Supplementary Material

Cholinergic dysfunction in the dorsal striatum promotes habit formation and maladaptive eating

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Supplementary Material and Methods

Human subjects

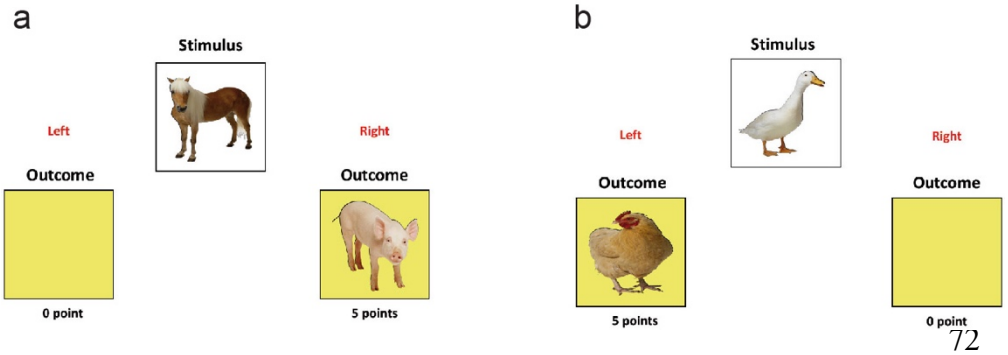
Patients were recruited at the "*Clinique des Maladies Mentales et de l'Encéphale*" (CMME, Hôpital Sainte-Anne), a care center specialized in eating disorders in Paris, France. Inpatients and outpatients included met the Diagnostic and Statistical Manual of Mental Disorders (DSM5) criteria for anorexia nervosa restricting type (AN-R), *anorexia nervosa* binge-eating purging type (AN-BP), *bulimia nervosa* (BN) or binge eating disorder (BED). In the present study, patients are stratified between AN-R and eating disorders binge-purge patients (which includes: AN-BP, BN and BED) (Supplementary Table 1).
Patients were older than 18 years of age at the time of inclusion (Supplementary Table 1). Healthy controls were seen by a senior psychiatrist using a semi-structured interview (MINI). Healthy controls had no cognitive, physical or psychiatric illness nor were receiving any psychotropic medication. In the group of eating disorder patients, one was suffering from atypical anorexia nervosa and one was diagnosed with a sub-syndromal eating disorder. Fifty-six individuals were included in the study (25 controls and 31 patients). Healthy controls were matched to patients with EDs according to sex, age and education level. Exclusion criteria included psychiatric diagnoses such as schizophrenia or Asperger, medical or neurological conditions that impact feeding behaviors.

The slip-of-action neurocognitive test

Designed to evaluate the balance between goal-directed behaviors and habits in humans, this computer-based neurocognitive task is subdivided into 4 phases: instrumental learning stage, outcome devaluation stage, "slip-of-action" stage and baseline stage of inhibitory control (1, 2). The instructions given to participants for each phase and a short description of the test are described below.

First phase (instrumental learning stage)

- For each picture/animal (stimulus), one correct response (right or left) is rewarded with points and a picture with another animal (outcome); an incorrect response is associated with no points and an empty picture.
- Measures: Rate of correct responses, reaction time.
- 10 blocks, stimuli in random order, 120 trials in total.



Instructions:

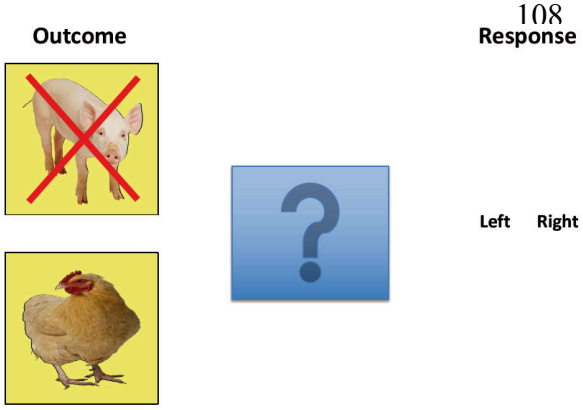
On the screen: In this game, **your aim is to earn as many points as possible**. **Points will be collected by pressing either the right OR left key when a picture of an animal is shown to you.** For each animal, a correct response will reveal a new animal picture, and you will get points. However, an incorrect response will lead to an empty picture, and you will get no points. **Your task is to learn the association between the animal picture and which key to press.** Sometimes the correct response will be the left key, and other times the right key. As such, the first animal picture should give you a clue as to which key you should press. **You should also learn the association between the first and second animal picture following a correct response.** The quicker you press the correct key, the more points will be accumulated. Your total number of points will be displayed at the top of the screen.

Oral explanation: You will see that animals always come by pairs: one animal on a white background followed by one animal on a yellow background. Only the animal on a white background allow you to get points, but it is also important to be careful in regards to the other animal (on a yellow background) that it is associated to. The animal on a yellow background does not give you any possibility to collect points, therefore it is not useful to click the animal on a yellow background. You will realize that the animal on a yellow background only appears when you click right or left on the keyboard, in response to a white animal. If you click on the wrong key, the yellow background will appear on the screen without any animal or point. In summary, for each animal that appears on a white background, you should try to remind yourself of the correct answer (right of left key press) and the animal on a yellow background that it is associated with (even if you don't see the interest at this point, this second animal will be important later)

Second Phase (outcome devaluation stage)

- Participants are tested on their outcome-action knowledge

- Two of the outcomes (animals) from the discrimination training phase are presented simultaneously on the screen, where one has a red X superimposed and the other does not
- Participants are instructed to make the response that have previously led to an outcome that did not have a red X superimposed
    - 36 trials
    - Measures: Rate of correct responses, reaction time



Instructions:

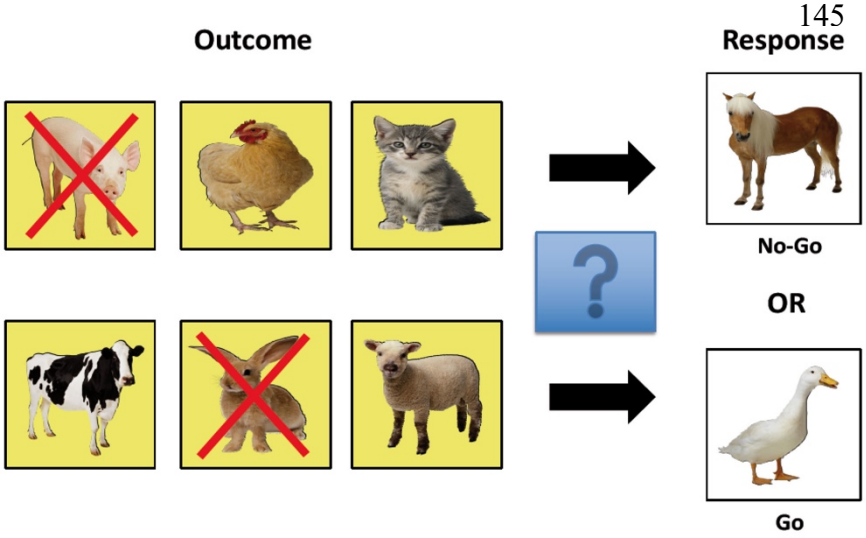
On the screen. For this phase, you will be shown two different pictures with two different animals. One animal will be associated with the left key press from the previous task, while the other will be associated with the right key press. **One of these pictures will now have a red cross displayed on it. This picture no longer has a value, and it will bring you no points. Your task consists of pressing the appropriate key that is associated with the animal that does *NOT* show a red cross (valuable animals).** Your total number of points will be displayed at the end of the stage.

Oral explanation. During this stage, it is no longer the animals on a white background that are presented on the screen, but instead the animals on the yellow background. On each trial, 2 animals on a yellow background will appear, one will have a red cross displayed on it and the other will not. You will now only see animals on a yellow background, but they allow you to get points by their association with animals on a white background (the association that you probably learned in the previous stage). In summary, you will earn points by clicking on the left or right key that was associated with a white background animal preceding a yellow background animal.

Third Phase ("slip-of-action" stage)

- At the onset of each block, participants first show all the outcomes from training, two of which had a red X superimposed, for 10 seconds.

- Stimuli are then presented alternatively.
- Participants are instructed to press the correct key for stimuli associated with still valuable outcomes in the pair (Go trials) and withhold the response for stimuli associated with devalued outcomes (No-go trials).
- 6 blocks, 144 trials in total. Each stimulus repeated 4 times in each block.
- Measures: Rate of responses associated with valuable and devaluated outcomes, response accuracy, reaction time.



Instructions:

On the screen. For this phase, you will once again have the chance to earn points by pressing keys in response to pictures of animals shown on the screen. However, remember that animals with a red cross will no longer give you points. **Your task is to press the key associated with pictures of the valuable animals but withhold the key press if the picture shows a red cross (devalued animal).** Correct key presses will be rewarded, incorrect key presses will not be rewarded, and one point will be deducted for every key press made to a picture of an animal with a red cross (devalued animal). You must press the keys quickly as the boxes will follow each other rapidly. You will be shown your cumulative number of points only at the end of the stage.

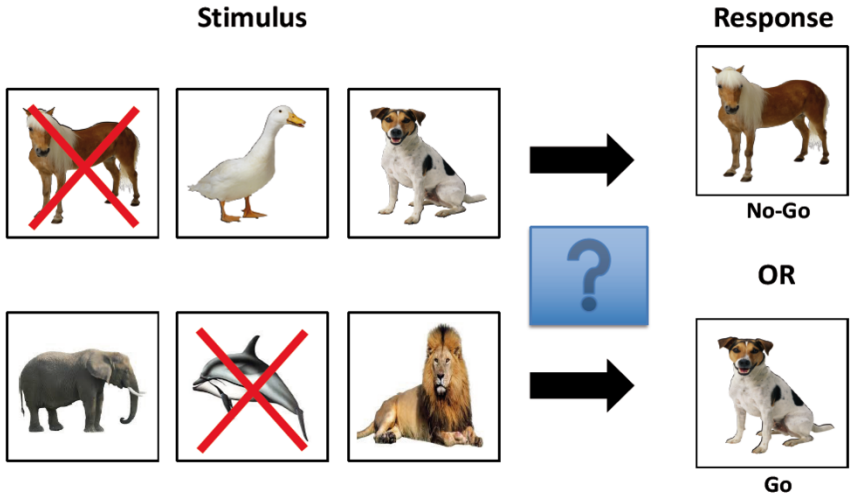
Oral explanation. In this step, additional working memory will be necessary. During a few seconds, you will see 6 animals on a yellow background, among them 2 will be red crossed. Try to remind yourself what are the animals on a white background that were associated to the red-crossed yellow animals and consider that those animals are now not rewarded: you should not press on any key when those animals appear on the screen. After few seconds, the 1st screen with 6 animals on a yellow background (with 2 red-crossed) will disappear and other animals will come quickly on the screen. All the animals that will be displayed on the screen will only be white background animals. You should click on the key (right or left) that was associated with each of those animals, except for temporary “forbidden” animals, which

means the animals that were associated with yellow background animals with a red cross on it (points will be substracted if you click on a key when the non-rewarded animals appear). The animals with the yellow background that are red-crossed will change for each trial, as you will see, which means that the "forbidden" white animals will change too. To get the most reward, speed is taken into account.

(these instructions were repeated as often as necessary until the beginning of a trial)

Fourth Phase (Baseline stage of inhibitory control)

- This stage is a control Go/No-Go task in which cueing stimuli themselves are devalued.
- At the onset of each block, the 6 stimuli are shown simultaneously for 10 seconds. Two of them are devalued, as indicated by a red X superimposed.
- Participants are instructed to provide correct key presses for valued stimuli (Go trials) and withhold the response for devalued stimuli (No-go trials).
- Same numbers of trials and blocks as in the slip-of-action stage.
- Measures: Rate of responses associated with valued and devalued stimuli, response accuracy, reaction time.



Instructions:

On the screen: For this phase, you will once again have the chance to earn points by pressing keys in response to pictures of animals shown on the screen. However, remember that animals with a red cross will no longer give you points. **Your task is to press the key associated with pictures of the valuable animals but withhold the key press if the picture shows a red cross (devalued animal).** Correct key presses will be rewarded, incorrect key presses will not be rewarded, and one point will be deducted for every key

press made to a picture of an animal with a red cross (devalued animal). You must press the keys quickly as the boxes will follow each other rapidly. You will be shown your cumulative number of points only at the end of the stage.

Oral explanation: This step is similar to the previous one, but a little bit easier as it only uses white background animals, which means those that were directly associated to a key (right or left). Within few seconds, you will see 6 animals on a white background, among them 2 are red crossed, these animals are now "forbidden". After few seconds, the 1st screen with white background animals will disappear and other animals on white background will quickly succeed on the screen. You must press the key (right or left) that was associated to each of the animals, except that you should refrain from pressing forbidden animals (if not, points will be removed). To get the maximal reward, speed of response is important.

Animals

Mice were housed in groups of two to four animals per cage (except when isolated for behavioral tasks as indicated) under standard conditions: 22±2 °C and a 12 h light/dark cycle (7:00-19:00 light period) with food (except when food-restricted for behavior as indicated) and water provided *ad libitum*. All precautions were taken to minimize the number of animals used and their suffering. Experiments were performed with 2- to 6-month-old male mice and their littermate controls. Animals were randomly allocated to experimental groups accordingly to their availability. Whenever possible, the investigator was blinded for experimental procedures.

The constitutive VGLUT3-null mouse line on C57BL/6N background was obtained as previously described (3, 4). Heterozygous mice were bred to generate VGLUT3$^{-/-}$ mice (*named VGLUT3-KO mice*) and wild-type (WT) littermates.

The VGLUT3$^{flox/flox}$ mice *Slc17a8* mutant mouse line was established at Phenomin - iCS (Phenomin- Institut Clinique de la Souris-, Illkirch, France; http://www.phenomin.fr/) (5). Mice lacking VGLUT3 selectively in striatal cholinergic interneurons (VGLUT3$^{Chat\text{-}IRES\text{-}Cre\text{-}flox/flox}$, *named VGLUT3cKO mice*) were generated by breeding ChAT$^{-ires\text{-}Cre}$ mice (B6N.129S6(B6)-*Chat*$^{tm2(cre)Lowl}$/J on a C57BL/6NJ-congenic background; The Jackson laboratory, stock No: 018957) with VGLUT3$^{flox/flox}$ mice. Mice used in our experiments were littermates VGLUT3$^{ChAT\text{-}IRES\text{-}Cre\text{-}flox/flox}$ and VGLUT3$^{flox/flox}$.

Selective ablation of VGLUT3 in ChIs was also obtained by crossing VGLUT3$^{flox/flox}$ mice with Drd2^{Cre} mice, expressing Cre in DRD2-positive neurons (*named VGLUT3cKO-D2CRE mice*). D2-Cre mice (Drd2, Tg(Drd2-cre)44Gsat/Mmcd; MMRRC stock# 017263-UCD) (7) were obtained from the GENSAT project via the mutant mouse regional resource centers. They were backcrossed to C57BL/6J for 5 generations in our laboratory.

VAChT$^{flox/flox}$ mice were obtained as previously described (6) and were backcrossed to C57BL/6J for 10 generations. VAChT$^{D2\text{-}Cre\text{-}flox/flox}$ mice (*named VAChTcKO mice*) were generated by crossing VAChT$^{flox/flox}$ with the D2-Cre hemizygous mice (7). ChIs preferentially express the DRD2 in the dorsal striatum (8, 9). Therefore VAChT$^{D2\text{-}Cre\text{-}flox/flox}$ mice allows to reach a high level of VAChT deletion throughout the dorsal striatum. Mice used in our experiments were littermates VAChT$^{D2\text{-}Cre\text{-}flox/flox}$ and VAChT$^{flox/flox}$.

Immunoautoradiography

Immunoautoradiography was performed as previously described (3, 4). Coronal sections (10 µm) were taken with a cryostat and incubated with rabbit polyclonal anti-VGLUT3 (dilution 1:10,000, Synaptic Systems, Goettingen, Germany, ref 135–203/26) or anti-VAChT antiserum (dilution 1:10,000, Synaptic Systems, Goettingen, Germany, ref 139-103/34) overnight at 4°C, followed by anti-rabbit [^{125}I]-IgG (GE Healthcare lifesciences, 100 mCi.ml^{-1}) for 2h. Sections were exposed to X-ray films (Biomax MR, Kodak, France) for 16 days. Autoradiograms were digitized using a powerLook 100 Umax scanner and analyzed with MCID Software.

***In situ* hybridization**

In situ hybridization was performed as recommended by Oramecell (Paris, France) and as previously described (4). Sections (10 µm) of fresh frozen brains were incubated with antisense oligonucleotides. Oligonucleotides were labeled with [^{35}S]dATP (GE Healthcare, Chalfont St. Giles, UK) using terminal transferase to a specific activity of 5x10^{8} dpm.µg^{-1}. Labeled sections were exposed to a BAS-SR Fujifilm imaging plate. The plates were scanned with a Fuji Bioimaging Analyzer BAS-5000.

Western blots

Immunoblotting was performed as previously described (10). Tissues were homogenized in RIPA lysis buffer containing protease inhibitor cocktail (Calbiochem). Samples were resolved in 10 % polyacrylamide gels and transferred to PVDF membranes. Membranes were probed with rabbit anti-VAChT (dilution 1:3000, Synaptic Systems, Goettingen, Germany, catalog ref 139-103/34), rabbit anti-VGLUT3 (dilution 1:1000, Synaptic Systems, Goettingen, Germany, catalog refs 135–203/26) and rabbit or mouse anti-Synaptophysin (Abcam Cambridge, UK, ref ab32594, dilution 1:1000, or Sigma-Aldrich Saint-Louis, USA, ref S5768, dilution 1:500, respectively). Synaptophysin immunoactivity was used as loading control. Band intensity was quantified using ImageJ software.

Immunofluorescence

Immunofluorescence was performed on fixed cryostat sections as previously described (3). Sections were incubated with VGLUT3 rabbit antiserum (dilution 1:2000, Synaptic Systems, Goettingen, Germany, catalog refs 135–203/26) and VAChT guinea pig antiserum (dilution 1:5000) (4). Primary antibodies were detected with secondary anti-rabbit or anti-guinea pig coupled to Alexa Fluor 488, Alexa Fluor 555 or Cy5 (dilution 1:2000, Invitrogen, Waltham, Massachusetts, USA). Images were acquired with a fluorescence microscope equipped with an Apotome module (Axiovert 200 M, Zeiss).

qPCR

For mRNA analysis tissue samples were frozen on dry ice and kept at -80°C until used. RNA was extracted and purified using the Aurum total RNA mini kit (BioRad) according the manufacturer's instructions. First-strand cDNA was synthesized using the High-Capacity cDNA transcription kit (Applied Biosystems, CA) according to the manufacturer's instructions. After reverse transcription, the cDNA was subjected to qPCR on a 7500 real-time PCR system (Applied Biosystems, CA) by using Power SYBR green PCR master mix (Applied Biosystems, CA). Amplification was carried out in a total volume of 5 µl containing 0.25 µM each primer, 2.5 µl of Power SYBR green master mix (2×), and 0.5 µl of cDNA. The PCRs were cycled 45 times after initial denaturation (95°C, 2 min) with the following parameters: 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. For each experiment, a nontemplate reaction was included as a negative control. Melting curve analysis of amplification products was performed by cooling the samples to 60°C and then increasing the temperature to 95°C at 0.1°C/s. Relative quantification of gene expression was done with the $2^{-\Delta\Delta CT}$ method, using the β-actin gene expression to normalize the data.

Touchscreen behavioral tasks

During the touchscreen behavioral experiments, mice were subjected to mild food restriction (85 % of their original weight or 24.5-25.0 g, which ever was lower). While on food restriction, mice were weighed daily and their weights were kept in a required range. Food-restricted mice were separated and housed individually (if they fought) or in groups of two per cage. Touchscreen-based tasks (Pairwise discrimination (PD) with reversal, extinction, 5-choice serial reaction time task (5-CSRTT) were conducted using automated Bussey-Saksida mouse touchscreen systems model 81426 (Campden Instruments). Schedules were designed and data were collected using the ABET II Touch software v.2.15 (Lafayette Instruments). Mice were trained 5 days a week (1 session per day). Tasks were performed in a battery in the following order: PD with reversal followed by extinction (VGLUT3cKO mice,

VGLUT3cKO-D2CRE mice and VAChTcKO mice) or PD with reversal followed by 5-CSRTT (AAV-injected VAChT$^{flox/flox}$ mice/VAChTcKO-DS).

Pretraining. Mice were habituated to the touchscreen apparatus at the beginning of the battery and then pretrained specifically for each task as needed. The habituation and pretraining procedure were done as previously described(11). In short, during the habituation (phase 1) mice were exposed to the touchscreen apparatus for 10-40 minutes per day and they were gradually habituated to the milkshake reward, with a tone playing whenever the mouse entered the reward magazine. The following pretraining phases (phases 2-5) were almost identical for PD, 5-CSRTT and extinction task, except the mask used on the touchscreen was different and only pretraining phases 2 and 3 were performed before the extinction task. The phase 2 of pretraining involved pairing the reward with the presentation of the stimulus on the touchscreen. The stimulus appeared randomly in one of the windows, and after 30 s it was removed and a reward was given paired with a tone. If the mouse touched the screen while the stimulus was displayed, it immediately received a reward. Once the mouse collected the reward a new trial was initiated. This phase was repeated until the mouse completed 30 trials within 60 min. In the following phase 3, the mouse was required to touch the stimulus on the screen in order to receive a reward paired with a tone. Once again, this was repeated until the mouse completed 30 trials within 60 min. Phase 4 was identical to phase 3, except that the mouse was required to initiate each trial by entering the reward magazine (same criterion). Finally, in the phase 5, the previous procedure was repeated but if the mouse touched an incorrect screen (with no stimulus displayed), it did not receive any reward plus a 5 s time-out, during which the house light was turned on. The final phase required the mouse to perform 30 trials in 60 min with at least 23 correct responses for 2 consecutive days.

Pairwise discrimination with reversal. Pairwise visual discrimination and reversal task were performed as described in published protocols (11). The mice were first trained to discriminate between two visual stimuli (Fig. 1a), which were presented simultaneously, with their spatial location randomized over a 30-trial session. If the mouse nosepoked the correct stimulus (S+), a tone was played and mouse received a reward, whereas if the incorrect stimulus (S−) was nosepoked, the mouse received a 5 s time-out, during which the house light was turned on, followed by a correction trial. The correction trial was repeated with the same configuration as the preceding incorrect trial until the mouse made a correct response. The correction trials were not included into total count of completed trials or in the final percentage of correct responses. Criterion was completing 30 trials in a session and 80% of the correct responses for 2 consecutive days. Once mice reached criteria, they were given two sessions to assess baseline performance on the task and then they were transferred to the reversal phase. In the reversal phase, the rule associated to each stimulus was switched

(Fig. 1d), that is, the rewarded image (S+) during acquisition became the (S−) image in reversal and was punished, while the (S−) image from acquisition became the correct stimulus (S+) and was rewarded.

5-CSRTT. The task was performed using a previously published protocol (11). Briefly, every session lasted max. 60 minutes and consisted of max. 50 trials. Each trial was initiated after the mouse entered the reward magazine. The stimulus (white square) was presented after a variable 5-10 s delay, during which the animal was required to attend to the screen. The stimulus duration was initially set to 4 s, followed by a limited holding period of 5 s, during which the stimulus was absent, but the mouse could still respond to the location (holding period). Responses to the stimulus window during stimulus presence or the holding period were recorded as correct while responses to any other window were recorded as incorrect. A correct choice was associated with a tone, reward magazine illumination and reward delivery. An incorrect response was punished with a 10 s time-out. A failure to respond to any window either during stimulus display, or during the holding period, was recorded as an omission and punished with a 10 s time-out. Once the performance of a mouse reached criterion at 4 s stimulus duration (completing minimum 30 trials within 60 minutes with minimum 80% accuracy and maximum 20% omissions for 3 consecutive days), the stimulus duration was reduced to 2 s. After reaching criterion with the 2 s stimulus, mice were transferred to probe trials. During the probe trials, each mouse was tested for 2 consecutive days at a given stimulus duration (1.5, 1.0, 0.8, and 0.6 s). After each test, the animal was re-tested onto the 2 s stimulus duration for 2 days, to assess baseline performance. The order of the probe trial sessions was semi-randomized across cohort. On all 5-CSRTT task sessions, accuracy was defined as the total number of correct responses, divided by the number of correct and incorrect (touches to a wrong window) responses and the rate of omissions was the proportion of omitted responses to total trials.

Extinction. We followed a protocol described by Nithianantharajah et al. (12) with slight modifications. During extinction acquisition phase, mice were required to respond to a white square stimulus presented in the center of the screen in order to obtain a reward. The stimulus remained on the screen until the response was made and was removed afterwards together with a tone, magazine illumination and reward delivery. The acquisition criterion was defined as completing 30 trials within 12.5 minutes on five consecutive days and ensured comparable performance across all groups of mice before entering probe sessions. After reaching this criterion, mice were transferred to extinction probe phase in which responses to the stimulus were no longer rewarded nor accompanied by any other feedback. During the probe sessions, the stimulus was displayed for 10 s and then it was automatically removed if no response was made. It was removed immediately if the response was made. After 10 s inter-trial interval, a new trial (stimulus presentation) was automatically initiated. Session was

terminated after 30 trials (maximum time of 1 session when no response was made was 12.5 minutes). The extinction probe phase was conducted over the course of six days.

Operant sucrose self-administration

Mice were single-housed and placed on a food deprivation schedule to reduce their weight to 85% of their baseline weight. They were weighed and fed with regular home chow daily after training. Water was available at all times in the home cages. Training and testing took place in eight operant chambers (21.6 cm length X 17.8 cm width X 12.7 cm height; MED Associates, Inc., Georgia, VT, USA) housed within light-resistant and sound-attenuating walls. Each chamber was equipped with a food magazine that received sucrose pellets (20mg chocolate flavor Dustless precision pellets, Bio-Serv, Canada) from a dispenser. Each chamber contained two holes with lights inside, on either side of the magazine, one randomly selected as the active hole and the other as the inactive, a house light (3 W/24 V) mounted on the wall opposite the magazine and a fan. A computer with the Med-PC-IV program was used to control the equipment and record behavior.

Nosepoke training. Mice were trained 7 days a week (1 session per day). At the beginning of each session, the house light, the lights inside the two holes and the fan were turned on. At the end of each session, the house light, the lights inside the two holes and the fan were turned off. Initial nosepoke training consisted of 12 consecutive days of continuous reinforcement (CRF), using a fixed-ratio 1 (FR1) procedure during which the animals received a pellet for each nosepoke. Sessions ended after 60 min without cut-off (except for VGLUT3-KO mice and control littermates, for this group, sessions ended after 60 min or 50 rewards, whichever came first). For the groups of mice used to test habitual behavior, after 12 days of CRF, mice were then trained with random interval (RI) schedules to generate habitual nosepoking (13). Mice were trained 2 days on RI-30 (on average one reinforcer delivered upon the first nosepoke after 30 seconds since the last reinforcer) and then switched to RI-60 for 6 additional days.

Devaluation tests. A sensory-specific satiety procedure was used for outcome devaluation (13). This procedure controls the overall level of satiety and motivational state while altering the current value of a specific reward. The devaluation test started 24 hours after the last training day and lasted 2 days. On each day, mice were given *ad libitum* food exposure for 1 hour in a separate clean cage. Mice were allowed to consume either the grain pellets (homechow) they had been exposed in their home cages (valued condition) or the reinforcer (sucrose pellets) used during nosepoke sessions (devalued condition). The amount of food consumed during the *ad libitum* session was recorded and mice that did not consume a minimum of 0.4 g of each food were not included in the analyses. We observed no significant

difference of regular food or sucrose pellets consumption between mutant mouse lines and their respective controls (Supplementary Figure 16).

Immediately after the *ad libitum* feeding session, mice were given a 5-minute test in extinction in the operant chambers, with house light, holes lights and fan on (same conditions as for nosepoke training) but no pellet was delivered. No extra training was conducted on probe days. The order of the valued and devalued condition tests (day 1 or day 2) was counterbalanced across animals, and the number of active and inactive nosepokes for each condition was recorded.

Binge-like sucrose overconsumption model

Mice were housed individually in Plexiglas cages. For habituation, water and food were available *ad libitum* during 2 days. Mice were then food deprived for 20-h (12PM-8AM) with free access to water. During 4 hours per day (8AM-12PM), mice had simultaneously access to a highly-concentrated sucrose solution (20%, Sigma) and water with the two-bottle method and food (Figure 2a). To control for side preference, the left/right position of the sucrose solution and water was alternated daily. Consumption of the sucrose solution during the first hour (8AM-9AM) and the total 4 hours (8AM-12PM) were recorded daily. Consumptions of water and food were recorded daily after 4 hours of access. Daily body weight (BW) was also measured before sucrose and food access. Another cohort of mice received saccharine (5 mM) instead of sucrose throughout the same regimen (14).

Sucrose preference

Mice were given 24-h concurrent access to two graduated plastic bottles for 3 days in their home cages. One of these bottles contained tap water, whereas the other one contained 20% sucrose solution. Bottles were weighed daily, with the position of the bottles (left/right) alternated to control for side preference. The first day was used as a habituation period. The volumes of sucrose solution and water consumed on the second and third days were averaged to determine sucrose, water and total fluid intake, and preference for sucrose over water (sucrose intake/total fluid intake).

Activity-based anorexia model

The ABA model was performed as previously described (15). For habituation, all mice were individually housed in cages (26 cm length X 12 cm width X 16 cm height) with running wheels (diameter: 14 cm; width: 9 cm) for 7 days prior to the start of the experiment (Figure 2i). This period was named "Baseline Conditions". Under baseline conditions, mice had unrestricted access to food, water and running wheel. After the adaptation period, all mice were maintained in the same running wheel cages for 8 additional days. Access to food was progressively restricted, from 8 hours (day 1) to 2 hours (day 8) per day. BW and food intake

were measured daily before and after food access, respectively. Animal were euthanized or « dropped » from experiments when they lost 70% of their initial body weight defined on the last day of baseline. Days until mice reached 75% of baseline BW provided a measure of survival. For running wheel activity recording, animals were individually housed in light-controlled (7:00-19:00 light period) cabinets and activity was recorded continuously (ClockLab, Actimetrics, Wilmette, IL). For pharmacological treatment experiment, mice received an intraperitoneal injection of Donepezil (0.3 $mg.kg^{-1}$, diluted in NaCl 0.9%), L-DOPA (15 $mg.kg^{-1}$ - benzerazide (7.5 $mg.kg^{-1}$) diluted in NaCl 0.9%) or NaCl 0.9%. Mice were treated daily 30 min. before the start of food access, during both baseline and food restriction phases (Donepezil) or only during food restriction phase (L-DOPA).

Stereotaxic surgery and intracerebral virus infusions

Virus injections for touchscreen experiments. VAChT-lox mice were anesthetized with ketamine (100 $mg.kg^{-1}$)-xylazine (10 $mg.kg^{-1}$) diluted in saline. Ophthalmic ointment was applied to prevent corneal drying. Mice were placed into stereotaxic apparatus and the same height of bregma and lambda was checked to ensure flat skull position. Incision was made to expose the skull, two symmetrical holes were drilled and the Hamilton syringe (28G, 10 µL) was placed at the following coordinates (in mm relative to bregma): AP, +0.8; ML, ±1.9; DV, -3.9 to target the dorsal striatum. Using a microsyringe pump (UltraMicroPump, World Precision Instruments), 2 µl of adeno associated virus (AAV) expressing Cre recombinase and GFP were injected. AAV from two different sources were used throughout the study: AAV8-CMV-Cre-GFP from Vector Biolabs and AAV5-CMV-Cre-GFP from UNC Vector Core together with their respective controls (AAV8-CMV-GFP, Vector Biolabs and AAV5-CMV-GFP, UNC Vector Core) (*mice named VAChTcKO-DS*). Injection rate was 500 $nl.min^{-1}$ and the needle was left in place 5 minutes after injection to allow the injected solution to be absorbed and to minimize the spread of the virus along the needle tract. The scalp incision was closed by tissue adhesive (Vetbond). Mice were kept on a heating pad during the surgery and meloxicam (10 $mg.kg^{-1}$) and warm saline was applied subcutaneously after the surgery for pain management and re-hydration. Behavioral testing was initiated 4 weeks after the surgery to allow for virus expression.

Virus injections for eating disorders models. VAChT-lox mice were anesthetized with isoflurane. Ophthalmic ointment was applied. Mice were placed into stereotaxic apparatus and the same height of bregma and lambda was checked. An incision was made to expose the skull and two symmetrical holes were drilled. The injection needle (NanoFil 34GA Beveled needle, World Precision Instruments) connected to the Hamilton syringe (28G, 10 µL) was placed at the following coordinates to target the dorsomedial striatum (in mm relative to bregma): AP, +1; ML, ±1; DV, -3.5 and -2.7. Using a microsyringe pump (UltraMicroPump,

World Precision Instruments), we injected twice (one injection per each DV coordinate) 0.4 µl of AAV5-CMV-Cre-GFP *(*mice named *VAChTcKO-DMS*) or AAV5-CMV-GFP (control mice). Both AAV were from UNC Vector Core (http://www.med.unc.edu/genetherapy/vectorcore). Injection rate was 100 nl.min^{-1} and the needle was left in place 5 minutes after injection. Scalp incisions were closed by nylon sutures (Ethicon). Mice were kept on a heating pad during the surgery and meloxicam (10 mg.kg^{-1}) and warm saline was applied subcutaneously after the surgery for re-hydration and pain management. Mice were used for behavioral testing 4 weeks post-surgery.

Histological evaluation of VAChT deletion after intracerebral virus infusions

Histological evaluation for touchscreen experiments. After completion of behavioral testing, mice were anesthetized with lethal dose of ketamine-xylazine and perfused with ice-cold 4% PFA in PBS. Brains were post-fixed in 4% PFA overnight and sectioned using a vibratome (40 µm thick). Free-floating sections were incubated in Tris-buffered saline (TBS) with 1.2 % Triton X-100 for 20 min and rinsed in TBS. Non-specific binding was blocked by incubation for 1 h with 10 % normal goat serum in TBS. After rinsing twice in TBS, sections were incubated 24 hours with anti-GFP (chicken, 1:1000, Abcam Catalog# ab13970), anti-VAChT (rabbit, 1:250, Synaptic Systems Catalog# 139 103) and anti-CHT (mouse, 1:100; Synaptic Systems Catalog# 216 011). All incubations were performed at room temperature. Antiserums were diluted in 0.2% Triton X-100 and 2% normal goat serum in TBS. After that sections were washed twice for 10 min in TBS and incubated with secondary fluorescent antibodies (anti-chicken Alexa 488, anti-rabbit Alexa 546 and anti-mouse Alexa 633, all Thermo Fisher Scientific), dilution 1:500 in 0.2% Triton X-100 and 2% normal goat serum in TBS. Sections were washed twice for 10 min in TBS then mounted on slides and visualized using either Zeiss ApoTome fluorescence microscope or Leica TCS SP8 confocal system. GFP expression was checked throughout the striatum in VAChTcKO-DS mice and control mice.

Histological evaluation for eating disorders models. After completion of behavioral testing, mice were anesthetized with a lethal dose of ketamine-xylazine and immunoautoradiography was performed on fresh frozen sections using VAChT and VGLUT3 antiserums as above-described (see « Immunoautoradiography »).

In vivo microdialysis

Mice were anesthetized with ketamine/xylazine (100 and 10 mg.kg^{-1}, respectively, in saline, i.p.) and a guide cannula (CMA7, CMA Microdialysis, Chelmsford, MA, USA) was stereotactically implanted into the striatum (in mm relative to bregma: AP, 0.0; ML, ±2.0; DV, -1.8) and fixed in place with two anchor screws (CMA) and carboxylate dental cement

(CMA). Operated mice were singly housed, treated with antibiotics (sulfamethoxazole 1.2 $mg.ml^{-1}$ and trimethoprim 0.24 $mg.ml^{-1}$) in the drinking water and allowed to recover 48-96 h post-surgery.

Dialysate collection: The microdialysis probe was inserted into the guide cannula approximately 16h before the start of sample collection to allow for the stabilization of DA_{Ext} levels. The mouse was gently restrained and the microdialysis probe (CMA7 7/2) was inserted into the guide cannula. The mouse was then placed in a circular chamber with bedding, chow and water available. A two-channel swivel (cat. no. 375/D/22QM; Instech, Plymouth Meeting, PA, USA) allowed for unimpeded movement of the mouse. Artificial CSF (147 mM NaCl, 2.7 mM KCl, 0.85 mM $MgCl_2$, 1.2 mM $CaCl_2$, CMA) was delivered at a flow rate of 0.45 $ml.min^{-1}$ from probe insertion until the end of experiment. Following the stabilization period, 4 baseline samples (30 min duration) were collected, where after artificial CSF with 10 µM nomifensine (Sigma) were perfused and 5 additional samples collected. Samples were collected on ice in the dark, then immediately frozen on dry ice and finally stored at -80°C. Dialysates were analyzed off-line. The first baseline sample was analyzed for Ach_{Ext} by LC-MS as previously described(16). The remaining dialysates were analyzed for DA_{Ext} using high-performance liquid chromatography-electro-chemical detection (HPLC-EC).

In vivo voltammetry

In vivo voltammetry was performed as previously described (17). Mice were anaesthetized with chloral hydrate (400 $mg.kg^{-1}$, i.p.) and voltammetric electrodes (Aldrich, Milwaukee, WI, USA) were implanted into the dorsomedial (DMS, stereotactic coordinates in mm relative to bregma AP, +1.1;, ML, ±1; relative to the dura, DV, -2.6) or dorsolateral striatum (DLS, AP, +1.1; ML, ±2; DV, -2.6). Voltammetric electrodes consisted of one 30-µm diameter carbon fiber coated with Nafion (Aldrich). Electrochemical measurements were performed using a high-speed chronoamperometric apparatus (Quanteon, Lexington, KY, USA) as previously described (18). DA release was evoked by local injections of 200-300 nl of KCl (120 mM) at 20-min interval between K1 and K2 and then 10-min intervals for next injections. The results are presented as the mean ± SEM of the difference in maximal DA variation after KCl ejection. The differences in DA release between the different groups of mice were assessed as a comparison with the differences in maximal variation for each group. The time to reach 80% of the maximal response (t80) was measured as an estimate of DA clearance.

Statistical analysis and mathematical modeling

For anatomical and behavioral experiments, GraphPad Prism 7 and MATLAB were used for statistical analysis. 2-tailed Student's t test or Mann Whitney test were used to compare two experimental groups. Anatomical experiments were repeated 2-3 times on 4-5 mice, in vivo

voltammetry measurements were performed once on 11-17 mice, AAV-GFP and AAV-CRE injections were performed once on 11 mice (each group) whereas some behavioral experiments were performed once with several cohorts of mice as depicted in Suppl Figure 8. Major behavioral experiments described in this study: evaluation of goal-directed behavior/habits balance and models of eating disorders (sucrose binge and activity-based anorexia) were performed at least 2 times, for both VAChT- and VGLUT3-deleted mice versus respective controls. Comparable results were obtained in the replicated experiments. Two-way analysis of variance (ANOVA) with or without repeated measure (depending on the design of the experiment) was used to compare several experimental groups. For post-hoc analysis, Bonferroni's post hoc comparison, the method of contrasts or Dunnett's test were used as indicated. For ABA model, survival analysis (comparison of the percentage of mice reaching 75% of their baseline body weight) was performed using the Kaplan–Meier test with log-rank (Mantel–Cox) and Gehan-Breslow-Wilcoxon post hoc tests. Dimensional analyses were performed by parametric simple linear regressions. All analyses were performed used two-sided statistical tests. All statistical comparisons were made by comparing distinct samples, except for devaluation tests where the same mice were compared in valued *vs* devalued conditions. We evaluated the associations between mice behavioral data using structural equation modeling with the R package lavaan. The meta-models were created based on expected and hypothesized associations to test the hypothesis demonstrated in the figures for controls and VAChTcKO mice. The models' estimates were generated with a bootstrap scheme repeated 1,000 times. The fit of the structural equation models was classified as good whether the root mean squared error of approximation (RMSEA) < 0.05, comparative fit index (CFI) > 0.97, and standardized root mean square residual (SRMR) < 0.05 (19). For logistic regression and ROC curve analysis, we used R (R Core Team (2017)) and IBM SPSS version 20 for Mac. Data were expressed as means ± SEM and p values < 0.05 were considered as statistically significant.

Statistics

Table S1. Related to Figure 1. Demographic data of healthy controls and patients included in the neurocognitive test.

	Healthy controls (n=25)	Eating disorders patients (n=31)	AN-R patients (n=21)	ED-BP patients (n=10)	HC *vs* EDs *P* value	HC *vs* AN-R *P* value	HC *vs* ED-BP *P* value	AN-R *vs* ED-BP *P* value
Age	29.2±1.6	28.8±1.7	27.8±2.0	31.2±2.9	0.8502	0.5787	0.5355	0.9286
Gender (F/M)	19/6	29/2	19/2	10/0				
Age at the beginning of the disease		19.3±1.8	22.9±2.8	16.8±1.2				0.0529
Duration of illness		12.0±2.1	8.5±1.8	14.7±2.9				0.0774
EDI-2		94.0±9.0	94.7±13.6	92.7±11.3				0.8322
ISCED	6.1±0.1	5.9±0.1	5.7±0.2	6.0±0.0	0.0239	0.0348	0.1267	0.1636
BMI*		21.3±2.0	17.3±0.5	25.7±3.0				0.0233
EAT-40*		43.5±4.3	60.0±3.8	27.0±3.4				0.0003
EDQ*		111.9±5.3	117.4±6.8	106.4±8.6				0.4366
HAD-anxiety*		11.8±1.2	13.7±1.1	9.2±1.4				0.0365
HAD-depression*		9.1±1.3	9.8±1.7	6.9±1.4				0.0883
OCI*		21.8±2.1	24.7±3.4	18.5±2.8				0.4554

Abbreviations: AN-R, restrictive anorexia nervosa; ED-BP, binge eating/purging eating disorders; F/M, female/male HC, healthy controls; EDI-2, Eating Disorder Inventory 2; ISCED, International Standard Classification of Education; BMI, Body Mass Index; EAT-40, Eating Attitudes Test 40; EDQ, Exercise Dependence Questionnaire; HAD, Hospital Anxiety and Depression; OCI, Obsessive-Compulsive Inventory.

621 **Table S2 related to Figure 1.**
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Instrumental learning stage (Phase 1) (Fig. 1A,B)		
Two-way ANOVA repeated measures – HC *vs* TCA (all patients) (Fig. 1A)		
	F-value	*p*-value
Group	$F_{1,54}$=0.5230	0.4727
Training blocks	$F_{9,496}$=87.89	<0.0001
Group x blocks	$F_{9,496}$=0.6751	0.7317
Two-way ANOVA repeated measures – HC *vs* AN-R *vs* ED-BP (Fig. 1B)		
	F-value	*p*-value
Group	$F_{2,53}$=0.3325	0.7186
Training blocks	$F_{18,477}$=77.58	<0.0001
Group x blocks	$F_{18,477}$=1.059	0.7009

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Outcome devaluation stage (Phase 2) (Fig. 1C,D)		
t test - HC *vs* TCA (all patients) (Fig. 1C)		
n		*p*-value
25-31		0.2691
One-way ANOVA - HC *vs* AN-R *vs* ED-BP (Fig. 1D)		
	F-value	*p*-value
Group	$F_{2,53}$=0.8307	0.4413

624

Slip-of-action stage (Phase 3) (Fig. 1E)		
Two-way ANOVA - HC *vs* TCA (all patients) (Fig. 1E)		
	F-value	*p*-value
Group	$F_{1,108}$=0.5471	0.4611
Condition	$F_{1,108}$=829.4	<0.0001
Group x Condition	$F_{1,108}$=4.121	0.0448
Two-way ANOVA - HC *vs* AN-R *vs* ED-BP (Fig. 1E)		
	F-value	*p*-value
Group	$F_{2,106}$=1.433	0.2432
Condition	$F_{1,106}$=729.8	<0.0001
Group x Condition	$F_{2,106}$=3.366	0.0382

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Slip-of-action stage (Phase 3) - heterogeneity		
Variances – ED-BP vs HC -Welch's test		
	n	*p*-value
	10-25	0.0123
Slip-of-action stage (Phase 3) - heterogeneity		
Variances – AN-R vs HC -Welch's test		
	n	*p*-value
	21-25	<0.0001
Slip-of-action stage (Phase 3) - heterogeneity		
Variances – ED-BP vs AN-R vs HC- Brown-Forsyte test		
	n	*p*-value
	10-21-25	0.0210

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Baseline stage of response inhibition (Phase 4) (Fig. 1F)		
Two-way ANOVA - HC *vs* TCA (all patients) (Fig. 1F)		
	F-value	*p*-value
Group	$F_{1,80}=8.069*10^{-6}$	0.9977
Value	$F_{1,80}=6147$	<0.0001
Group x Value	$F_{1,80}=6.339$	0.0133
Two-way ANOVA - HC *vs* AN-R *vs* ED-BP (Fig. 1F)		
	F-value	*p*-value
Group	$F_{2,106}=0.006$	0.9939
Value	$F_{1,106}=5391$	<0.0001
Group x Value	$F_{2,106}=5.122$	0.0075

628

Correlation cognitive flexibility-% of responses for devalued outcome			
Simple linear regression test			
All eating disorders patients (Fig. 1G)			
n	*p*-value		
31	<0.0001		
AN-R *vs* ED-BP			
AN-R patients (Fig. 1H)		ED-BP patients (Fig. 1I)	
n	*p*-value	n	*p*-value
21	0.0011	10	0.2513

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Table S3 related to Figure 2.

Pairwise Discrimination task with Reversal (Fig. 2A-H)				
	VGLUT3cKO mice		VAChTcKO mice	
	unpaired t-test			
	Controls *vs* mutant littermates			
Task phase	n	*p*-value	n	*p*-value
Acquisition (Fig. 2B,C)	12-14	0.0652	14-15	0.6739
Reversal (Fig. 2E,G)	12-14	0.2858	14-15	0.0058
	Two-way ANOVA (Fig. 2F,H)			
	Controls *vs* mutant littermates			
	F-value	*p*-value	F-value	*p*-value
Genotype	$F_{1,24}$=0.5246	0.4724	$F_{1,27}$=19.43	0.0001
Session	$F_{1,48}$=34.81	<0.0001	$F_{1,54}$=94.43	<0.0001
Genotype x Session	$F_{1,48}$=0.6359	0.4291	$F_{1,54}$=9.714	0.0043

Pairwise Discrimination task with Reversal (Fig. 2R-T)		
VAChTcKO-DS mice		
	unpaired t-test	
	AAV-Cre-GFP injected *vs* AAV-GFP injected mice	
Task phase	n	*p*-value
Acquisition (Fig. 2R)	11-15	0.329
Reversal (Fig. 2S)	11-15	0.0357
	Two-way ANOVA (Fig. 2T)	
	Controls *vs* mutant littermates (n=11-15)	
	F-value	*p*-value
Genotype	$F_{1,24}$=8.939	0.0044
Session	$F_{1,48}$=21.8	<0.0001
Genotype x Session	$F_{1,48}$=0.9268	0.3405

636 **Table S4 related to Figure 3.**
637

Sucrose self-administration - FR1 training – male VGLUT3cKO mice (Fig. 3B)		
	VGLUT3cKO mice (n=13-11)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,22}$=2.262	0.1468
Session	$F_{11,242}$=16.52	<0.0001
Genotype x Session	$F_{11,242}$=1.392	0.1769

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Devaluation after FR1 training (Fig. 3C)			
Control male *vs* VGLUT3cKO male			
Two-way ANOVA			
	F-value		*p*-value
Genotype	$F_{1,42}$=0.1656		0.6861
Value	$F_{1,42}$=17.36		0.0002
Genotype x Value	$F_{1,42}$=0.1267		0.7237
Paired t test			
Valued *vs* devalued condition			
Control male		VGLUT3cKO male	
n	*p*-value	n	*p*-value
12	0.0103	11	0.0006

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Sucrose self-administration - 2 last days FR1 + RI training (Fig. 3D)		
	VGLUT3cKO mice (n=13-11)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,22}$=5.132	0.0337
Session	$F_{9,198}$=13.31	<0.0001
Genotype x Session	$F_{9,198}$=4.024	<0.0001

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Devaluation after RI training – Log 10 (Fig. 3E)			
Control male *vs* VGLUT3cKO male			
Two-way ANOVA			
	F-value		*p*-value
Genotype	$F_{1,42}$=0.9588		0.3331
Value	$F_{1,42}$=3.470		0.0695
Genotype x Value	$F_{1,42}$=0.1339		0.7193
Paired t test			
Valued *vs* devalued condition			
Control male		VGLUT3cKO male	
n	*p*-value	n	*p*-value
13	0.1093	10	0.0094

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Devaluation after RI training - Raw number of nosepokes (Fig. 3E)			
Control male *vs* VGLUT3cKO male			
Two-way ANOVA			
	F-value		*p*-value
Genotype	$F_{1,42}$=3.260		0.0782
Value	$F_{1,42}$=0.7523		0.3907
Genotype x Value	$F_{1,42}$=0.0002		0.9890
Paired t test			
Valued *vs* devalued condition			
Control male		VGLUT3cKO male	
n	*p*-value	n	*p*-value
13	0.2276	10	0.0336

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Sucrose self-administration - FR1 training - male VAChTcKO mice (Fig. 3F)		
Controls *vs* VAChTcKO (male) (n=12-12)		
Two-way ANOVA repeated measures		
	F-value	*p*-value
Genotype	$F_{1,22}$=9.732	0.0050
Session	$F_{11,242}$=42.30	<0.0001
Genotype x Session	$F_{11,242}$=5.530	<0.0001

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Devaluation after FR1 training (Fig. 3G)			
Control male *vs* VAChTcKO male			
Two-way ANOVA			
	F-value		*p*-value
Genotype	$F_{1,44}$=4.979		0.0362
Value	$F_{1,44}$=4.648		0.0423
Genotype x Value	$F_{1,44}$=1.328		0.2554
Paired t test			
Valued *vs* devalued condition			
Control male		VAChTcKO male	
n	*p*-value	n	*p*-value
12	0.0209	12	0.5316

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Progressive ratio after FR1 training (Fig. 3H)	
Controls *vs* VAChTcKO (male)	
Unpaired t test	
n	*p*-value
12-12	0.0076

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Sucrose self-administration - FR1 training – female VAChTcKO mice (Fig. 3I)		
Controls *vs* VAChTcKO (female) (n=11-13)		
Two-way ANOVA repeated measures		
	F-value	*p*-value
Genotype	$F_{1,22}$=0.6295	0.4360
Session	$F_{11,242}$=26.72	<0.0001
Genotype x Session	$F_{11,242}$=0.7364	0.7029

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Devaluation after FR1 training (Fig. 3J)			
Control female *vs* VAChTcKO female			
Two-way ANOVA			
	F-value		*p*-value
Genotype	$F_{1,44}$=0.1477		0.7026
Value	$F_{1,44}$=11.63		0.0014
Genotype x Value	$F_{1,44}$=5.613		0.0223
Paired t test			
Valued *vs* devalued condition			
Control female		VAChTcKO female	
n	*p*-value	n	*p*-value
11	0.0055	13	0.2363

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Progressive ratio after FR1 training (Fig. 3K)	
Controls *vs* VAChTcKO (female)	
Unpaired t test	
n	*p*-value
11-13	0.3290

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Table S5 related to Fig. 4.

Binge-like sucrose overconsumption model (Fig. 4B-G)		
Controls *vs* VAChTcKO (male) (n=12-12)		
Two-way ANOVA repeated measures		
Sucrose consumption - H0-H4 (Fig. 4B)	F-value	*p*-value
Genotype	$F_{1,22}$=8.7	0.0074
Time (Days)	$F_{15,330}$=64.28	<0.0001
Genotype x Time	$F_{15,330}$=1.994	0.0152
Sucrose consumption - H0-H1 (Fig. 4C)	F-value	*p*-value
Genotype	$F_{1,22}$=11.20	0.0029
Time (Days)	$F_{15,330}$=32.78	<0.0001
Genotype x Time	$F_{15,330}$=1.564	0.0818
Sucrose consumption - H1-H4 (Fig. 4D)	F-value	*p*-value
Genotype	$F_{1,22}$=2.467	0.1305
Time (Days)	$F_{15,330}$=28.38	<0.0001
Genotype x Time	$F_{15,330}$=2.498	0.0017
Controls *vs* VAChTcKO (female) (n=11-13)		
Two-way ANOVA repeated measures		
Sucrose consumption - H0-H4 (Fig. 4E)	F-value	p-value
Genotype	$F_{1,22}$=15.28	0.0008
Time (Days)	$F_{15,330}$=78.93	<0.0001
Genotype x Time	$F_{15,330}$=4.709	<0.0001
Sucrose consumption - H0-H1 (Fig. 4F)	F-value	p-value
Genotype	$F_{1,22}$=6.20	0.0165
Time (Days)	$F_{15,330}$=37.25	<0.0001
Genotype x Time	$F_{15,330}$=4.174	<0.0001
Sucrose consumption - H1-H4 (Fig. 4G)	F-value	p-value
Genotype	$F_{1,22}$=1.589	0.2207
Time (Days)	$F_{15,330}$=23.07	<0.0001
Genotype x Time	$F_{15,330}$=0.8198	0.6555

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Activity-based anorexia model - survival (Fig. 4I)		
Controls *vs* VAChTcKO (male)		
Kaplan–Meier test		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	12-12	0.0314
Gehan-Breslow-Wilcoxon post hoc comparison	12-12	0.0416
Activity-based anorexia model - survival (Fig. 4J)		
Controls *vs* VAChTcKO (female)		
Kaplan–Meier test		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	11-13	0.0039
Gehan-Breslow-Wilcoxon post hoc comparison	11-13	0.0197

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Table S6 related to Fig. 5.

Logistic Regression analysis for Binge-like sucrose overconsumption model (related to Figure 5G)			
Tests for Model Coefficients			
	Chi-square	df	*p*-value
Block	30.953	5	0.000
Model	30.953	5	0.000

Model Summary		
Log Likelihood	Cox & Snell R Square	Nagelkerke R Square
23.031	0.475	0.704

Hosmer and Lemeshow Test		
Chi-square	df	*p*-value
2.091	8	0.978

Classification Table			
	Wildtype	ABA	%
Wildtype	34	2	94.44
Binge	2	10	83.33
		Overall Percentage	91.667

Variables in the Logistic Regression Equation						
Variable	B	Standard error	Wald	df	*p*-value	Exp(B)
Valued Accuracy	*-0.189*	*0.076*	*6.154*	1	*0.013*	*0.828*
Devalued Accuracy	0.112	0.065	2.939	1	0.086	1.119
Sex	*-4.113*	*1.517*	*7.357*	1	*0.007*	*0.016*
Genotype	-0.133	1.262	0.011	1	0.916	0.876
Motivation Nosepokes	0.002	0.010	0.055	1	0.814	1.002
Constant	2.204	2.042	1.164	1	0.281	9.057

ROC analysis

N-positive = 13, N-negative = 35
AUC = 92.1%, $p < 0.001$

Logistic Regression analysis for ABA model (related to Figure 5H)			
Tests for Model Coefficients			
	Chi-square	df	*p*-value
Block	29.134	5	0.001
Model	29.134	5	0.001

Model Summary		
Log Likelihood	Cox & Snell R Square	Nagelkerke R Square
26.938	0.455	0.660

Hosmer and Lemeshow Test		
Chi-square	df	*p*-value
5.967	8	0.651

Classification Table			
	Wildtype	ABA	%
Wildtype	31	4	88.57
Binge	3	10	76.92
		Overall Percentage	84.417

Variables in the Logistic Regression Equation						
Variable	B	Standard error	Wald	df	*p*-value	Exp(B)
Valued Accuracy	-0.040	0.027	2.250	1	0.134	0.961
Devalued Accuracy	*0.089*	*0.042*	*4.425*	1	*0.035*	*1.093*
Sex	1.647	1.098	2.250	1	0.134	5.193
Genotype	-22.964	7006.104	0.000	1	0.997	0.000
Motivation Nosepokes	0.005	0.007	0.481	1	0.488	1.005
Constant	-2.121	1.786	1.410	1	0.235	0.120

ROC analysis

N-positive = 13, N-negative = 35
AUC = 92.1%, $p < 0.001$

Table S6 related to Fig. 6.

Voltammetry - DA release - dorsomedial striatum (DMS) (Fig. 6B)		
	Controls *vs* VAChTcKO mice (n=11-16)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,25}$=5.933	0.0223
Injections	$F_{3,75}$=54.70	<0.0001
Genotype x Injections	$F_{3,75}$=3.501	0.0195

Voltammetry - DA release - dorsomedial striatum (DMS) (Fig. 6C)		
	VAChTcKO mice	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	11-16	0.0300

Voltammetry - DA release - dorsolateral striatum (DLS) (Fig. 6F)		
	Controls *vs* VAChTcKO mice (n=16-17)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,31}$=0.0617	0.8054
Injections	$F_{3,93}$=64.16	0.0001
Genotype x Injections	$F_{3,93}$=0.7421	0.5296

Voltammetry - DA release - dorsolateral striatum (DLS) (Fig. 6G)		
	VAChTcKO mice	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	16-17	0.4827

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Immunoautoradiography (Fig. 6I)	
VAChTcKO-DMS mice	
Unpaired t-test	
AAV-GFP injected *vs* AAV-Cre-GFP injected mice (n=11-11)	
VAChT expression	
Brain area	*p*-value
NAc	0.1827
DMS	0.0007
DLS	0.2033
VGLUT3 expression	
Brain area	*p*-value
NAc	0.4131
DMS	0.3176
DLS	0.5648

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Binge-like sucrose overconsumption model (Fig. 6J-L)		
	VAChTcKO-DMS mice (n=11-11)	
	Two-way ANOVA repeated measures	
	AAV-GFP injected *vs* AAV-Cre-GFP injected mice	
Sucrose consumption - H0-H4 (Fig. 6J)	F-value	*p*-value
Genotype	$F_{1,20}$=14.92	0.0006
Time (Days)	$F_{15,300}$=74.23	<0.0001
Genotype x Time	$F_{15,300}$=1.484	0.1096
Sucrose consumption - H0-H1 (Fig. 6K)	F-value	*p*-value
Genotype	$F_{1,20}$=16.65	0.001
Time (Days)	$F_{15,300}$=37.11	<0.0001
Genotype x Time	$F_{15,300}$=0.8331	0.6404
Sucrose consumption – H1-H4 (Fig. 6L)	F-value	*p*-value
Genotype	$F_{1,20}$=8.815	0.0076
Time (Days)	$F_{15,300}$=32.36	<0.0001
Genotype x Time	$F_{15,300}$=1.406	0.1426

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Activity-based anorexia model – survival (Fig. 6M)		
VAChTcKO-DMS mice		
Kaplan–Meier test		
AAV-GFP injected *vs* AAV-Cre-GFP injected mice		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	11-11	0.0189
Gehan-Breslow-Wilcoxon post hoc comparison	11-11	0.0176

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Table S7 related to Fig. 7.

Activity-based anorexia model - survival (Fig. 7A,B)		
Kaplan-Meier test		
Controls-saline *vs* mutant littermates-saline		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	10-10	0.0131
Gehan-Breslow-Wilcoxon post hoc comparison	10-10	0.0259
Controls-saline *vs* mutant littermates-L-Dopa		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	10-10	0.1369
Gehan-Breslow-Wilcoxon post hoc comparison	10-10	0.0712
Controls-saline *vs* Controls-L-Dopa		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	10-10	0.0432
Gehan-Breslow-Wilcoxon post hoc comparison	10-10	0.0257
Mutant littermates-saline *vs* mutant littermates-L-Dopa		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	10-10	0.0113
Gehan-Breslow-Wilcoxon post hoc comparison	10-10	0.0283

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Activity-based anorexia model - survival (Fig. 7C,D)		
Kaplan-Meier test		
Controls-saline *vs* mutant littermates-saline		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	9-9	0.0215
Gehan-Breslow-Wilcoxon post hoc comparison	9-9	0.0381
Controls-saline *vs* mutant littermates-donepezil		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	9-9	0.7196
Gehan-Breslow-Wilcoxon post hoc comparison	9-9	0.7252
Controls-saline *vs* Controls-donepezil		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	9-9	0.6900
Gehan-Breslow-Wilcoxon post hoc comparison	9-9	0.7251
Mutant littermates-saline *vs* mutant littermates-donepezil		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	9-9	0.0382
Gehan-Breslow-Wilcoxon post hoc comparison	9-9	0.0670

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Statistics for supplementary figures

Table S8: Statistics for Supplementary Figure 1.

Instrumental learning (Phase 1) – Reaction time (Supplementary Fig.1A)		
Two-way ANOVA – HC *vs* TCA (all patients)		
	F-value	*p*-value
Group	$F_{1,54}$=1.800	0.1853
Training blocks	$F_{9,486}$=38.73	<0.0001
Group x blocks	$F_{9,486}$=0.9070	0.5187

Instrumental learning (Phase 1) – Reaction time (Supplementary Fig.1B)		
Two-way ANOVA – HC *vs* ED-BP *vs* AN-R		
	F-value	*p*-value
Group	$F_{2,53}$=0.9873	0.3793
Training blocks	$F_{18,477}$=35.86	<0.0001
Group x blocks	$F_{18,477}$=1.332	0.1625

Outcome devaluation (Phase 2) – Reaction time (Supplementary Fig.1C)
Unpaired t-test – HC *vs* TCA (all patients)
p-value
0.3492

Outcome devaluation (Phase 2) – Reaction time (Supplementary Fig.1D)
One way ANOVA – HC *vs* ED-BP *vs* AN-R
p-value
0.0685

Table S9: Statistics for Supplementary Figure 2

Immunoautoradiography (Supplementary Fig. 2C,D)				
	VGLUT3 in VGLUT3cKO mice		VAChT in VGLUT3cKO mice	
	Unpaired t-test			
	Controls *vs* mutant littermates			
Brain area	n	p-value	n	*p*-value
NAc	5-5	<0.0001	5-5	0.7777
DMS	5-5	<0.0001	5-5	0.4534
DLS	5-5	<0.0001	5-5	0.6541

Immunoautoradiography (Supplementary Fig. 2E,F)				
	VAChT in VAChTcKO mice		VGLUT3 in VAChTcKO mice	
	Unpaired t-test			
	Controls *vs* mutant littermates			
Brain area	n	*p*-value	n	*p*-value
NAc	6-5	0.0015	6-5	0.8886
DMS	6-5	<0.0001	6-5	0.5429
DLS	6-5	<0.0001	6-5	0.1437

Microdialysis (Supplementary Fig. 2G)		
	ACh in VAChTcKO mice	
	unpaired t-test	
	Controls *vs* mutant littermates	
Brain area	n	*p*-value
Dorsal striatum	7-7	0.0221

729 **Table S10: Statistics for Supplementary Figure 3**

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Western blots (Supplementary Fig. 3A-F and Supplementary Fig. 3I-K)						
	VGLUT3 in VGLUT3cKO mice Supplementary Fig. 3A-C		VAChT in VAChTcKO mice Supplementary Fig. 3D-F		VGLUT3 in VGLUT3cKO-D2CRE mice Supplementary Fig. 3I-K	
	Unpaired t-test					
	Controls *vs* mutant littermates					
Brain area	n	*p*-value	n	*p*-value	n	*p*-value
Striatum	4-4	0.0083	4-4	0.0013	4-4	0.0012
Cortex	4-4	0.9545	4-4	0.0004	4-4	0.2561
Hippocampus	4-4	0.8596	4-4	0.7807	4-4	0.842

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qPCR (Supplementary Fig. 3G,H)				
	VGLUT3cKO mice (Supplementary Fig. 3G)		VGLUT3cKO-D2CRE mice (Supplementary Fig. 3H)	
	Unpaired t-test			
	Controls vs. mutant littermates			
Transcripts	n	*p*-value	n	*p*-value
VGLUT3	4-4	0.002	4-6	0.0391
VAChT	4-4	0.8645	4-6	0.6059
ChAT	4-4	0.5635	4-6	0.3183
CHT1	4-4	0.3906	4-6	0.183
DRD1	4-4	0.2503	4-6	0.1106
DRD2	4-4	0.681	4-6	0.5849

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Immunoautoradiography (Supplementary Fig. 3L)		
	VGLUT3 in VGLUT3cKO-D2CRE mice	
	Unpaired t-test	
	Controls *vs* mutant littermates	
Brain area	n	*p*-value
NAc	5-5	0.0358
DMS	5-5	<0.0001
DLS	5-5	<0.0001

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Table S11: Statistics for Supplementary Figure 4

Pairwise Discrimination task with Reversal (Supplementary Fig. 4A-C)		
VGLUT3cKO-D2CRE mice		
	unpaired t-test	
	Controls *vs* mutant littermates	
Task phase	n	*p*-value
Acquisition (Supplementary Fig. 4A)	13-13	0.8398
Reversal (Supplementary Fig. 4B)	13-13	0.1583
	Two-way ANOVA (Supplementary Fig. 4C)	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,24}$=1.853	0.1797
Session	$F_{1,48}$=39.06	<0.0001
Genotype x Session	$F_{1,48}$=2.441	0.1248

738 **Table S12: Statistics for Supplementary Figure 5**

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5-Choice Serial Reaction Time Task (5-CSRTT, Supplementary Fig. 5A-C)		
VAChTcKO-DS mice		
	Two-way ANOVA	
	AAV-Cre-GFP injected *vs* AAV-GFP injected mice (n=9-9)	
Number of training sessions (Supplementary Fig. 5A)	F-value	*p*-value
Genotype	$F_{1,16}$=0.03184	0.8595
Session	$F_{1,32}$=0.9284	0.3425
Genotype x Session	$F_{1,32}$=1.071	0.3085
Accuracy (Supplementary Fig. 5B)		
Genotype	$F_{1,64}$=0.7635	0.3855
Session	$F_{3,64}$=18.11	<0.0001
Genotype x Session	$F_{3,64}$=0.3216	0.8097
Omission (Supplementary Fig. 5C)		
Genotype	$F_{1,64}$=0.1663	0.6848
Session	$F_{3,64}$=25.56	<0.0001
Genotype x Session	$F_{3,64}$=0.3155	0.9258

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5-CSRTT (Supplementary Fig. 5D,E)		
VAChTcKO mice - reward collection latency (Supplementary Fig. 5D, n=11-9)		
	Two-way ANOVA	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,72}$=0.0004	0.9846
Stimulus duration	$F_{7,72}$=1.107	0.3521
Genotype x Stimulus duration	$F_{7,72}$=0.2045	0.8929
VAChTcKO mice – premature responses (Supplementary Fig. 5E, n=11-9)		
	Two-way ANOVA	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,72}$=2.141	0.1477
Stimulus duration	$F_{7,72}$=0.8809	0.4552
Genotype x Stimulus duration	$F_{7,72}$=0.5226	0.6681

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Table S13: Statistics for Supplementary Figure 6

Extinction tests (Supplementary Fig. 6A-F)				
	VGLUT3cKO overtrained mice (Supplementary Fig. 6A,B)		VGLUT3cKO-D2CRE overtrained mice (Supplementary Fig. 6C,D)	
	Unpaired t-test			
	Controls *vs* mutant littermates			
	n	*p*-value	n	*p*-value
Training	12-14	0.7053	13-13	0.0219
	Two-way ANOVA repeated measures			
	Controls *vs* mutant littermates			
Probe sessions	F-value	*p*-value	F-value	*p*-value
Genotype	$F_{1,24}$=0.2363	0.6313	$F_{1,24}$=0.6877	0.4151
Time	$F_{5,120}$=45.57	<0.0001	$F_{5,120}$=57.48	<0.0001
Genotype x Time	$F_{5,120}$=0.3684	0.8694	$F_{5,120}$=1.328	0.257
	VAChTcKO overtrained mice (Supplementary Fig. 6E,F)			
	Unpaired t-test			
	Controls *vs* mutant littermates			
	n	*p*-value		
Training	14-11	0.4086		
	Two-way ANOVA repeated measures			
	Controls *vs* mutant littermates			
Probe sessions	F-value	*p*-value		
Genotype	$F_{1,23}$=13.4	0.0013		
Time	$F_{5,115}$=38.88	<0.0001		
Genotype x Time	$F_{5,115}$=2.77	0.0212		

745 **Table S14: Statistics for Supplementary Figure 7**

Devaluation after FR1 training – normalized data (Supplementary Fig. 7A)	
Controls	VGLUT3cKO mice
One sample t-test again chance (0.5)	
	p-value
Controls valued	<0.0001
Controls devalued	<0.0001
VGLUT3cKO valued	0.0003
VGLUT3cKO valued	0.0003

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Sucrose self-administration - FR1 training (Supplementary Fig. 7B)		
VGLUT3-KO mice (n=12-12)		
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,22}$=3.630	0.0699
Session	$F_{11,242}$=26.16	<0.0001
Genotype x Session	$F_{11,242}$=2.173	0.0165

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Devaluation after FR1 training (Supplementary Fig. 7C)			
Controls		VGLUT3-KO mice	
Paired t test			
Valued *vs* devalued condition			
n	*p*-value	n	*p*-value
12	0.0053	11	0.0131

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Devaluation after FR1 training – normalized data (Supplementary Fig. 7D)	
Controls	VGLUT3-KO mice
One sample t-test again chance (0.5)	
	p-value
Controls valued	0.0021
Controls devalued	0.0022
VGLUT3-KO valued	0.0014
VGLUT3-KO valued	0.0015

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Devaluation after FR1 training – normalized data (Supplementary Fig. 7E)	
Controls	VAChTcKO male mice
One sample t-test again chance (0.5)	
	p-value
Controls valued	0.0222
Controls devalued	0.0222
VAChTcKO valued	0.2613
VAChTcKO valued	0.2613

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Devaluation after FR1 training (Fig. 7F)		
Control male mice *vs* VAChTcKO male mice		
Two-way ANOVA		
	F-value	*p*-value
Genotype	$F_{1,44}$=1.881	0.1772
Value	$F_{1,44}$=8.943	0.0045
Genotype x Value	$F_{1,44}$=4.735	0.0350

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Devaluation after FR1 training (Supplementary Fig. 7F)			
Controls		VAChTcKO male mice	
Paired t test			
Valued *vs* devalued condition			
n	*p*-value	n	*p*-value
12	0.0146	12	0.2756

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Devaluation after FR1 training – normalized data (Supplementary Fig. 7G)	
Controls	VAChTcKO female mice
One sample t-test again chance (0.5)	
	p-value
Controls valued	0.0046
Controls devalued	0.0046
VAChTcKO valued	0.1388
VAChTcKO valued	0.1449

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Sucrose self-administration - 2 last days FR1 + RI training (Supplementary Fig. 7H)		
VGLUT3-KO mice (n=12-12)		
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,22}$=8.352	0.0085
Session	$F_{9,198}$=49.03	<0.0001
Genotype x Session	$F_{9,198}$=4.225	<0.0001

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Devaluation after RI training – normalized data (Supplementary Fig. 7I)	
Controls	VGLUT3cKO mice
One sample t-test again chance (0.5)	
	p-value
Controls valued	0.1093
Controls devalued	0.1093
VGLUT3cKO valued	0.0063
VGLUT3cKO valued	0.0065

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Devaluation after RI training (Supplementary Fig. 7J)			
Controls		VGLUT3-KO mice	
Paired t test			
Valued *vs* devalued condition			
n	*p*-value	n	*p*-value
12	0.7049	11	0.0042

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Sucrose self-administration - 2 last days FR1 + RI training (Supplementary Fig. 7K)		
	VAChTcKO mice (n=11-12)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,21}$=3.627	0.0706
Session	$F_{9,189}$=25.49	<0.0001
Genotype x Session	$F_{9,189}$=2.247	0.0208

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Devaluation after RI training (Supplementary Fig. 7L)			
Controls		VAChTcKO mice	
Paired t test			
Valued *vs* devalued condition			
n	*p*-value	n	*p*-value
11	0.7461	11	0.8594

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Devaluation after RI training – normalized data (Supplementary Fig. 7M)	
Controls	VAChTcKO mice
One sample t-test again chance (0.5)	
	p-value
Controls valued	0.8326
Controls devalued	0.8326
VGLUT3cKO valued	0.4942
VGLUT3cKO valued	0.4942

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Locomotion (Supplementary Fig. 7N)		
Unpaired t test		
	Controls vs. VAChTcKO littermates	
	n	*p*-value
Day 1	10-10	0.9760
Day 2	10-10	0.7735
Day 3	10-10	0.2527
Night	10-10	0.2129

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Progressive ratio test (Supplementary Fig. 7O)		
Unpaired t test		
	Controls vs. VGLUT3-KO littermates	
	n	*p*-value
Total number of nosepokes during session	12-12	0.9822
Breaking point	12-12	0.8863

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Table S15: Statistics for Supplementary Figure 9

Binge-like sucrose overconsumption model (Supplementary Fig. 9A-D)		
VGLUT3cKO mice (n=10-10)		
Two-way ANOVA repeated measures		
Controls *vs* mutant littermates		
Sucrose consumption - H0-H1 (Supplementary *Fig.9A)*	F-value	*p*-value
Genotype	$F_{1,18}$=0.3360	0.5693
Time (Days)	$F_{11,198}$=32.24	<0.0001
Genotype x Time	$F_{11,198}$=1.496	0.1352
Sucrose consumption - H0-H4 (Supplementary *Fig. 9B)*	F-value	*p*-value
Genotype	$F_{1,18}$=1.090	0.3104
Time (Days)	$F_{11,198}$=52.59	<0.0001
Genotype x Time	$F_{11,198}$=0.9488	0.4948
Water consumption (Supplementary *Fig. 9C)*	F-value	*p*-value
Genotype	$F_{1,18}$=0.3424	0.5657
Time (Days)	$F_{11,198}$=10.21	<0.0001
Genotype x Time	$F_{11,198}$=1.559	0.1133
Food consumption (Supplementary *Fig. 9D)*	F-value	*p*-value
Genotype	$F_{1,18}$=1.221	0.2837
Time (Days)	$F_{11,198}$=41.98	<0.0001
Genotype x Time	$F_{11,198}$=0.7811	0.6587

773 **Table S16: Statistics for Supplementary Figure 10**

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Binge-like sucrose overconsumption model (Supplementary Fig. 10A-D)		
VAChTcKO mice		
Two-way ANOVA repeated measures		
Controls *vs* mutant littermates		
*Food consumption - H0-H4 - male (*Supplementary *Fig. 10A, n=12-12)*	F-value	*p*-value
Genotype	$F_{1,22}$=0.0856	0.7727
Time (Days)	$F_{15,330}$=51.34	<0.0001
Genotype x Time	$F_{15,330}$=1.512	0.0988
*Food consumption - H0-H4 - female (*Supplementary *Fig. 10B, n=13-11)*	F-value	*p*-value
Genotype	$F_{1,22}$=0.2988	0.5901
Time (Days)	$F_{15,330}$=25.37	<0.0001
Genotype x Time	$F_{15,30}$=0.9188	0.5433
*Water consumption - H0-H4 - male (*Supplementary *Fig. 10C, n=12-12)*	F-value	p-value
Genotype	$F_{1,22}$=0.6309	0.4355
Time (Days)	$F_{15,330}$=11.66	<0.0001
Genotype x Time	$F_{15,330}$=0.5939	0.8795
*Water consumption - H0-H4 - female (*Supplementary *Fig. 10D, n=13-11)*	F-value	p-value
Genotype	$F_{1,22}$=1.366	0.2549
Time (Days)	$F_{15,330}$=15.30	<0.0001
Genotype x Time	$F_{15,330}$=0.5527	0.9091

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Food and water consumption H0-H1-male (Supplementary Fig. 10E-F)		
VAChTcKO mice		
Unpaired t-test		
Controls *vs* mutant littermates		
	n	*p*-value
Food consumption (Supplementary Fig. 10E)	12-12	0.4467
Water consumption (Supplementary Fig. 10F)	12-12	0.8836

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Food and water consumption H0-H1-female (Supplementary Fig. 10G-H)		
VAChTcKO mice		
Unpaired t-test		
Controls *vs* mutant littermates		
	n	*p*-value
Food consumption (Supplementary Fig. 10G)	11-13	0.5232
Water consumption (Supplementary Fig. 10H)	11-13	0.5279

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Binge-like saccharine overconsumption model (Supplementary Fig. 10I-M)		
	VAChTcKO mice (n=11-9)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
Saccharine consumption - H0-H4 (Supplementary Fig. 10I)	F-value	*p*-value
Genotype	$F_{1,18}$=2.675	0.1193
Time (Days)	$F_{8,144}$=21.96	<0.0001
Genotype x Time	$F_{8,144}$=0.8928	0.5243
Saccharine consumption - H0-H1 (Supplementary Fig. 10J)	F-value	*p*-value
Genotype	$F_{1,18}$=5.051	0.0374
Time (Days)	$F_{8,144}$=18.62	<0.0001
Genotype x Time	$F_{8,144}$=0.6513	0.7334
Saccharine consumption - H1-H4 (Supplementary Fig. 10K)	F-value	p-value
Genotype	$F_{1,18}$=0.3801	0.5453
Time (Days)	$F_{8,144}$=13.31	<0.0001
Genotype x Time	$F_{8,144}$=1.501	0.1618
Food consumption (Supplementary Fig. 10L)	F-value	p-value
Genotype	$F_{1,18}$=47.53	<0.0001
Time (Days)	$F_{8,144}$=23.06	<0.0001
Genotype x Time	$F_{8,144}$=0.9925	0.4445
Water consumption (Supplementary Fig. 10M)	F-value	p-value
Genotype	$F_{1,18}$=3.234	0.0889
Time (Days)	$F_{8,144}$=21.53	<0.0001
Genotype x Time	$F_{8,144}$=1.413	0.1957

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Sucrose preference (Supplementary Fig. 10N)	
VAChTcKO mice	
Unpaired t-test	
Controls *vs* mutant littermates	
n	*p*-value
11-9	0.6742

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Sucrose consumption after glucose injection (Supplementary Fig. 10O)		
VAChTcKO mice		
Unpaired t-test		
Controls vs mutant littermates		
	n	*p*-value
H0-H4	11-9	0.0065
H0-H1	11-9	0.0280
H1-H4	11-9	0.1621

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Sucrose consumption after chow preload (Supplementary Fig. 10P)		
VAChTcKO mice		
Unpaired t-test		
Controls *vs* mutant littermates		
	n	*p*-value
H0-H4	12-12	0.0303
H0-H1	12-12	0.0049
H1-H4	12-12	0.7266
Food consumption during chow preload (Supplementary Fig. 10Q)		
p-value		
0.3153		
Sucrose consumption with high-caloric food (Supplementary Fig. 10R)		
VAChTcKO mice		
Unpaired t-test		
Controls *vs* mutant littermates		
	n	*p*-value
H0-H4	12-12	0.0012
H0-H1	12-12	0.0019
H1-H4	12-12	0.0594
High-caloric food consumption (Supplementary Fig. 10S)		
p-value		
0.5297		

788 **Table S17: Statistics for Supplementary Figure 11**

Activity-based anorexia model - baseline (Supplementary Fig. 11A,B)		
VGLUT3cKO mice (n=10-10)		
Two-way ANOVA repeated measures		
Controls *vs* mutant littermates		
Food intake (Supplementary Fig. 11A)	F-value	*p*-value
Genotype	$F_{1,18}$=0.032	0.8594
Time (Days)	$F_{6,108}$=43.14	<0.0001
Genotype x Time	$F_{6,108}$=1.392	0.2242
Body weight (Supplementary Fig. 11B)	F-value	*p*-value
Genotype	$F_{1,18}$=0.013	0.9089
Time (Days)	$F_{6,108}$=72.91	<0.0001
Genotype x Time	$F_{6,108}$=0.2056	0.9744

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Food intake during baseline of ABA model (Supplementary Fig. 11C,D)		
	VAChTcKO mice	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
Male (n=12-12) *(*Supplementary Fig. 11C*)*	F-value	*p*-value
Genotype	$F_{1,22}$=0.0095	0.9231
Time (Days)	$F_{6,132}$=84.69	<0.0001
Genotype x Time	$F_{6,132}$=0.6359	0.7013
Female (n=11-13) *(*Supplementary Fig. 11D*)*	F-value	*p*-value
Genotype	$F_{1,22}$=0.0337	0.8560
Time (Days)	$F_{6,132}$=135.4	<0.0001
Genotype x Time	$F_{6,132}$=2.097	0.0577

790

Body weight during baseline of ABA model (Supplementary Fig. 11E,F)		
	VAChTcKO mice	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
Male (n=12-12) *(*Supplementary Fig. 11E*)*	F-value	*p*-value
Genotype	$F_{1,22}$=0.9828	0.3323
Time (Days)	$F_{6,132}$=17.23	<0.0001
Genotype x Time	$F_{6,132}$=0.9299	0.4758
Female (n=11-13) *(*Supplementary Fig. 11F*)*	F-value	*p*-value
Genotype	$F_{1,22}$=0.0119	0.9138
Time (Days)	$F_{6,132}$=14.53	<0.0001
Genotype x Time	$F_{6,132}$=0.3201	0.9255

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Activity-based anorexia model (Supplementary Fig. 11G)		
VGLUT3cKO mice (n=10-10)		
Two-way ANOVA repeated measures		
Controls *vs* mutant littermates		
Food intake (Supplementary Fig. 11G)	F-value	*p*-value
Genotype	$F_{1,18}$=1.227	0.2825
Time (Days)	$F_{7,126}$=97.84	<0.0001
Genotype x Time	$F_{7,126}$=1.380	0.2194

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Activity-based anorexia model – survival (Supplementary Fig. 11H)		
VGLUT3cKO mice		
Kaplan–Meier test		
Controls *vs* mutant littermates		
	n	*p*-value
Log-rank (Mantel-Cox) post hoc comparison	n=10-10	0.9558
Gehan-Breslow-Wilcoxon post hoc comparison	n=10-10	0.9674

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Activity-based anorexia model (Supplementary Fig. 11I,J)		
Controls *vs* VAChTcKO (n=12-12 for males and 11-13 for females)		
Two-way ANOVA repeated measures		
Food intake - males (Supplementary Fig. 11I)	F-value	*p*-value
Genotype	$F_{1,22}$=29.30	<0.0001
Time (Days)	$F_{7,154}$=62.68	<0.0001
Genotype x Time	$F_{7,154}$=2.365	0.0253
Food intake - females (Supplementary Fig. 11J)	F-value	*p*-value
Genotype	$F_{1,22}$=14.20	0.0011
Time (Days)	$F_{7,154}$=34.92	<0.0001
Genotype x Time	$F_{7,154}$=3.537	0.0015

Table S18: Statistics for Supplementary Figure 13

Microdialysis (Supplementary Fig. 13A)		
	DA in VAChTcKO mice	
	Unpaired t-test	
	Controls *vs* mutant littermates	
Brain area	n	*p*-value
Dorsal striatum	7-7	0.0221

Voltammetry - DA release - dorsomedial striatum (DMS) (Supplementary Fig. 13B)		
	VGLUT3cKO mice (n=9-9)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,16}$=7.099	0.0170
Injections	$F_{3,48}$=55.52	<0.0001
Genotype x Injections	$F_{3,48}$=5.155	0.0038

Voltammetry - DA release - dorsomedial striatum (DMS) (Supplementary Fig. 13C)		
	VGLUT3cKO mice	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	9-9	0.0188

Voltammetry - DA release - dorsolateral striatum (DLS) (Supplementary Fig. 13D)		
	VGLUT3cKO mice (n=9-9)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,16}$=0.1099	0.7466
Injections	$F_{3,48}$=52.85	<0.0001
Genotype x Injections	$F_{3,48}$=0.2886	0.8334

Voltammetry - DA release - dorsolateral striatum (DLS) (Supplementary Fig. 13E)		
	VGLUT3cKO mice	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	9-9	0.7137

Voltammetry - DA release - dorsomedial striatum (DMS) (Supplementary Fig. 13F)		
	VGLUT3-KO mice (n=16-14)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,28}$=6.711	0.0150
Injections	$F_{3,84}$=113.2	<0.0001
Genotype x Injections	$F_{3,84}$=3.801	0.0131

Voltammetry - DA release - dorsomedial striatum (DMS) (Supplementary Fig. 13G)		
	VGLUT3-KO mice	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	16-14	0.0103
K2	16-14	0.3083
K3	16-14	0.4919
K4	16-14	0.0620

Voltammetry - T80 - dorsomedial striatum (DMS) (Supplementary Fig. 13H)		
	VGLUT3-KO mice (n=16-14)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,28}$=0.7270	0.4011
Injections	$F_{3,84}$=42.21	0.0001
Genotype x Injections	$F_{3,84}$=0.0956	0.9623

Voltammetry - T80 – dorsomedial striatum (DMS) (Supplementary Fig. 13H)		
	VGLUT3-KO mice (n=16-14)	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	16-14	0.9100
K2	16-14	0.0316
K3	16-14	0.0392
K4	16-14	0.0013

Voltammetry - DA release - dorsolateral striatum (DLS) (Supplementary Fig. 13I)		
	VGLUT3-KO mice (n=16-17)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,31}$=0.3360	0.1112
Injections	$F_{3,93}$=75.75	0.0001
Genotype x Injections	$F_{3,93}$=0.3360	0.7993

Voltammetry - DA release - dorsolateral striatum (DLS) (Supplementary Fig. 13J)		
	VGLUT3-KO mice	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	16-17	0.3985
K2	16-17	0.0106
K3	16-17	0.2006
K4	16-17	0.2072

Voltammetry - T80 - dorsolateral striatum (DLS) (Supplementary Fig. 13K)		
	VGLUT3-KO mice (n=16-16)	
	Two-way ANOVA repeated measures	
	Controls *vs* mutant littermates	
	F-value	*p*-value
Genotype	$F_{1,30}$=0.5997	0.4448
Injections	$F_{3,90}$=70.39	0.0001
Genotype x Injections	$F_{3,90}$=0.3169	0.8131

Voltammetry - T80 - dorsolateral striatum (DLS) (Supplementary Fig. 13K)		
	VGLUT3-KO mice	
	Mann Whitney	
	Controls *vs* mutant littermates	
KCl injection	n	*p*-value
K1	16-16	0.8001
K2	16-16	0.1036
K3	16-16	0.8307
K4	16-16	0.6619

Voltammetry - T80 - dorsomedial striatum (DMS) (Supplementary Fig. 13L,M)				
	VGLUT3cKO mice (n=9-9) (Supplementary Fig. 13L)		VAChTcKO mice (n=11-16) (Supplementary Fig. 13L)	
	Two-way ANOVA repeated measures			
	Controls *vs* mutant littermates			
	F-value	*p*-value	F-value	*p*-value
Genotype	$F_{1,16}$=0.1153	0.7386	$F_{1,25}$=6.148	0.0217
Injections	$F_{3,48}$=20.98	<0.0001	$F_{3,75}$=51.73	<0.0001
Genotype x Injections	$F_{3,48}$=0.0919	0.9642	$F_{3,75}$=6.148	0.0471

Voltammetry - T80 - dorsomedial striatum (DMS) (Supplementary Fig. 13L,M)				
	VGLUT3cKO mice		VAChTcKO mice	
	Mann Whitney			
	Controls *vs* mutant littermates			
KCl injection	n	*p*-value	n	*p*-value
K1	9-9	0.9090	10-13	0.0492
K2	9-9	0.6502	10-13	0.4734
K3	9-9	0.6517	10-13	0.3051
K4	9-9	0.8421	10-13	0.8433

Voltammetry - T80 - dorsolateral striatum (DLS) (Supplementary Fig. 13N,O)				
	VGLUT3cKO mice (n=8-9)		VAChTcKO mice (n=16-17)	
	Two-way repeated measures			
	Controls *vs* mutant littermates			
	F-value	*p*-value	F-value	*p*-value
Genotype	$F_{1,15}$=1.310	0.2704	$F_{1,31}$=3.323	0.0787
Injections	$F_{3,45}$ =27.30	<0.0001	$F_{3,93}$=43.16	<0.0001
Genotype x Injections	$F_{3,45}$=1.230	0.3098	$F_{3,93}$=2.957	0.0368

Voltammetry - T80 - dorsolateral striatum (DLS) (Supplementary Fig. 12N,O)				
	VGLUT3cKO mice		VAChTcKO mice	
	Mann Whitney			
	Controls *vs* mutant littermates			
KCl injection	n	*p*-value	n	*p*-value
K1	9-9	0.3088	16-17	0.0713
K2	9-9	0.3568	16-17	0.9147
K3	9-9	0.9056	16-17	0.0766
K4	9-9	0.1316	16-17	0.0482

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Voltammetry - DA release - dorsomedial striatum (DMS) (Supplementary Fig. 12P)				
	VGLUT3cKO mice		VAChTcKO mice	
	Mann Whitney			
	Controls *vs* mutant littermates			
KCl injection	n	*p*-value	n	*p*-value
K2	9-9	0.9090	11-16	0.0271
K3	9-9	>0.9999	11-16	0.0484
K4	9-9	0.8142	11-16	0.0045

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Voltammetry - DA release - dorsolateral striatum (DLS) (Supplementary Fig. 12Q)				
	VGLUT3cKO mice		VAChTcKO mice	
	Mann Whitney			
	Controls *vs* mutant littermates			
KCl injection	n	*p*-value	n	*p*-value
K2	9-9	0.7475	16-17	0.7285
K3	9-9	0.9518	16-17	0.5633
K4	9-9	0.1883	16-17	0.4073

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Table S19: Statistics for Supplementary Figure 14

Binge-like sucrose overconsumption model (Supplementary Fig. 14A,B)		
VAChTcKO-DMS mice (n=11-11)		
Food consumption H0-H4 (Supplementary Fig. 14A)	F-value	*p*-value
Genotype	$F_{1,20}$=0.4283	0.5203
Time (Days)	$F_{15,300}$=9.975	<0.0001
Genotype x Time	$F_{15,300}$=0.7358	0.7475
Water consumption H0-H4 (Supplementary Fig. 14B)	F-value	*p*-value
Genotype	$F_{15,300}$=0.7430	0.3989
Time (Days)	$F_{15,300}$=1.838	0.0292
Genotype x Time	$F_{15,300}$=0.5433	0.9149

Sucrose preference (Supplementary Fig. 14C)	
VAChTcKO-DMS mice	
Unpaired t-test	
AAV-GFP injected *vs* AAV-Cre-GFP injected mice	
n	*p*-value
11-11	0.7067

Sucrose consumption after chow preload (Supplementary Fig. 14D)		
VAChTcKO-DMS mice		
unpaired t-test		
AAV-GFP injected *vs* AAV-Cre-GFP injected mice		
	n	*p*-value
H0-H4	11-11	0.0151
H0-H1	11-11	0.0140
H1-H4	11-11	0.136

Food consumption during chow preload (Supplementary Fig. 14E)		
VAChTcKO-DMS mice		
Unpaired t-test		
AAV-GFP injected *vs* AAV-Cre-GFP injected mice		
	n	*p*-value
H0-H4	n=11-11	0.8752

827 **Table S20: Statistics for Supplementary Figure 15**

Activity-based anorexia model – L-DOPA treatment – BASELINE (Supplementary Figure 15A,B)		
Two-way ANOVA repeated measures		
Controls-saline *vs* mutant littermates-saline, Controls-L-Dopa and mutant littermates-L-Dopa (n=10 for each group)		
Body weight (Supplementary Fig. 15A)	F-value	*p*-value
Group	$F_{3,36}$=0.1780	0.9106
Time (Days)	$F_{18,216}$=51.66	<0.0001
Group x Time	$F_{18,216}$=0.6168	0.8846
Food intake (Supplementary Fig. 15B)	F-value	*p*-value
Group	$F_{3,36}$=0.0866	0.9669
Time (Days)	$F_{18,216}$=242.1	<0.0001
Group x Time	$F_{18,216}$=1.265	0.2132

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Activity-based anorexia model – Donepezil treatment – BASELINE (Supplementary Figure 15C,D)		
Two-way ANOVA repeated measures		
Controls-saline *vs* mutant littermates-saline, Controls-L-Dopa and mutant littermates-L-Dopa (n=10 for each group)		
Body weight (Supplementary Fig. 15C)	F-value	*p*-value
Group	$F_{3,36}$=0.1342	0.9391
Time (Days)	$F_{18,216}$=12.84	<0.0001
Group x Time	$F_{18,216}$=0.5735	0.9161
Food intake (Supplementary Fig. 15D)	F-value	*p*-value
Group	$F_{3,36}$=0.1038	0.9573
Time (Days)	$F_{18,216}$=40.90	<0.0001
Group x Time	$F_{18,216}$=0.9838	0.4795

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Activity-based anorexia model – L-DOPA treatment (Supplementary Figure 15E)		
Two-way ANOVA repeated measures		
Controls-saline *vs* mutant littermates-saline, Controls-L-Dopa and mutant littermates-L-Dopa (n=10 for each group)		
Food intake (Supplementary Fig. 15E)	F-value	*p*-value
Group	$F_{3,36}$=5.217	0.0043
Time (Days)	$F_{7,252}$=27.73	<0.0001
Group x Time	$F_{21,252}$=1.417	0.1101

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Activity-based anorexia model – L-DOPA treatment (Supplementary Figure 15E)		
Three-way ANOVA		
Controls-saline *vs* mutant littermates-saline, Controls-L-Dopa and mutant littermates-L-Dopa (n=10 for each group)		
Food intake (Supplementary Fig. 15F)	F-value	*p*-value
Group	$F_{1,160}$=26.96	<0.0001
Time (Days)	$F_{7,160}$=10.03	<0.0001
Drug	$F_{1,160}$=30.41	<0.0001
Group x Time	$F_{7,160}$=1.705	0.1112
Drug x Time	$F_{7,160}$=0.8561	0.5427
Group x Drug	$F_{1,160}$=4.419	0.0371
Group x Drug x Time	$F_{7,160}$=1.005	0.4297

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Activity-based anorexia model - Donepezil treatment (Supplementary Figure 15F)		
Two-way ANOVA repeated measures		
Controls-saline *vs* mutant littermates-saline, Controls-donepezil and mutant littermates-donepezil (n=9 for each group)		
Food intake (Supplementary Fig. 15F)	F-value	*p*-value
Group	$F_{3,32}$=4.682	0.0080
Time (Days)	$F_{7,224}$=40.88	<0.0001
Group x Time	$F_{21,224}$=2.800	0.0522

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Activity-based anorexia model – Donepezil treatment (Supplementary Figure 15F)		
Three-way ANOVA		
Controls-saline *vs* mutant littermates-saline, Controls-L-Dopa and mutant littermates-L-Dopa (n=10 for each group)		
Food intake (Supplementary Fig. 15F)	F-value	*p*-value
Group	$F_{1,256}$=35.48	<0.0001
Time (Days)	$F_{7,256}$=21.00	<0.0001
Drug	$F_{1,256}$=14.58	0.0002
Group x Time	$F_{7,256}$=0.9648	0.4575
Drug x Time	$F_{7,256}$=0.5554	0.7916
Group x Drug	$F_{1,256}$=11.71	0.0007
Group x Drug x Time	$F_{7,256}$=0.9354	0.4796

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838 **Table S21: Statistics for Supplementary Figure 16**

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Food consumption during chow preload (Supplementary Figure 16A,B)		
VGLUT3cKO mice		
Unpaired t-test		
Controls vs mutant littermates		
	n	*p*-value
Conso. regular food during prefeeding -(after FR1) Supplementary Figure 16A	n=11-12	0.6168
Conso. sucrose pellets during prefeeding (after FR1) Supplementary Figure 16A	n=11-12	0.1794
Conso. sucrose pellets during prefeeding -(after FR1) Supplementary Figure 16B	n=10-13	0.8055
Conso. sucrose pellets during prefeeding (after RI) Supplementary Figure 16B	n=10-13	0.3818

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Food consumption during chow preload (Supplementary Figure 16C,D)		
VGLUT3-KO mice		
Unpaired t-test		
Controls vs mutant littermates		
	n	*p*-value
Conso. regular food during prefeeding -(after FR1) Supplementary Figure 16C	n=11-12	0.2959
Conso. sucrose pellets during prefeeding (after FR1) Supplementary Figure 16C	n=11-12	0.9969
Conso. sucrose pellets during prefeeding -(after FR1) Supplementary Figure 16D	n=11-12	0.8260
Conso. sucrose pellets during prefeeding (after RI) Supplementary Figure 16D	n=11-12	0.7733

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Food consumption during chow preload (Supplementary Figure 16E,F)		
VAChTcKO male mice		
Unpaired t-test		
Controls vs mutant littermates		
	N	*p*-value
Conso. regular food during prefeeding -(after FR1) Supplementary Figure 16E	n=12-12	0.3357
Conso. sucrose pellets during prefeeding (after FR1) Supplementary Figure 16E	n=12-12	0.5599
Conso. sucrose pellets during prefeeding -(after FR1) Supplementary Figure 16F	n=11-11	0.3576
Conso. sucrose pellets during prefeeding (after RI) Supplementary Figure 16F	n=11-11	0.7237

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Food consumption during chow preload (Supplementary Figure 16G)		
VAChTcKO female mice		
Unpaired t-test		
Controls vs mutant littermates		
	N	*p*-value
Conso. regular food during prefeeding -(after FR1) Supplementary Figure 16E	n=11-13	0.6211
Conso. sucrose pellets during prefeeding (after FR1) Supplementary Figure 16E	n=11-13	0.8820

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Ratio sucrose/(sucrose+food) during chow preload (Supplementary Figure 16 H,I,J)		
Unpaired t-test		
Controls vs mutant littermates		
	N	*p*-value
VAChTcKO females (after FR1) Supplementary Figure 16H	n=11-13	0.4816
VAChTcKO males (after FR1) Supplementary Figure 16I	n=12-12	0.5232
VAChTcKO males (after RI) Supplementary Figure 16J	n=11-11	0.6102

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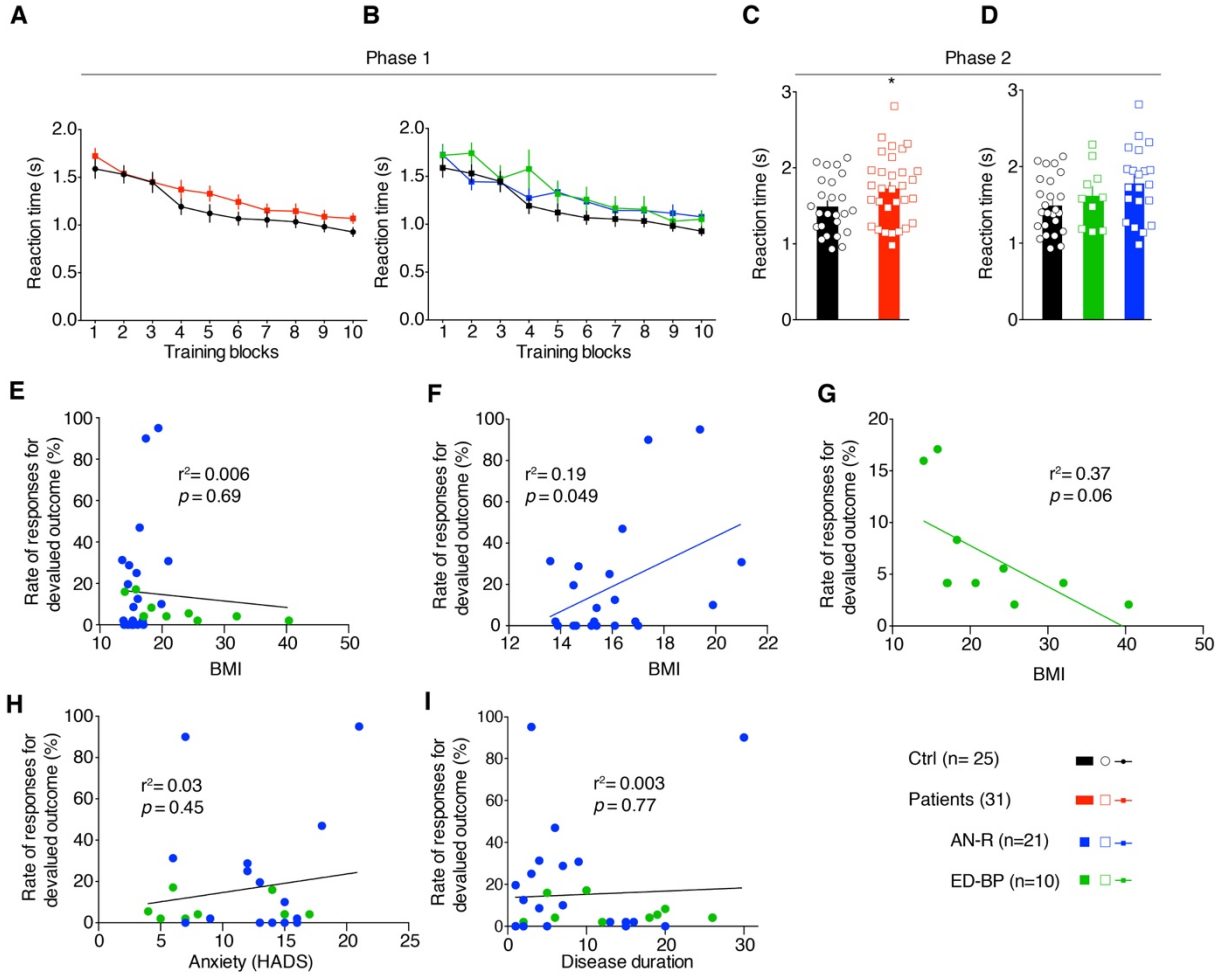
Supplementary Figures, Text and Tables



Supplementary Figure 1.

Analysis of eating disorder patients and heatly controls using a computer-based neurocognitive task.

(A) Mean reaction time across the training blocks of phase-1 (instrumental learning stage) for patients with eating disorders and healthy controls. (B) Mean reaction time across the training blocks of phase-1 (instrumental learning stage) for AN-R patients, ED-BP patients and healthy controls. (C) Mean reaction time during phase-2 (outcome devaluation stage) for patients with eating disorders and healthy controls. (D) Mean reaction time during phase-2 (outcome devaluation stage) for AN-R patients, ED-BP patients and healthy controls. (E-G) Simple linear regression between rate of responses for devalued outcome (phase-3) and the body mass index (BMI) of ED patients. (H) Simple linear regression between rate of responses for devalued outcome (phase-3) and anxiety (HADS) of ED patients. (I) Simple linear regression between rate of responses for devalued outcome (phase-3) and disease duration of ED patients. Two-way ANOVA repeated measures for (A), (B) unpaired t-test for

865 (C), one-way ANOVA followed by Dunnett post-hoc test for (D). Pearson linear regression for
866 (E) to (I).
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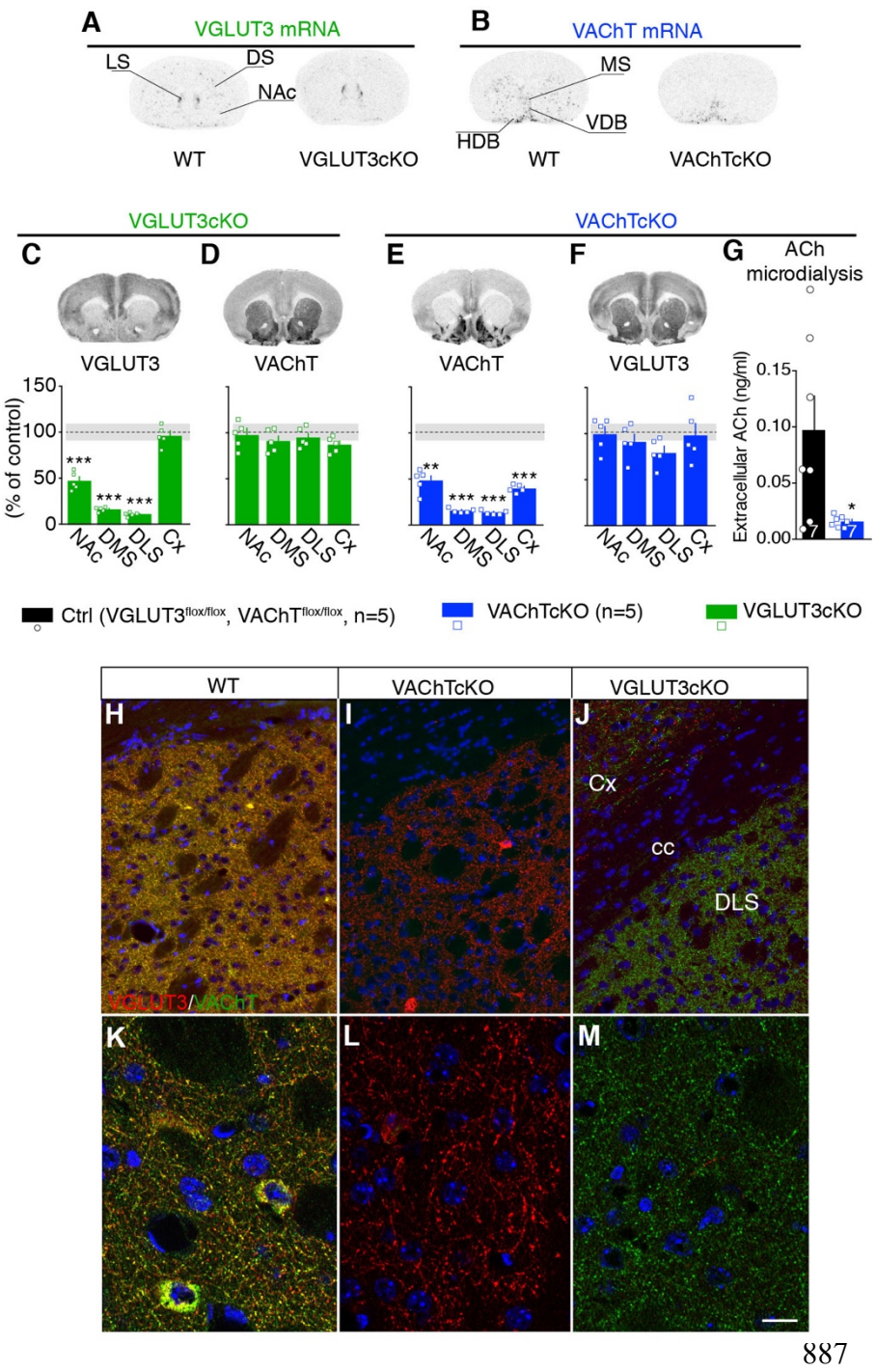
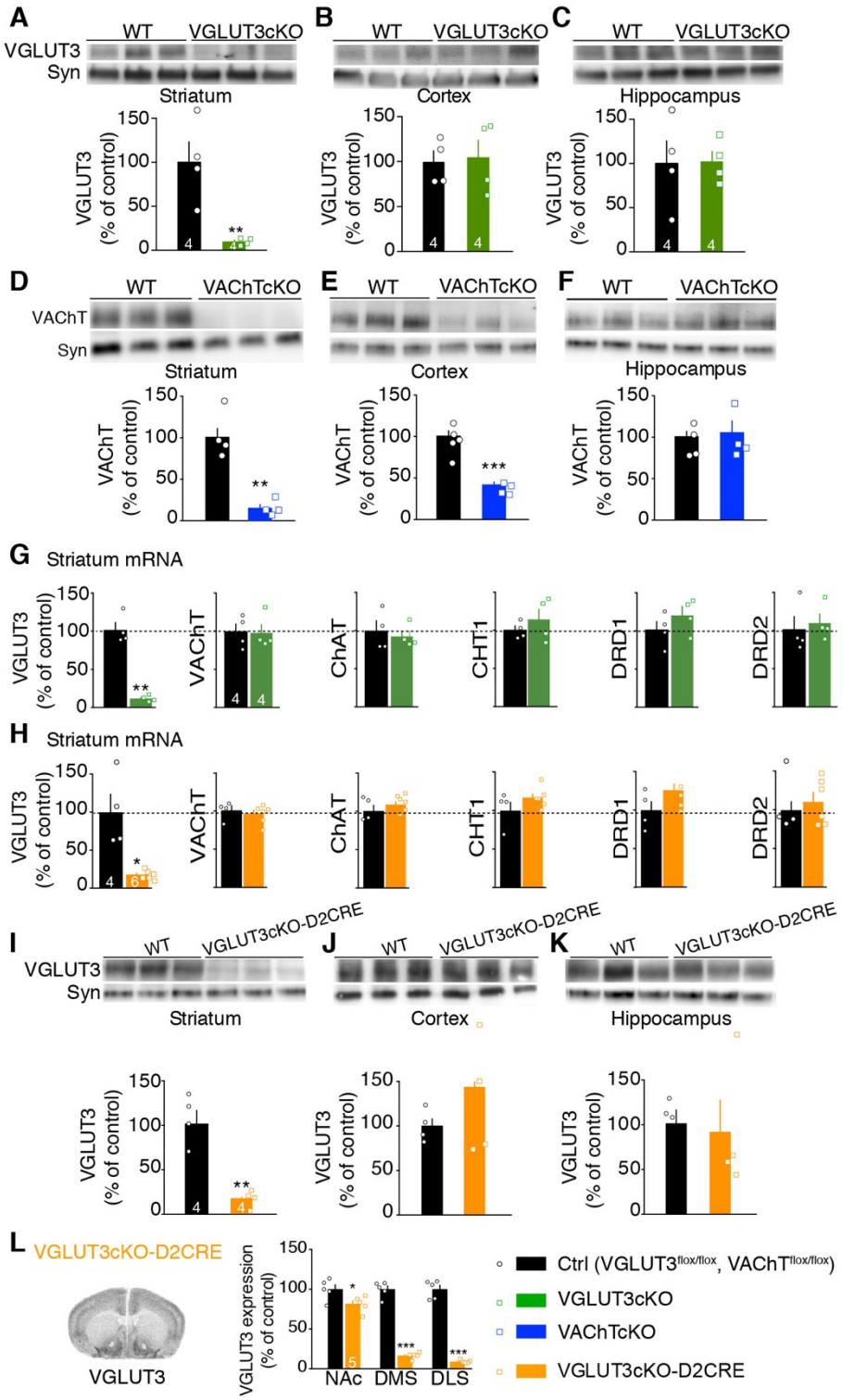


Supplementary Figure 2.

Anatomical validation of VGLUT3cKO mice and VAChTcKO mice.

(**A**-**G**) VAChT or VGLUT3 expressions were characterized by *in situ* hybridization (**A**,**B**) and immunoautoradiography (**C**-**G**) in the striatum of VAChTcKO mice and VGLUT3cKO mice. (**A**) VGLUT3 transcript was deleted from the striatum of VGLUT3cKO mice but was still present in other areas (septum, cortex or basal forebrain). (**B**) VAChT mRNA was absent

from the dorsal striatum (DS) and nucleus accumbens (NAc) of VAChTcKO mice but was still detected in the diagonal band (HDB). (**C**) At the protein level, VGLUT3 expression was almost completely deleted in the DMS and in the DLS of VGLUT3cKo mice. However, 50% of the protein was still present in the ventral striatum or NAc of mutant mice. (**D**) VAChT expression was not affected in the striatum of VGLUT3cKO mice. (**E**) VAChT expression was removed from the dorsal striatum of VAChTcKO mice. Around 50% of the protein was still expressed in the NAc and ≈ 40% in the cortex of VAChTcKO mice. (**F**) In contrast, VGLUT3 expression was not affected in VAChTcKO mice. (**G**) Extracellular ACh release in control mice and VAChTcKO mice measured by *in vivo* microdialysis. (**H**-**M**) Immunofluorescent labelling of VGLUT3 (in red) or VAChT (in green) in the dorsal striatum of VGLUT3cKO mice or VAChTcKO mice demonstrating specificity of transporter deletion. Nuclei are stained in blue (DAPI). Scale bars (in M): 50µm for h-j and 15µm for k-m. cc, corpus callosum; Cx, cerebral cortex; DMS, dorsomedial striatum. DLS, dorsolateral striatum; DS, dorsal striatum; HDB, horizontal diagonal band; NAc, nucleus accumbens; VDB, Vertical diagonal band. Unpaired t-test for (C-G). *p<0.05, **p<0.01, ***p<0.001. All data are mean ± SEM.

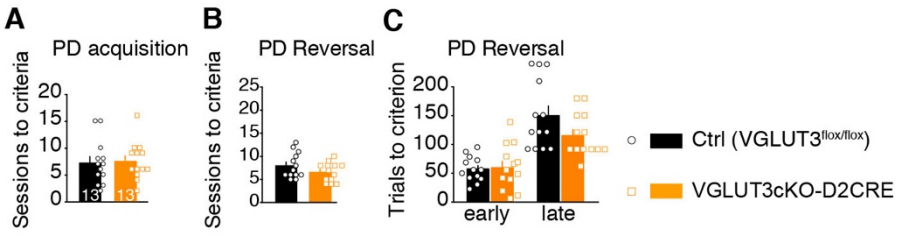


Supplementary Figure 3.

Additional anatomical validation of VGLUT3 or VAChT deletion.

(**A-C**) Quantification by Western blot of VGLUT3 in the striatum (**A**), cortex (**B**) and hippocampus (**C**) of VGLUT3cKO mice and controls. VGLUT3 was dramatically reduced

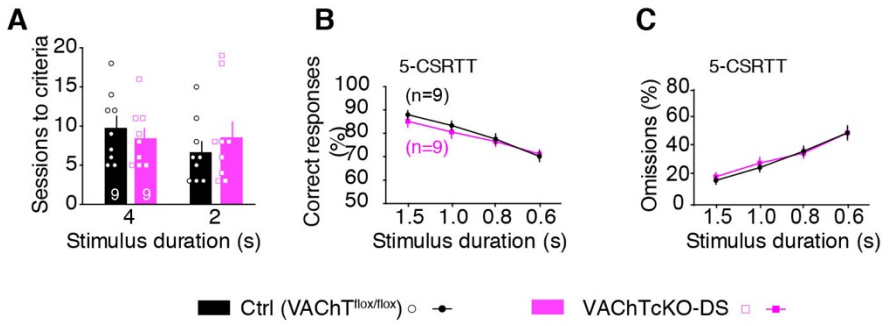
from the striatum of VGLUT3cKO mice whereas no modifications were observed in the cortex or hippocampus. (**D-F**) Quantification of VAChT in the striatum (**D**), cortex (**E**) and hippocampus (**F**) of VAChTcKO mice and controls. VAChT protein was almost completely deleted from the striatum whereas its expression was not modified in the hippocampus. However, a 50% decrease of VAChT expression was detected in the cortex of VAChTcKO mice suggesting that cholinergic neurons from the basal forebrain (projecting to the cortex) express the D2 receptor. (**G**-**H**) Quantification by RT-qPCR of transcripts coding for VGLUT3, VAChT, ChAT, CHT1, DRD1 and DRD2 in VGLUT3cKO (**G**) and VGLUT3cKO-D2CRE mice (**H**). VGLUT3 mRNA was selectively removed from the striatum of both mutant lines. VGLUT3 deletion did not impact the expression of cholinergic markers like VAChT, ChAT, CHT1 neither impacted DRD2 or DRD1 receptors expression. Quantifications are expressed as % of littermate controls. (**I-K**) Quantification by Western blot of VGLUT3 in VGLUT3cKO-D2CRE mice and controls in the striatum (**I**), cortex (**J**) and hippocampus (**K**). VGLUT3 was selectively removed from the striatum of VGLUT3cKO-D2CRE mice. (**L**) Quantification of VGLUT3 expression in the striatum of VGLUT3cKO-D2CRE mice by immunoautoradiography. As observed with VGLUT3cKO mice, VGLUT3 expression was markedly decreased in the DMS and in the DLS but more moderately in the NAc of VGLUT3cKO-D2CRE mice. Unpaired t-test for (A-L). *p<0.05, **p<0.01, ***p<0.001. All data are mean ± SEM.



Supplementary Figure 4.

Pairwise visual discrimination task in VGLUT3cKO-D2CRE.

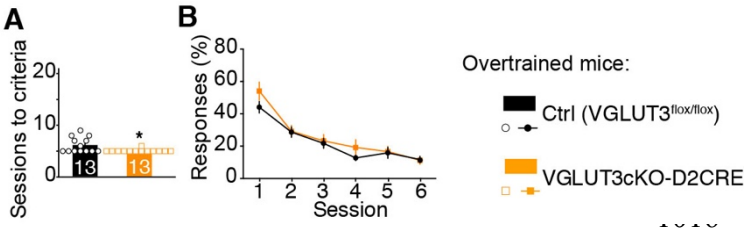
(**A-C**) Performance of VGLUT3cKO-D2CRE mice (n=13) and littermate controls (n=13) during pairwise discrimination task with reversal. (**A**) Performance in the acquisition phase of the task expressed as the number of training sessions required until the mice reached criteria (80% correct responses out of 30 completed trials in two consecutive sessions). (**B**) Performance in the reversal phase of the task. Number of training sessions required for mice to criteria (same as for acquisition). (**C**) Performance of mice during the early and late phases of reversal. Unpaired t-test for (A) and (B). Two-way ANOVA for (C) and Bonferroni's post hoc comparison. All data are mean ± SEM.



Supplementary Figure 5.

Cognitive evaluation of mice lacking VAChT in the dorsal striatum using the 5-CSRTT and impact of VAChT genetic deletion on inhibitory control in mice.

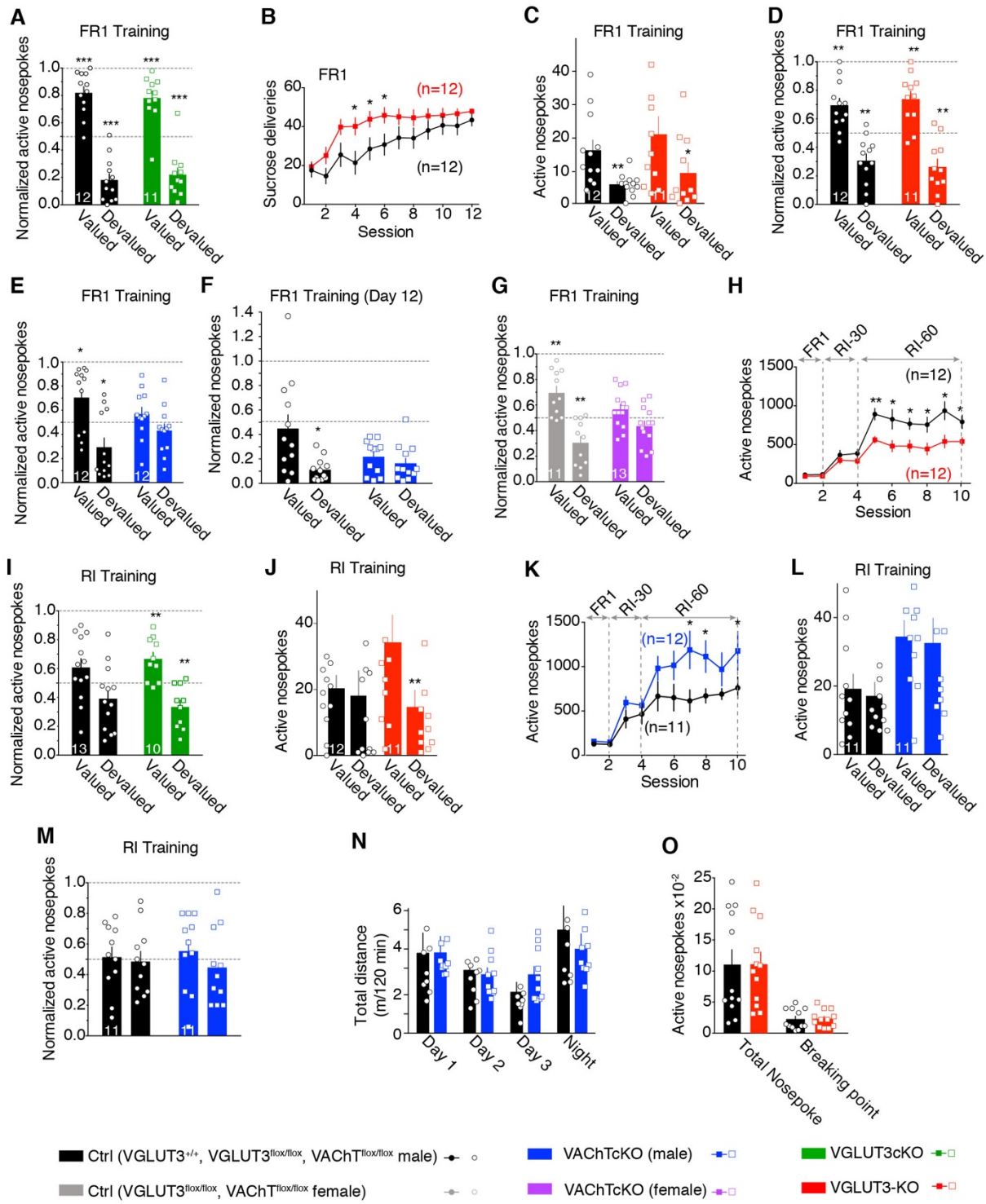
(**A-C**) Five-choice serial-reaction time task (5-CSRTT) was used to assess attention in VAChTcKO-DS mice (n=9) and controls (n=9). (**A**) Number of training sessions required to reach criteria. (**B**) Response accuracy during probe sessions with decreasing stimulus duration. (**C**) Percentage of omitted responses during probe sessions. VAChTcKO-DS mice performed similarly as controls during 5-CSRTT, showing that these mice did not suffer from any attentional deficit. Two-way ANOVA for (A-C) and Bonferroni's post hoc comparison. All data are mean ± SEM.



Supplementary Figure 6.

Extinction tests using touchscreen tasks with overtrained mice

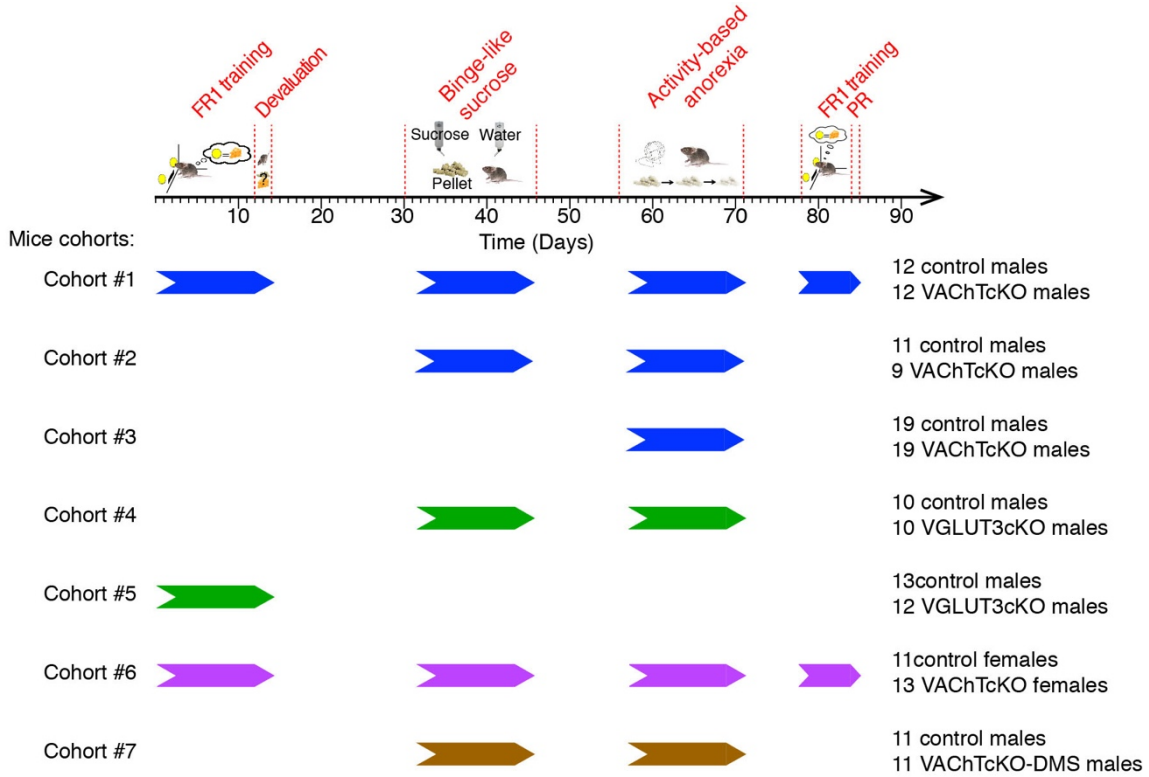
(**A, B**) Extinction tests were performed in mice that were trained in other touchscreen tasks previously. (**A**) Number of training sessions required for VGLUT3cKO-D2CRE mice learn the task. (**B**) Extinction response for VGLUT3cKO-D2CRE mice. Unpaired t-test for (A), Two-way ANOVA repeated measures for (B) and Bonferroni's post hoc comparison. *p<0.05. All data are mean ± SEM.



Supplementary Figure 7.

Normalized performance devaluation tests for all lines, VGLUT3-KO constitutive mice and VAChTcKO mice performance in RI training compared to control littermates, locomotor activity of VAChTcKO mice *vs* controls and motivation (PR) of VGLUT3-KO mice.

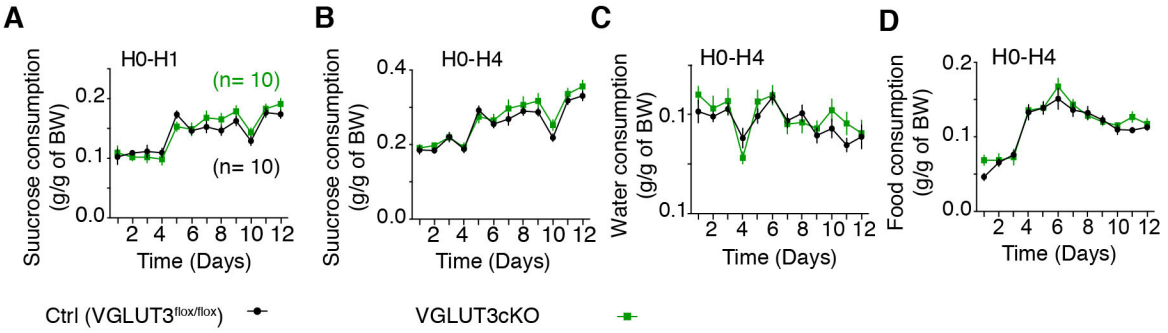
(**A**) Normalized performance of VGLUT3cKO mice and controls during devaluation test after FR1 training. (**B**) Number of sucrose deliveries during the initial instrumental training (FR1) for VGLUT3-KO mice and control littermates. (**C**) Number of active nosepokes during devaluation tests after FR1 initial training for VGLUT3-KO mice and controls, showing they are both sensitive to devaluation (goal-directed behavior).(**D**) Normalized performance of VGLUT3-KO mice and controls during devaluation test after FR1 training. (**E**) Normalized performance of VAChTcKO mice and controls during devaluation test after FR1 training. (**F**) Normalized performance by last day training performance of male VAChTcKO mice and controls during devaluation test after FR1 training. (**G**) Normalized performance of female VAChTcKO mice and controls during devaluation test after FR1 training. (**H**) Number of active nosepokes during the initial and late phases of random-interval (RI) training (RI-30 and RI-60) for VGLUT3-KO mice and control littermates. Note the decreased nosepoke activity during RI training for VGLUT3-KO mice compared to controls. (**I**) Normalized performance of VGLUT3cKO mice and controls during devaluation test after RI training. (**J**) Number of active nosepokes during devaluation tests after RI training for VGLUT3-KO mice and controls, showing that controls developed habits (same performance in valued and devalued conditions), whereas VGLUT3-KO mice are stuck in goal-directed behaviors. (**K**) Number of active nosepokes during the initial and late phases of random-interval (RI) training (RI-30 and RI-60) for VAChTcKO mice and control littermates. Note the increased nosepoke activity during RI training for VAChTcKO mice compared to controls. (**L**) Number of active nosepokes during devaluation tests after RI training for AChTcKO mice and controls, showing that both genotypes developed habitual behavior. (**M**) Normalized performance of VAChTcKO mice and controls during devaluation test after RI training. (**N**) Locomotor activity of VAChTcKO mice and control littermates in a new or a familiar environment (Day 1, 2 and 3), and during day and night periods. (**O**) Progressive ratio for VGLUT3-KO mice and controls. Two-way ANOVA repeated measures for (B), (H), (K) and post hoc comparison with the method of contrasts. One way sample t-test for (A), (D), (E), (G), (I), (M). (B), (D), (F). Paired t-test for (C), (J), (L). Unpaired t-test for (N), (O). *p<0.05, **p<0.01, **p<0.001. All data are mean ± SEM.



Supplementary Figure 8.

Experimental design and major cohorts of mice used in behavioral experiments.

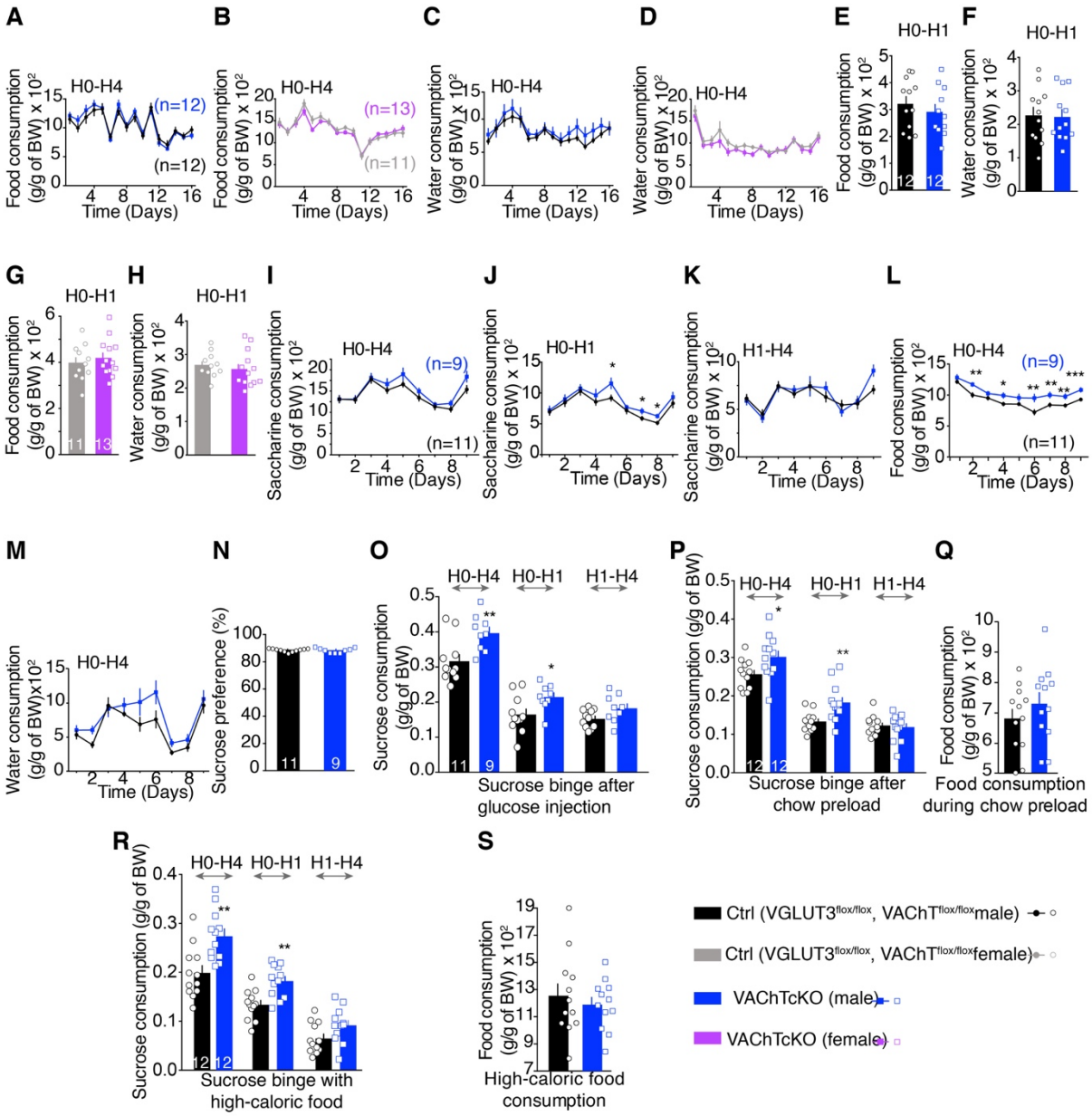
- Cohorts 1 and 6 were used to perform longitudinal experiments (goal-directed behaviors/habits evaluation, sucrose binge, ABA) described on Figure 3F-H and Fig. 4B-D, I. Results obtained with these animals were also used to perform statistical analyses shown on Figure 5.
- Cohort 2 was used to perform controls for binge and activity-based anorexia models. Results collected with these mice are shown on Supplementary Figures 10 and 11
- Cohort 3 was used to perform pharmacological experiments described on Figure 7.
- Cohort 4 was used for experiments described on Supplementary Fig. 9.
- Cohort 5 was used for experiments described on Figure 3B-E.
- Cohort 7: AAV-CRE (VAChTcKO-DMS) or AAV-GFP (controls) were used for experiments described on Figure 6I-M.



Supplementary Figure 9.

Performance of VGLUT3cKO mice during binge-like sucrose overconsumption model.

(**A-D**) Binge-like sucrose overconsumption model. (**A**) Sucrose consumption during the first hour of access (H0-H1) for VGLUT3cKO mice (n=10) and littermates controls (n=10). (**B**) Sucrose consumption during the total duration of access (H0-H4) for VGLUT3cKO mice and controls. (**C**) Water consumption during the total duration of access for VGLUT3cKO mice and controls. (**D**) Food consumption during the total duration of access for VGLUT3cKO mice and controls. Two-way ANOVA repeated measures for (A) to (D) and post hoc comparison with the method of contrasts. All data are mean ± SEM.

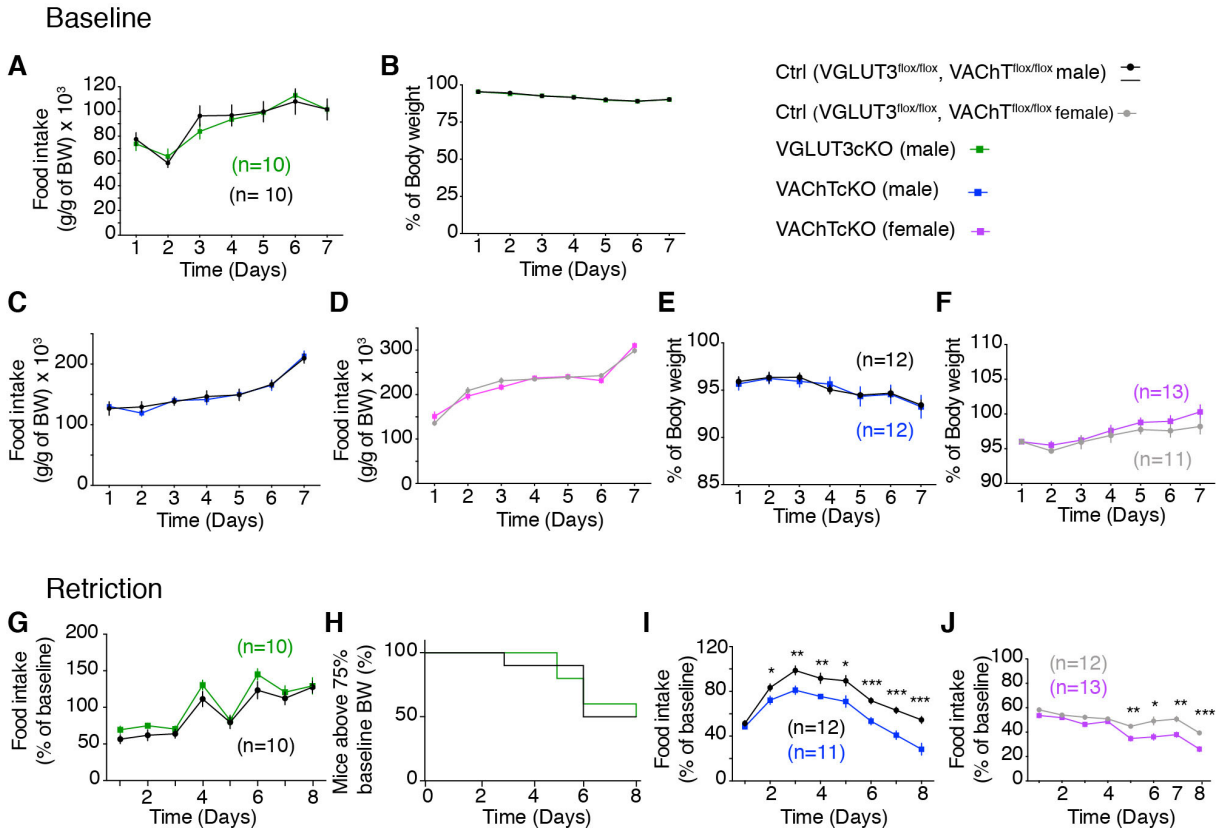


Supplementary Figure 10.

VAChTcKO mice and littermate controls during sucrose binge-like overconsumption.

(**A-H**) Binge-like sucrose overconsumption model. (**A**) Food consumption during the total duration of access (H0-H4) for male VAChTcKO mice and control mice. (**B**) Food consumption during the total duration of access (H0-H4) for female VAChTcKO mice and control mice. (**C**) Water consumption during the total duration of access for male VAChTcKO mice and controls. (**D**) Water consumption during the total duration of access for female VAChTcKO mice and control mice. (**E**) Food consumption during the 1st hour of access (H0-H1) for male VAChTcKO mice and control mice after 16 days of sucrose binge-like model. (**F**) Water consumption during the 1st hour of access (H0-H1) for male VAChTcKO mice and control

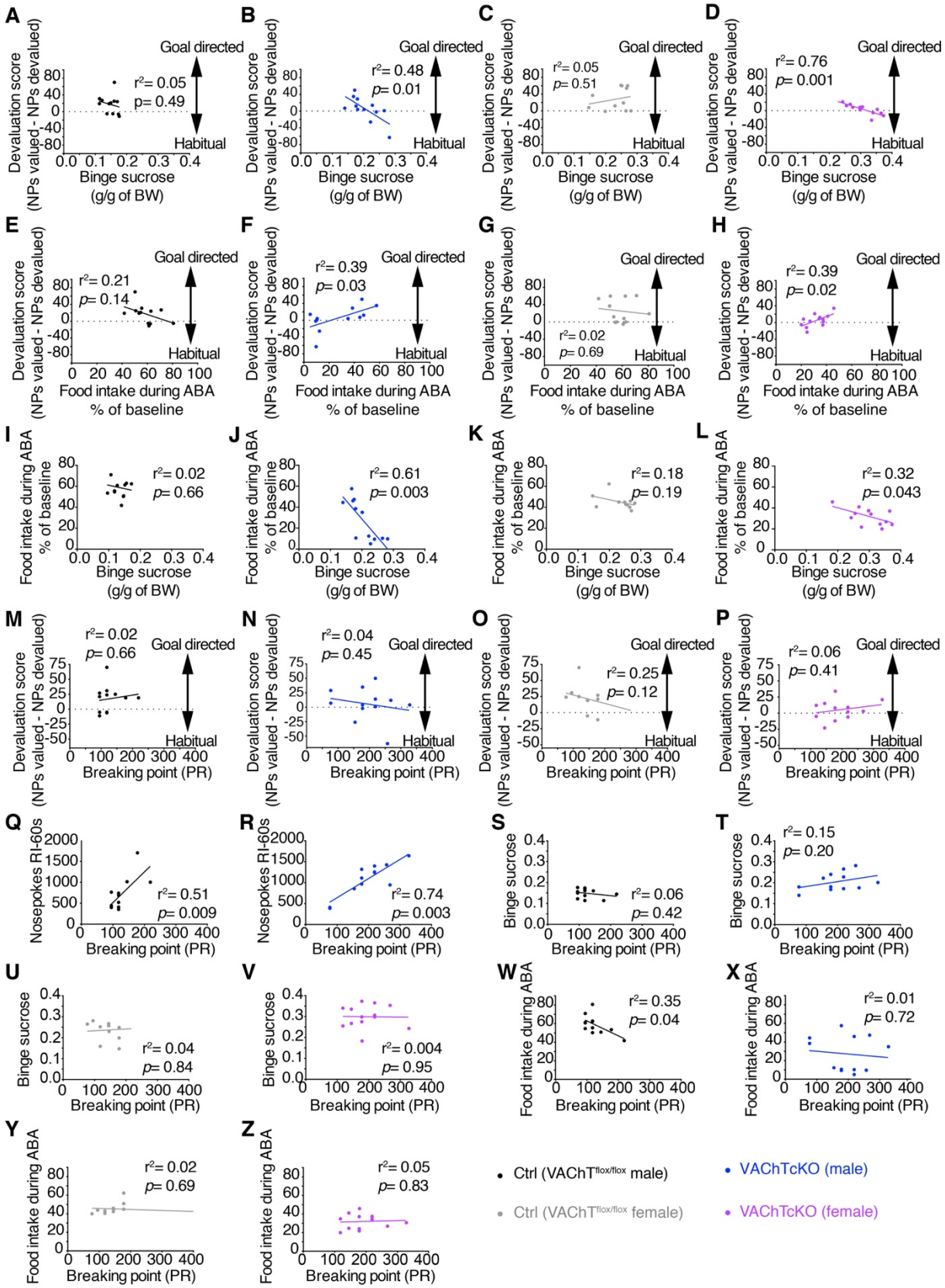
mice after 16 days of sucrose binge-like model. (**G**) Food consumption during the 1st hour of access (H0-H1) for female VAChTcKO mice and control mice after 16 days of sucrose binge-like model. (**H**) Water consumption during the 1st hour of access (H0-H1) for female VAChTcKO mice and control mice after 16 days of sucrose binge-like model. (**I-M**) Saccharine binge-like overconsumption model. Saccharine (a non-caloric sweetener) was tested instead of sucrose in the binge-like overconsumption model (**I**) Saccharine consumption during the total duration of access for VAChTcKO males and control mice (H0-H4) (**J**) Saccharine consumption during the 1st hour of access (H0-H1) for VAChTcKO males and control mice. (**K**) Saccharine consumption during the 3 last hours of access (H1-H4) for VAChTcKO mice and control mice. (**L**) Food consumption during the total duration of access for VAChTcKO mice and control mice (H0-H4). (**M**) Water consumption during the total duration of access for VAChTcKO mice and control mice (H0-H4). Note that VAChTcKO males consumed significantly more saccharine than controls during the 1st hour of access and more food during the total duration of the test. (**N**) Two-bottle choice procedure to determine preference for sucrose solution over water with VAChTcKO mice and littermate controls. (**O-Q**) Sucrose consumption by mice trained to sucrose binge for 16 days was measured after exposing animals to an intraperitoneal injection of glucose solution 30 minutes before the session or after *ad libitum* food for one hour ("chow preload"). (**O**) Sucrose consumption during the total duration of access (H0-H4) in glucose injected male mice. (**P**) Sucrose consumption after chow pre-load in different periods of testing (H0-H4). (**Q**) Food consumption during chow preload for male VAChTcKO mice and controls. Note that after glucose injection or chow preload, VAChTcKO mice binged significantly more sucrose than control mice. (**R**) Sucrose consumption when appetitive food (high fat/high sugar pellets) was used instead of regular food for VAChTcKO male mice and controls. (**S**) Consumption of high-caloric food for VAChTcKO male mice and controls. Two-way ANOVA repeated measures for (A) to (D), (I) to (M) and post hoc comparison with the method of contrasts. Unpaired t-test for (E) to (H), and (N) to (S). *p<0.05, **p<0.01, ***p<0.001. All data are mean ± SEM.



Supplementary Figure 11.

VGLUT3cKO mice, VAChTcKO mice and littermate controls during ABA model.

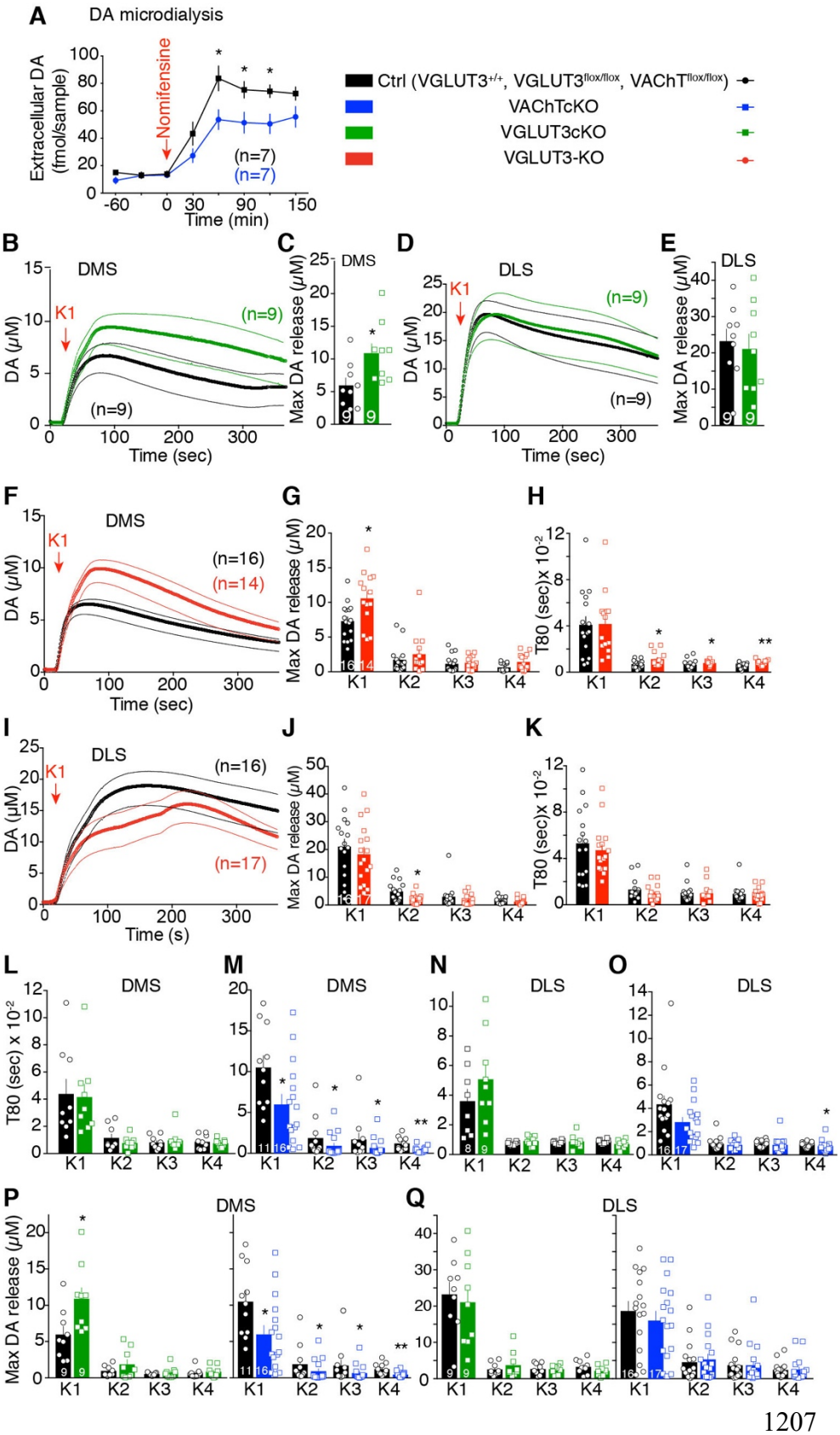
(**A**) Daily food intake and (**B**) body weight of VGLUT3cKO male mice (n=10) and respective controls (n=10) during baseline period before the ABA test. (**C, D**) Daily food intake and (**E, F**) body weight of VAChTcKO male (n=12) and female (n=13) mice and respective controls (n=11-12) during baseline period before the ABA test. (**G**) VGLUT3cKO mice (n=10) and controls (n=10) food intake during the restriction period of the activity-based anorexia model. (**H**) Percentage of VGLUT3cKO mice and controls that reached less than 75% of their baseline body weight (BW) during ABA restriction period. (**I**, **J**) Daily food consumption during food-restriction period of the ABA test for VAChTcKO male (n=11) and female (n=13) mice compared to littermate controls (n=11-12), expressed as a percentage of baseline food intake. Two-way ANOVA repeated measures for (A-G, I, J) and post hoc comparison with the method of contrasts. Kaplan-Meier test for (H) and post hoc comparison with log-rank Mantel–Cox and Gehan-Breslow-Wilcoxon tests. *p<0.05, **p<0.01, ***p<0.001. All data are mean ± SEM.



Supplementary Figure 12.

Two-by-two Pearson correlation analyses reveal that propensity for habits, but not motivation, predicts vulnerability to maladaptive eating in VAChTcKO mice.

Four groups of mice were used: male control mice, male VAChTcKO mice, female control mice and female VAChTcKO mice. These mice performed successively: instrumental training and outcome devaluation tests to evaluate the balance between goal-directed behaviors and habits, motivation using the progressive ratio procedure, binge-like sucrose overconsumption test and activity-based anorexia model. This design allowed us to run correlation analyses between these 4 behavioral components. (**A-D**) A significant correlation was found between the propensity to form habits and sucrose intake in the binge-like sucrose overconsumption model for both (**B**) male and (**D**) female VAChTcKO mice. (**A,C**) On the other hand, this correlation was not present in control mice. (**F,H**) The more VAChTcKO mice (males and females) used habitual behavior, the more they developed dramatic food-restriction in the activity-based anorexia model. (**E,G**) This correlation was not observed in control mice. (**J,L**) The vulnerability of VAChTcKO mice for binge-like sucrose overconsumption was correlated with the tendency to develop self-starvation in the activity-based anorexia model. (**I,K**) This was not the observed in control mice. As shown in Fig. 1o, motivation was increased in VAChTcKO male mice compared to controls. (**M-O**) However, motivation was not correlated with propensity to develop habits, either in controls (**M,O**) or in mutant mice (**N,P**). (**Q, R**) Higher breaking points during the progressive ratio test was correlated with the number of nosepokes during sucrose self-administration using the random-interval procedure, for both controls (**Q**) and VAChTcKO mice (**R**). (**U-Z**) Importantly, motivation was not correlated with the vulnerability to develop maladaptive eating behaviors**,** with the exception of food intake in male controls during activity-based anorexia (**W**).

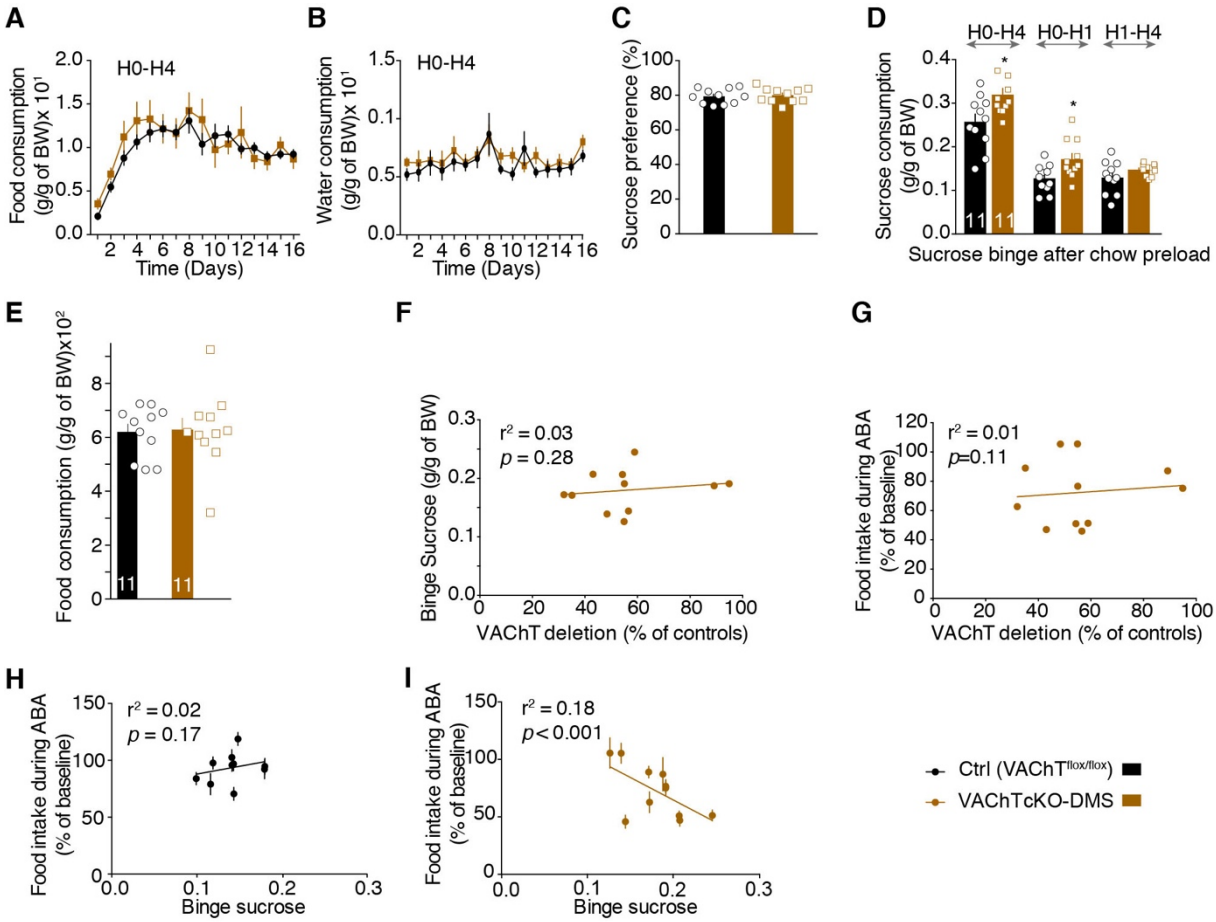


1207

1208 **Supplementary Figure 13.**

DA release in the dorsal striatum of VAChTcKO mice, VGLUT3cKO mice and constitutive VGLUT3-KO mice compared with control littermates.

(A) Extracellular DA levels measured by *in vivo* microdialysis in the dorsal striatum of VAChTcKO mice (n=7) and controls (n=7) in baseline condition (-60 - 0 min) and after a single local infusion of nomifensine (10µM, a dopamine reuptake inhibitor ; 0 - 150 min) *via* the microdialysis probe. **(B)** DA release measured by *in vivo* voltammetry after KCl-induced depolarization (K1) in the dorsomedial striatum (DMS) of VGLUT3cKO mice (n=9) and control littermates (n=9). **(C)** Maximum DA release after KCl depolarization in the DMS of VGLUT3cKO and controls. **(D)** DA release measured by *in vivo* voltammetry in the dorsolateral (DLS) of VGLUT3cKO mice and controls after KCl-induced depolarization (K1). **(E)** Maximum DA release after KCl depolarization in the DLS of VGLUT3cKO mice and controls. **(F)** DA release measured by *in vivo* voltammetry in DMS of VGLUT3-KO mice (n=14) and controls (n=16) after KCl-induced depolarization (K1). **(G)** Maximum DA release after KCl-induced depolarization (K1-K4) in the DMS for VGLUT3-KO mice and controls. **(H)** Accommodation of DA efflux (T80) after consecutive KCl stimulation in the DMS of VGLUT3cKO mice and controls. **(I)** DA release measured by *in vivo* voltammetry in DLS of.VGLUT3-KO (n=17) and controls (n=16) after KCl-induced depolarization (K1). **(J)** Maximum DA release after KCl-induced depolarization (K1-K4) in the DLS for VGLUT3-KO mice and controls. **(K)** Accommodation of DA efflux (T80) after consecutive KCl stimulation (K1-K4) in the DLS of VGLUT3-KO mice and controls. **(L)** Accommodation of DA efflux (T80) after consecutive KCl stimulation (K1-K4) in the DMS of VGLUT3cKO mice and controls. **(M)** Accommodation of DA efflux (T80) after consecutive KCl stimulation (K1-K4) in the DMS of VAChTcKO mice (n=16) and controls (n=11). **(N)** Accommodation of DA efflux (T80) after consecutive KCl stimulation (K1-K4) in the DLS of VGLUT3cKO mice and controls. **(O)** Accommodation of DA efflux (T80) after consecutive KCl stimulation (K1-K4) in the DLS of VAChTcKO mice (n=17) and controls (n=16). **(P)** Maximum DA release after KCl-induced depolarization (K1-K4) in the DMS for VGLUT3cKO and control mice or VAChTcKO mice and controls. **(Q)** Maximum DA release after KCl-induced depolarization (K1-K4) in the DLS for VGLUT3cKO mice and controls or VAChTcKO mice and controls. Note that regulation of DA release was only found in the DMS of VAChTcKO mice, VGLUT3cKO mice or VGLUT3-KO mice, but not in the DLS. Two-way ANOVA repeated measures for (A), (B), (D), (F), (I) and Mann Whitney for (C), (E), (G), (H), (J), (K), and (L) to (Q). Bonferroni's post hoc comparison for (A). *p<0.05, **p<0.01. All data are mean ± SEM.

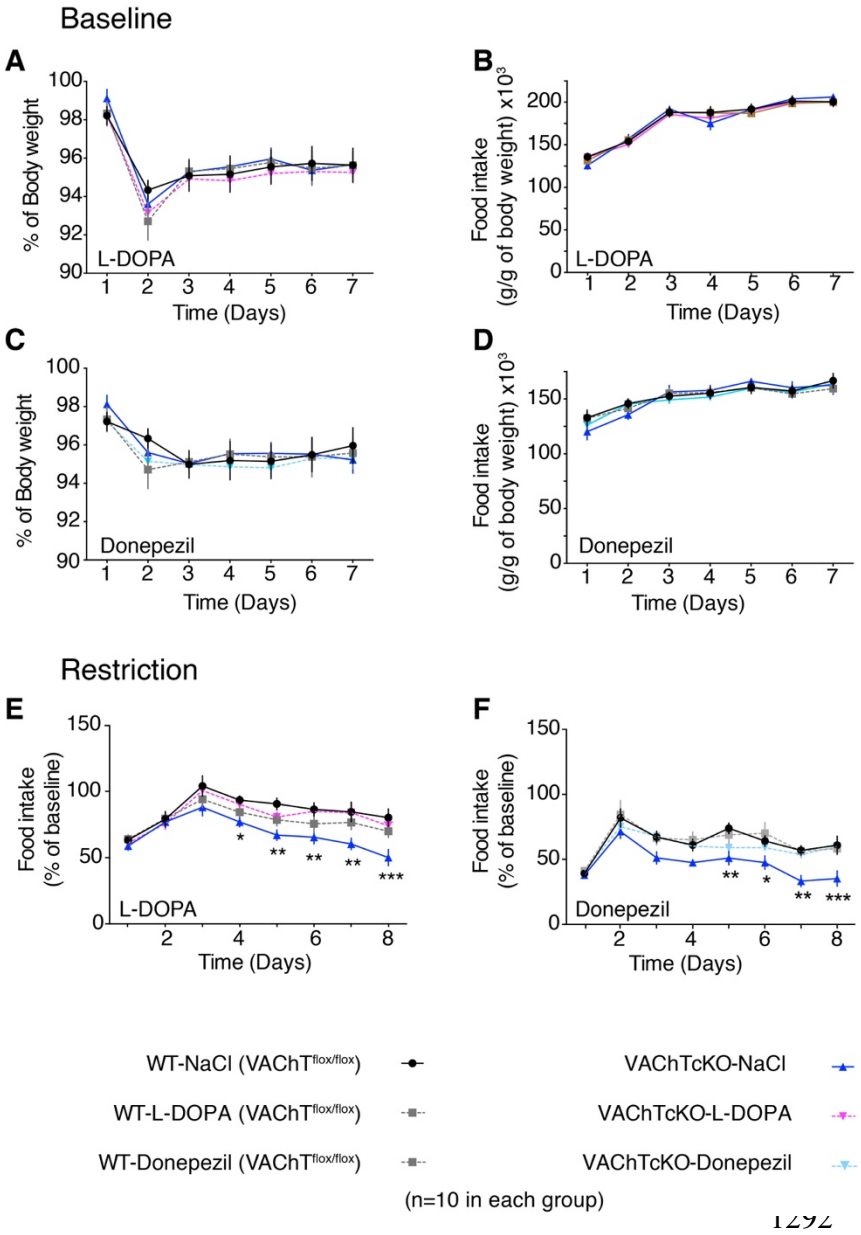


Supplementary Figure 14.

Additional results on the performance of VAChTcKO-DMS mice and control littermates during binge-like sucrose overconsumption and activity-based anorexia models.

(**A**) Food consumption of VAChTcKO-DMS mice and control littermates along the 16 days of binge-like testing during the total period of access (H0-H4. (**B**) Water consumption during the total duration of access (H0-H4) of VAChTcKO-DMS mice and controls. (**C**) Sucrose preference of VAChTcKO-DMS mice and controls. (**D**) Sucrose consumption during the total duration of sucrose access (H0-H4), the first hour of access (H0-H1) and the 3 last hours (H1-H4) for VAChTcKO-DMS mice and controls after mice have been exposed for 1 hour to *ad libitum* food (chow pre-load). (**E**) Food intake of VAChTcKO-DMS mice and controls during chow preload. (**F**) Pearson linear regression comparing the extent of VAChT deletion (measured by the density of the labelling using immunoautoradiography) in individual VAChTcKO-DMS mice (25-100%) with the quantity of sucrose consumed in the first hour of access to sucrose during binge-like model. **(G)** Pearson linear regression comparing the extent of VAChT deletion in individual VAChTcKO-DMS mice with food intake measured during food-restriction period of ABA model. Note that no significant correlation was observed between VAChT deletion and binge or ABA phenotypes, suggesting that even a

partial disruption of VAChT expression in the DMS is sufficient to precipitate maladaptive eating. (**H**) Correlation analysis between sucrose consumption in the first hour of access to sucrose during binge-like model and food intake during food-restriction period of ABA model for control mice. (**i**) Correlation analysis between sucrose consumption in the first hour of access to sucrose during binge-like model and food intake during food-restriction period of ABA model for VAChTcKO-DMS mice. Note that a significant correlation between sucrose bingeing and decreased food intake is specifically observed in VAChTcKO-DMS mice. Two-way ANOVA repeated measure for (A), (B) and post hoc comparison with the method of contrasts. Unpaired t-test for (C) to (E). Dimensional analyses were performed by parametric simple linear regressions for (F) to (I). *$p<0.05$. All data are mean ± SEM.

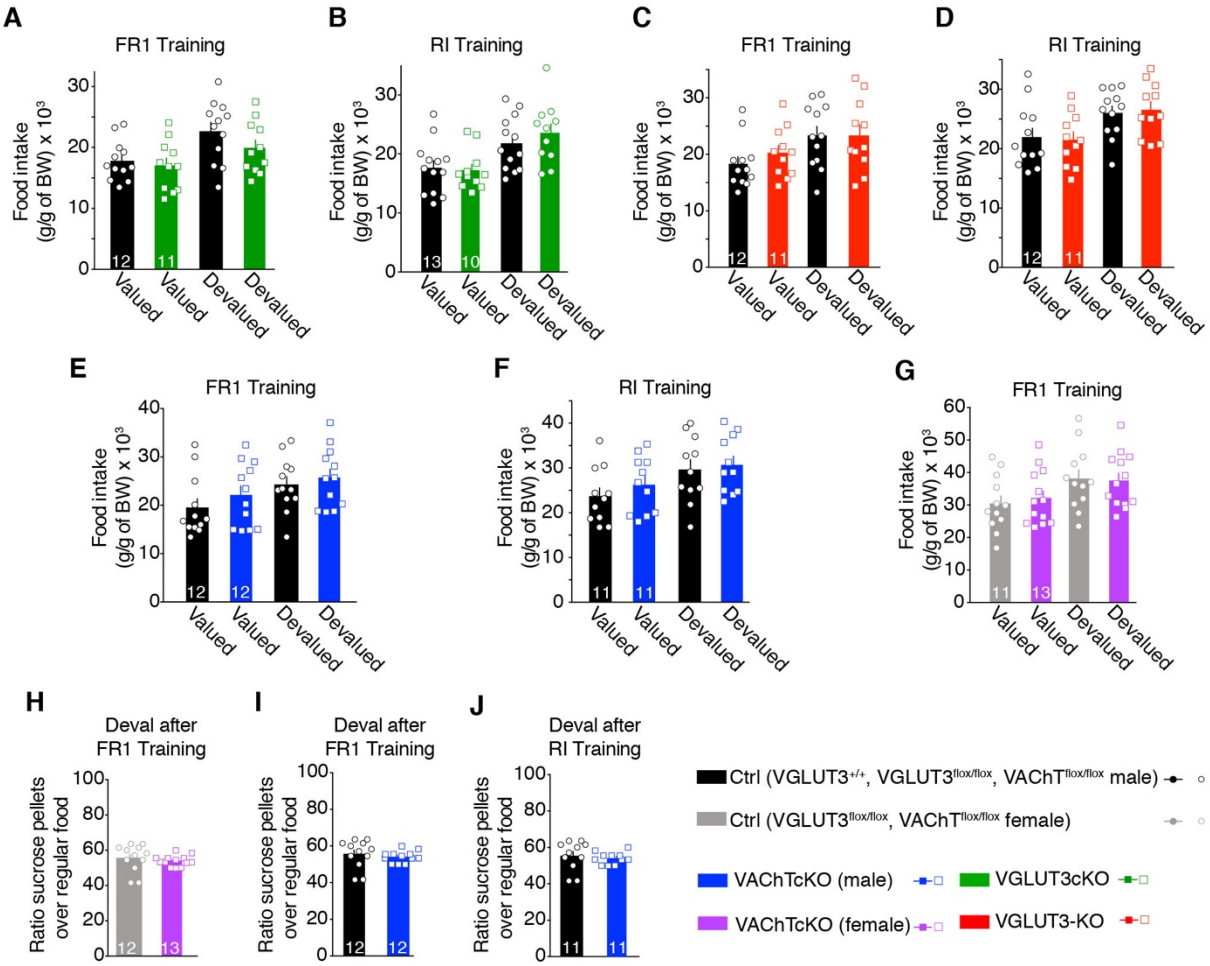


Supplementary Figure 15.

Additional results on the performance of control and VAChTcKO mice in the activity-based anorexia model in the presence or absence of chronic treatment with L-DOPA or donepezil.

(**A**,**B**) Chronic pharmacological treatment (IP injection) with L-DOPA (15mg.kg^{-1}) (**A**) Daily food intake for VAChTcKO or control mice treated with saline or L-DOPA during baseline of the ABA test. (**B**) Mean daily BW change during baseline of the ABA test for VAChTcKO mice or control mice treated with saline or L-DOPA. **(C,D**). Chronic pharmacological

treatment (IP injection) with Donepezil (0.3mg.kg^{-1}). (**C**) Daily food intake for VAChTcKO mice or control mice treated with saline or Donepezil during baseline of the ABA test. (**D**) Mean daily BW change during baseline of the ABA test for VAChTcKO mice or control mice treated with saline or Donepezil. (**E**, **F**) Daily food consumption during food-restriction period of ABA for VAChTcKO male and female mice treated with L-DOPA (**E**) or Donepezil (**F**) compared to littermate controls. Two-way ANOVA repeated measure for (A-F) and post hoc comparison with Dunnett's test. *p<0.05, **p<0.01, ***p<0.001. All data are mean ± SEM.

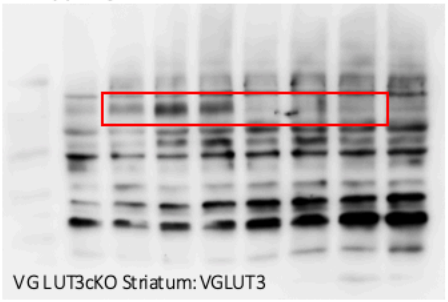


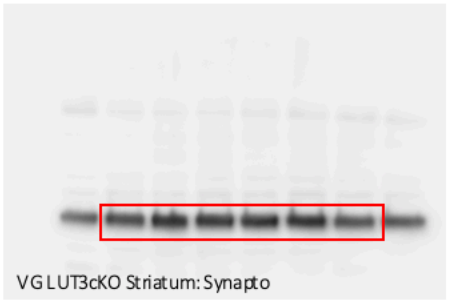
Supplementary Figure 16.

Food consumption of control and mutant mouse lines during the 1-hour pre-feeding period preceeding devaluation tests.

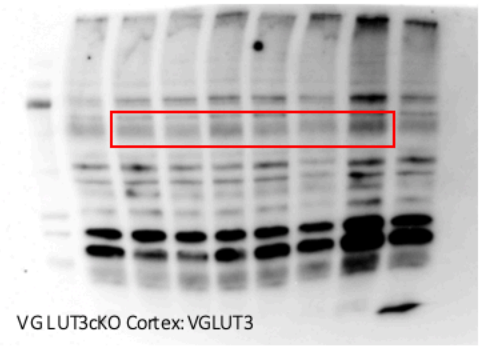
(**A-G**). Mean food intake of VGLUT3cKO mice (**A,B**), VGLUT3-KO mice (**C,D**), male VAChTcKO mice (**E,F**) and female VAChTcKO mice (**G**) during the 60-min prefeeding period with free access to either regular home chow (valued) or sucrose pellets (devalued) before devaluation test. (**H-J**) Ratio between the quantity of sucrose pellets versus regular home chow consumed over 2 days of devaluations tests for VAChTcKO mice, females (**H**) and males (**I,J**). Unpaired t-test for (A) to (J).

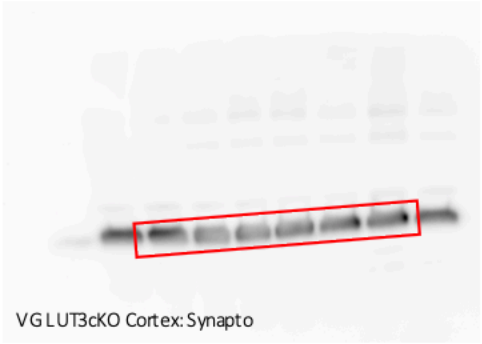
Suppl Figure 3A



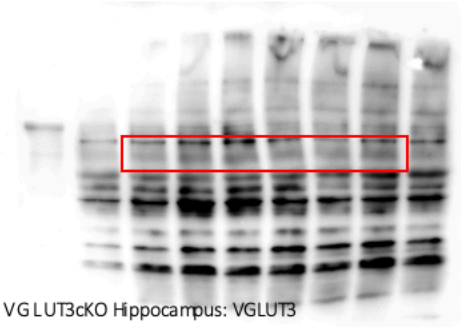


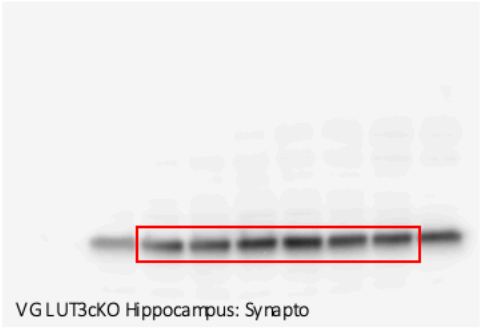
Suppl Figure 3B



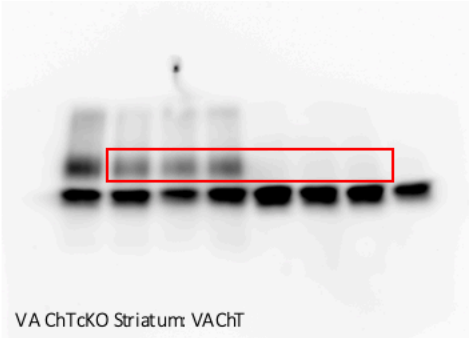


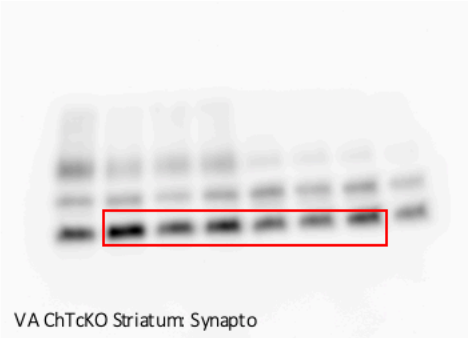
Suppl Figure 3C





Suppl Figure 3D





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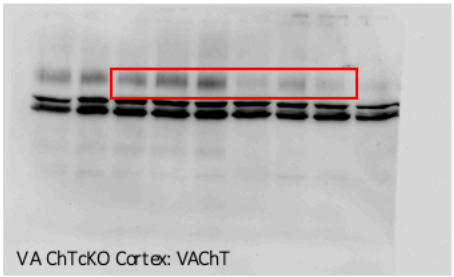

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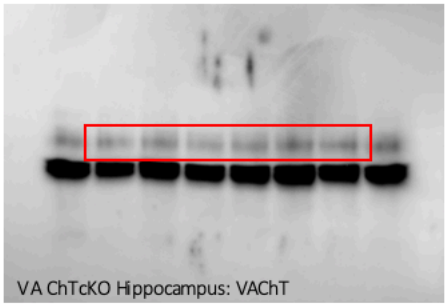
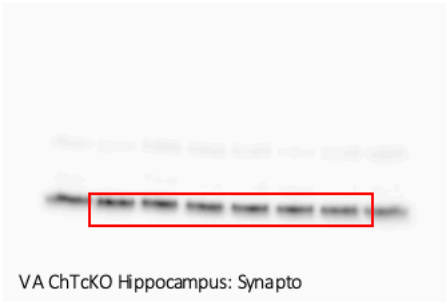
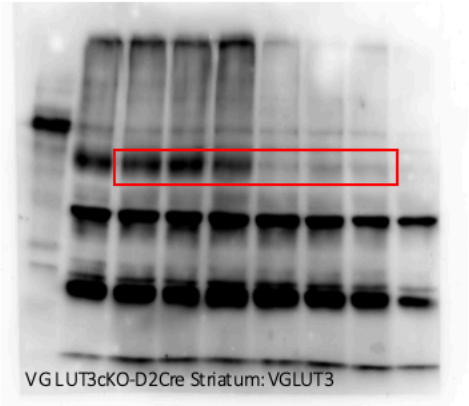
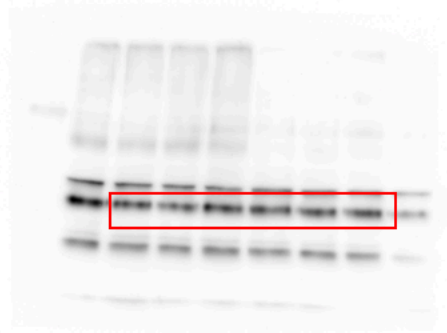


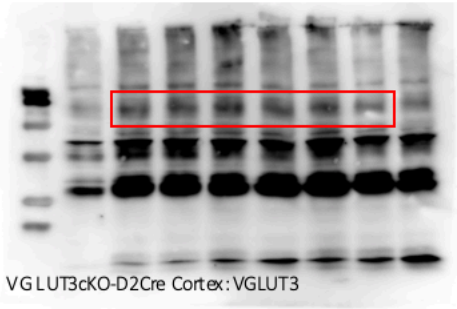


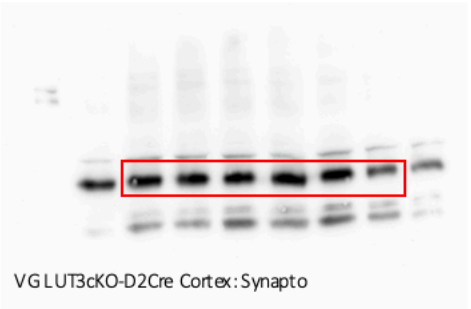
Suppl Figure 3I



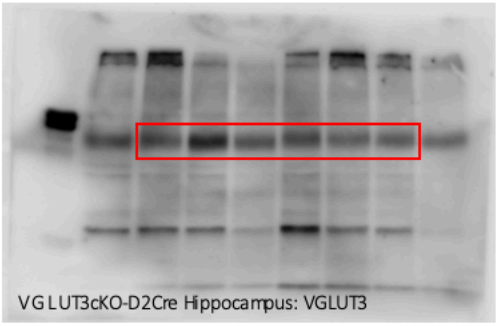


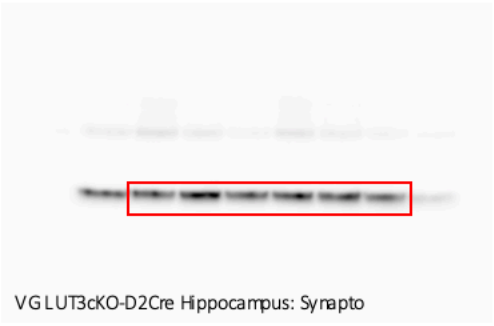
Suppl Figure 3J





Suppl Figure 3K





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