10 s clicker that ended with the delivery of sucrose pellets (C+), except on trials where the clicker was presented without the sucrose reward (C-)sFN training: 10 second tone (3000 Hz) which ended with sucrose reward at the end of each 4 min trial (T+), except on trials where the tone was preceded by the presentation of a 4 min light (T-) and no sucrose reward followedProbe test began 4 d and 12 d after diet and drug initiationMean nose pokes | Liraglutide did not show any significant effect on body weight; however, the body fat gain of liraglutide injected rats were significantly lower compared to saline-injected ratsAfter the transfer cue training, the rats responded the Target+ (T+) trials more in comparison to non-target trialsMean nose pokes of rats treated with liraglutide were lower than rats treated with saline only on C- trials where there were not different in C+ trialsMean nose pokes of rats treated with chow liraglutide were lower than rats treated with chow saline in both T+ and T- trialsMean nose pokes of rats treated with WD + liraglutide were lower than rats treated with WD + saline in both T+ and T- trialsBoth sex and diet type were independent of the effects of liraglutideThese results showed that liraglutide successfully enhanced the effect of inhibition to suppress responding to a reward cue/ However, it did not have a significant impact when the inhibitory cue was absent |
| (López-Ferreras et al., 2019) | Male and female Sprague-Dawley rats (3 weeks old) | High fat and sugar choice diet (HFHS) (a choice of lard, 30% sucrose solution, and chow)/Ex-4 into the supramammillary nucleus (SuM) (0.01 and 0.03 μg)Ex-9 (10ug)GLP-1R knockdown in the SuM, AAV-GLP-1R-shRNA | Exp1:1. Male2. FemaleExp2:1. Vehicle2. 0.01 μg Ex-43. 0.03 μg Ex-4Exp3:1. Vehicle2. Ex-9Exp4:1. Vehicle2. Ex-4Exp5:1. Control2. GLP1rKD | Operant response testing Locomotor activityChow, high caloric food intake (1 and 24 h)Body weight (24 h)/IHC for mCherryNeural tract tracingBlue-fluorescent DNA stain for cell countRNAscope Multiplex Fluorescent kitIn situ hybridization for GLP-1RqPCR for GLP-1 mRNA expressionHematoxylin and eosin stainingAdipose tissue collection | SuM bundle of fibers innervated the LH and dense GLP-1R mRNA expression was seen throughout the SuM fibers that projected to LH in both males and femalesSuM Ex-4 reduced ad libitum intake of chow in both male (both doses) and female rats (only the higher dose)/ along with reduced body weight at 24 hSuM Ex-4 reduced high-calorie food intake in both males and femalesSuM Ex-4 reduced the number of sucrose rewards and food-seeking behavior only in male ratsSuM-Ex-4 did not change food-motivated behavior in females at all cyclesIntra-VTA Ex-4 reduced motivation and food-seeking in femalesFood-seeking and adiposity in obese male rats was increased by LH, VTA, SuM GLP-1R KD without altering food intake, body weight, or food motivation in lean or obese, females were not affected as much as males in SUM, but they were similar in VTA injections |
| (Gabery et al., 2020) | Diet-induced obese (DIO) mice and rats | High-fat diet/Semaglutide (1, 3, 10, 30,100 nmol/kg/day) s.c.VitoTag750-S-labeled semaglutide (acute: 6 hours i.v., steady-state: 5 days, once daily s.c.) | Exp1:1. Vehicle2. SemaglutideExp2:1. Vt750-semaglutide2. Vt750-liraglutide | Body weight, fat massFood intakePalatable food preferenceCalorimetry/Fluorescent immunostaining and fluorescently labeled semaglutide and liraglutideWhole-brain c-Fos, GLP-1R immunoreactivityIHC for GLP-1R, SST, TH, CART, MCH, orexin, prolactin-releasing hormone (PrLH) , p-CREBqPCRConnectivity analysis/Light sheet fluorescence microscopyElectron microscopy | In DOI mice, semaglutide reduced food intake, body weight and fat mass dose-dependentlyIn DOI rats, semaglutide transiently reduced food intake and decreased body weight dose-dependentlyIn DOI mice, semaglutide significantly decreased chocolate intake compared to vehicle, but total chow intake was similar between groupsSemaglutide targeted GLP-1R positive neurons in hypothalamus and hindbrain which were colocalized with other genes involved in control of appetite (CART, SST, TH)Semaglutide induced neuronal activation overlapped with neuronal pathways involved in meal termination controlled via lateral parabrachial nucleusIn DOI rats, semaglutide increased PrLH and TH levels in area postrema |
| (Pierce-Messick and Pratt, 2020) | 16 male Sprague-Dawley rats | Ex-4 (0.05, 0.10 μg per side) intra-NAc coreEx-9 (2.5, 5.0 μg per side) intra-NAc core | Exp1:1. Ex-42. Ex-4 + DAMGO (μ-opioid receptor agonist)Exp2:1. Ex-92. Ex-9 + DAMGO | Food and water intakeLocomotor activity | DAMGO significantly increased food intake and this effect was reduced by both doses of Ex-4DAMGO enhanced ambulation, rearing, and locomotion and were blocked by Ex-4DAMGO and Ex-9 combination significantly increased the length of food intakeEx-9 did not change the DAMGO induced ambulation and locomotor measurements |
| (Konanur et al., 2020) | 22 Long Evans rats  | Fiber photometry surgery/Ex4 (0.05, 0.1 μg) into lateral ventricle | 1. Vehicle 2. Ex-4 | Licking behavior/IHC for GFP and THFiber photometry for measuring the activity of dopamine neurons | Ex-4 decreased the sucrose directed licking behavior and cue evoked dopamine activity in the VTASucrose directed behaviors was found to be associated with magnitude of cue evoked dopamine activity |
| (Vestlund et al., 2020) | Male Wistar Rcc Han rats (160-190 g) | Ex-4 (1.2 μg/kg) i.p.Ex-4 (0.05 μg per side) NAc shellLiraglutide (0.1 mg/kg) s.c.Dulaglutide (0.1 mg/kg) s.c. | Exp1:1. Ex-42. Liraglutide3. DulaglutideExp2:1. Intra-NAc shell Ex-4 | Montoya staircase test (measures skilled reach foraging for sucrose pellets)Skilled reach performance testBody weightRotarod test/Ex vivo field potential recordingsWhole cell recordings | Ex-4 and liraglutide significantly decreased motivation for consumption of sucrose pellets in rats with acquired skilled reach performance (trained) but this was not seen with dulaglutideIntra-NAc shell Ex-4 also significantly reduced motivation in trained ratsEx-4 and liraglutide suppressed evoked field potentials in NAc shellDulaglutide increased learning of skilled reach foraging (increased sucrose pellet consumption and better success rate) in untrained rats but this was not seen with Ex-4 or liraglutide |
| **1.b. Mice** |
| (Wang et al., 2015) | Phox2b-Cre BAC transgenic mice  | HFD (45 kcal%)/Chemogenic activation of GLP-1 expressing neurons in NTS by clozapine-N-oxide (CNO) injectionEx-4 (2.4 μg/kg, IP. and 0.24 μg/kg for intracranial)Exn-9 (8.4 μg/kg) IP | Exp1:1. Control 2. CNOExp2:1. Control2. hM3Dq (activates neuronal burst firing)3. hM4Di (inhibits neuronal firing)Exp3: 1. Control 2. Ex-4  | Chow and HFD intake at 2, 5, and 24 h post-injectionBody weight/IHC for TH, vGlT1, vGLuT2, and GLP-1 levelsRetrograde labeling of VTA-to-NAc medial-shell-projecting neurons/Electrophysiological recordings from adult mouse midbrain | Highly palatable HF food intake was decreased by activation of NTS GLP-1 neurons by releasing GLP-1GLP-1 release into the VTA caused decreased HF food intakeEx-4 application TH+ VTA-to-NAc projecting DA neurons suppressed AMPA-R-mediated EPSCs without changing NMDA EPSCsIn the presence of TTX, Ex-4 significantly decreased the amplitude of DA neuronsThere were no changes recorded in mEPSCs of non-DA neurons |
| (Sirohi et al., 2016) | GLP-1R KDNestin mice FLOX mice | Ad libidum chow or HFD/GLP-1R KD Nestin (GLP-1R selectively abolished from CNS)Ex-4 (30 μg/kg) IP | 1. Saline2. Ex-4 | Locomotor activityFood intake (HF or chow) after 21 h of deprivationBody weight each hour for 2 h | Ex-4 completely blocked chow intake in both groups at 2nd hour and HFD intake in FLOX mice entirely and only to a certain extent in GLP-1R KD Nestin miceLocomotor activity did not change in both groups after Ex-4No weight change was detected in both groups |
| (Yamaguchi et al., 2017) | C57/BL6J male mice (9 weeks old) | Binge-like sucrose overconsumption model:7–8 day acclimation periodPre-training, ‘limited access’ training, and post-training phasesIn all three phases, 0.5 M sucrose, water and chow were provided dailyIn 'limited access' training (10 days), only water was given at night without chowIn the pre and post-training, chow and water was given between 17:00-9:00, but sucrose was not/Lower GLP-1 group (500 nmol/kg IP BW) and higher GLP-1 group (1000 nmol/kg IP BW) | 1. Saline2. Lower PYY (12.5 nmol/kg BW IP)3. Middle PYY (25 nmol/kg BW IP)4. Lower CCK-8 (2 μg/kg BW IP) 5. Higher CCK-8 (4 μg/kg BW IP)6. Lower GLP-1 (500 nmol/kg BW IP)7. Higher GLP-1 (1000 nmol/kg BW. IP) | Taste aversion conditioning performed at day 11Sucrose and saccharin intake (pre-training, day 1, 10, 11)/PYY, GLP-1, CCK-8 levels | Exogenous PYY, CCK-8, and GLP-1 (both high and low doses) suppressed the consumption of sucrose compared to saline injectionsHigher doses of PYY, CCK-8 and GLP-1 did not result in conditioned taste aversion for saccharin, indicating these molecules did not cause visceral distress |
| (Mella et al., 2017) | 8-12 weeks old BALB/C male mice (20-25 g at arrival) | Cafeteria (CAF) diet (chow ad libidum, rotation of 10 sweet and 10 savory foods) or chow diet (ad libitum chow) for 12 weeks/Ex-4 injection of (0.1, 1, 5, and 25 μg/kg) IP | 1. CAF diet 2. Control diet  | Preferred food intake during weeks 1 to 8 of the CAF dietChow intake at 1, 3, 6, and 24 hours post-injectionBody composition analysis Conditioned place preference (CPP) test for chocolate at 10 weeksChow and kaolin intake 3 hours after injection/Plasma GLP-1 levelsHypothalamic GLP-1R mRNA expression | Ex-4 non-significantly decreased chow intake at 1, 3, and 6 hours but not at 24 hours post-injectionMice fed the CAF diet showed higher weight gain, fat mass and developed preference patterns of palatable food over timeCAF diet mice had decreased anorectic effect of Ex-4 on blocking of novel palatable food intakeWhile there was palatable food present, the effect of Ex-4 decreased in terms of food inhibitionBoth CAF and chow diet mice showed a CPP for chocolate and Ex-4 only successfully blocked this effect on chow diet miceMice fed the CAF diet showed a decrease in GLP1R mRNA expression in the hypothalamus compared to chow mice, but did not change active plasma GLP-1 levelsEx-4 decreased chow intake, but it did not increase kaolin intakeEx-4 doses used did not produce malaise |
| (Williams et al., 2018) | Naïve male C57Bl6J mice (22 g) | Cre-expressing miceHFD (60% fat) for 2 weeks prior to injections/Intra-BNST GLP-1 (0.1, 0.3, or 1.0 μg)Intra-BNST and intra-NAc Ex-9 (3 or 10 μg) | 1. Chow diet, intra-BNST GLP-1 (0.1, 0.3, or 1.0 mg) or saline2. Chow diet, intra-BNST Ex-9 (3 or 10 μg) or saline3. HFD, GLP-1 + saline or Ex-9 + saline | Food intake at 1, 2, 4 and 22 hBody weight dailyRestraint stress (30 min)/Fourth generation neuroanatomical tracing: Distribution of PPG axons, NTS-PPG neuron projections to BNST/BNST electrophysiologyResting fire frequency recordings | NTS-PPG neuronal projections to BNST and other areas were similar with the YFP-PPG projections/ which showed strong projections between these regionsIntra-BNST GLP-1 decreased chow intake at 1,2 and 4 h at all doses dose-dependently while Ex-9 increased itIntra-BNST GLP-1 less effectively suppressed feeding in HFD at 1 and 2 h (more significant at higher doses) and Ex-9 did not change intake in HFD miceIntra-NAc Ex-9 did not affect chow intakeIntra-BNST Ex-9 attenuated restraint stress-induced hypophagia as food intake was higher at all time points compared to vehicle100 nM GLP-1 reversibly depolarized or hyperpolarized BNST neurons, which was opposite to the response when dopamine was applied |
| (Terrill et al., 2019) | Naive male and female C57Bl6J mice or transgenic mice | Transgenic mice that express the YFP variant under the control of mGLU on a C57Bl/6 backgroundExp2: Large meal of chocolate Ensure 15 min prior to dark onset for 20 days (training period), access to Ensure for the 15 min just before lights out (experiment days)/Intra-LS GLP-1 (0.3 and 1.0 μg) iBilateral intra-dorsal subregion of LS (dLS) GLP-1 (1.0 μg)Intra-LS Ex-9 (3 and 10 μg) Bilateral intra-dLS Ex-9 (10 μg) | Exp1:1. Intra-LS saline vehicle 2. Intra-LS GLP-1 (0.3 and 1.0 μg)3. Intra-LS Ex-9 (3, 10 μg)Exp2-41. Intra-LS saline vehicle 2. Intra-LS Ex-9 (10 μg)Exp5:1. Bilateral intra-dLS saline vehicle2. Bilateral intra-dLS GLP-1 (1.0 μg)3. Bilateral intra-dLS Ex-9 (10 μg)Exp6:1. Saline vehicle2. GLP-1 (1.0 μg)3. Ex-9 (10 μg)  | Body weight Cumulative chow intake for 1, 2 and 4 h after dark onsetTotal session lick counts for sucrose daily (120 min)FR on operant responding 45 min after injectionsMeal patterns (meal duration, ingestion rate)/GFP immunoreactivity in hindbrain PPG neurons | Chow intake of mice was reduced (both in the dark phase and overnight intake) with intra-LS GLP-1 at subthreshold dosesIntra-LS GLP-1 with Ex-9 (at subthreshold doses) did not change chow intakeEnsure intake (large meal) before test sessions decreased subsequent chow intake and this effect was reserved with intra-LS GLP-1R Ex-9, but it did not affect body weightIn the saline group, the acute stress significantly decreased food intake, but there was no effect of Ex-9 to the food intake during acute stressWhole intra-dLS GLP-1 decreased active lever presses; they were increased with bilateral dLS Ex-9 in operant responding (this effect remained even after mice were returned to ad libidum food intake)Lick rate during the first minute of the meal was suppressed by intra-dLS GLP-1 in ad libidum fed miceIntra-dLS GLP-1 suppressed burst number, whereas Ex-9 increased it, but there was no effect on burst size and durationAfter dLS GLP-1, the ingestion rate was decreased significantly |
| (Decarie-Spain et al., 2019) | 8-week-old C57BL/6J male mice | HF-HS for 12 weeksWithdrawal: HF-HS for 6 weeks, washout for 2 weeks (treatments)/GLP-1/Dexa (100 nmol/kg) s.c.GLP-1 analogue (100 nmol/kg) s.c.Daily for 11 days (last 2 weeks of HF-HS diet)For 3 weeks on operant conditioning tests | 1. Saline 2. GLP-1/Dexa3. GLP-1 analogue alone4. Pair-fed | PR operant respondingForced swim test (FST)EPMOpen field test (OFT)Novel object recognition (NOR)/Quantitative PCR for D1r, D2rsh, d2rlg, Th, Dat, Dor, Kor, Mor, Gr, Mr in NAc core and shell | GLP-1/dexa significantly decreased food-motivated behavior for 20 hours, whereas GLP-1 analogue only showed a transient reduction (4 hours) in motivation, but the vehicle did not have a significant change in food motivationAfter withdrawal from the HF-HS diet, the mice which were treated with GLP-1/dexa showed less motivation for food reward comparison to mice treated with vehicleGLP-1/dexa decreased the expression of reward-related genes in the NAc such as D1r, D2rlg, Kor, Gr, whereas others were also reduced but non-significantly GLP-1/dexa induced weight loss without changing anxiety and depressive-like behaviors in HF-HS diet mice |