Figure S1

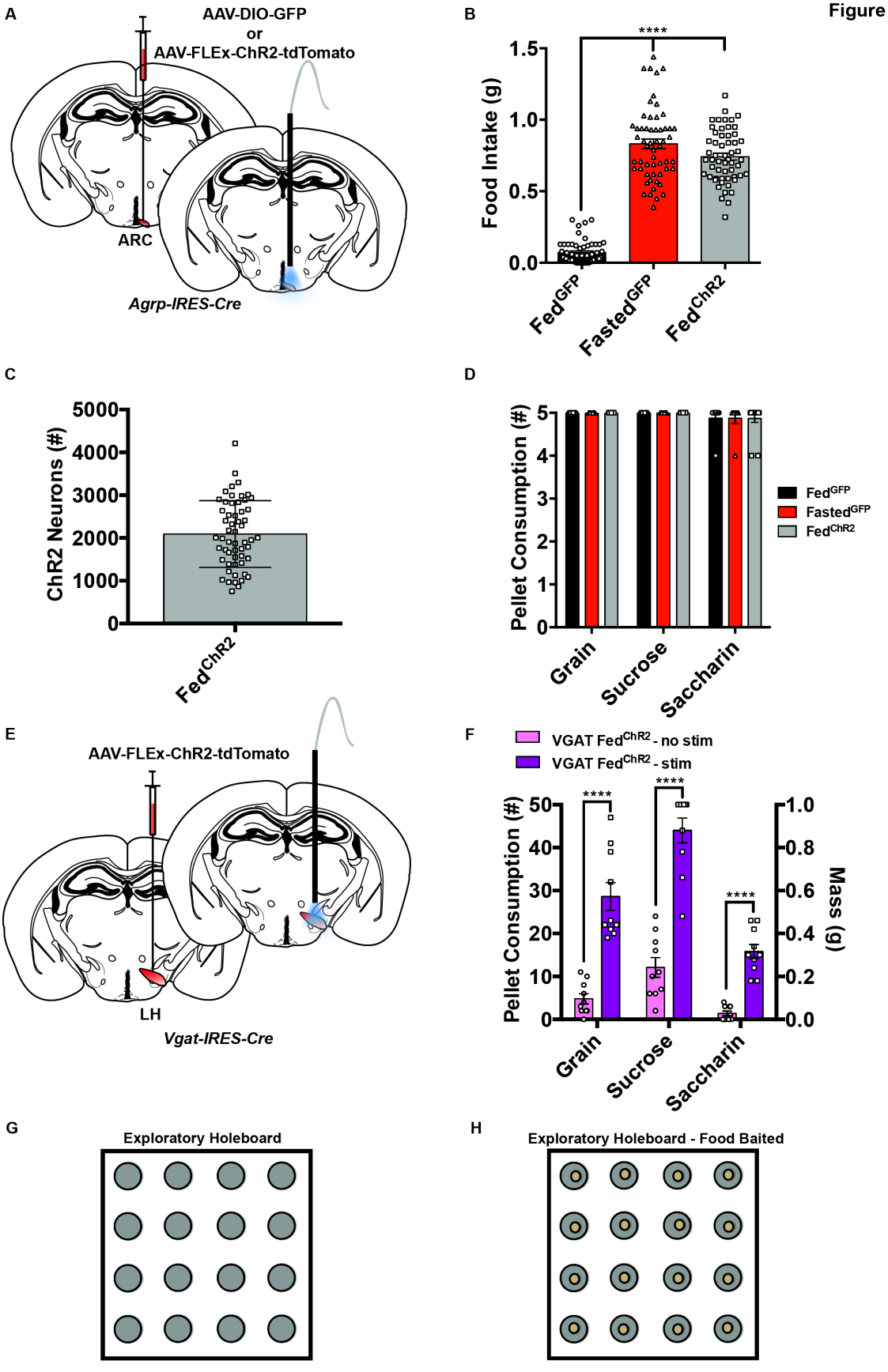

A
AAV-DIO-GFP
or
AAV-FLEx-ChR2-tdTomato
ARC
Agrp-IRES-Cre
B
Food Intake (g)
1.5
1.0
0.5
0.0
****
Fed^GFP
Fasted^GFP
Fed^ChR2
C
ChR2 Neurons (#)
5000
4000
3000
2000
1000
0
Fed^ChR2
D
Pellet Consumption (#)
5
4
3
2
1
0
Grain
Sucrose
Saccharin
Fed^GFP
Fasted^GFP
Fed^ChR2
E
AAV-FLEx-ChR2-tdTomato
LH
Vgat-IRES-Cre
F
VGAT Fed^ChR2 - no stim
VGAT Fed^ChR2 - stim
Pellet Consumption (#)
50
40
30
20
10
0
Mass (g)
1.0
0.8
0.6
0.4
0.2
0.0
****
****
****
Grain
Sucrose
Saccharin
G
Exploratory Holeboard
H
Exploratory Holeboard - Food Baited

Figure S2

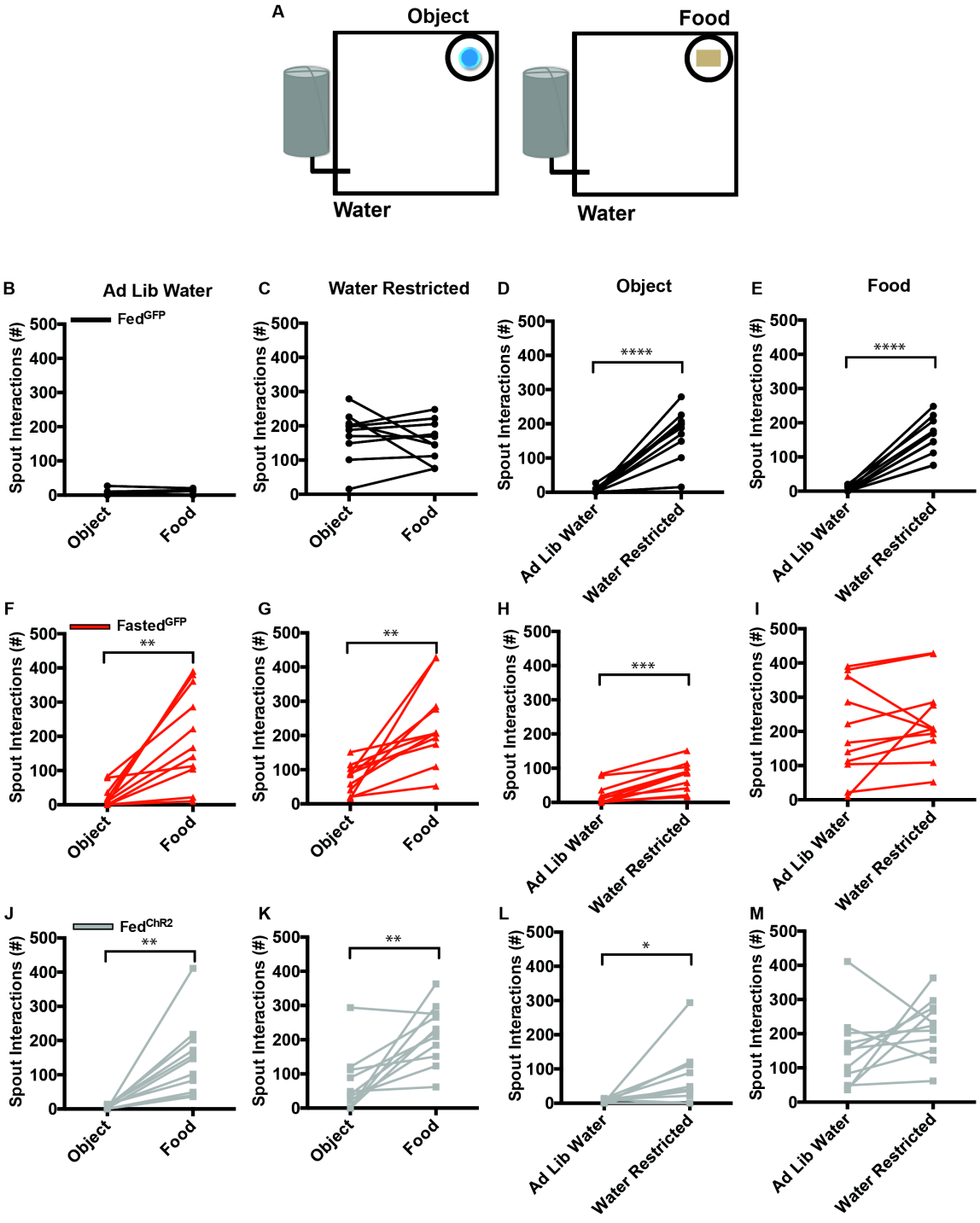

A
Object
Food
Water
Water
B
Ad Lib Water
C
Water Restricted
D
Object
E
Food
Fed^GFP
Spout Interactions (#)
Object
Food
Ad Lib Water
Water Restricted
****
F
Fasted^GFP
G
H
I
**
***
J
Fed^ChR2
K
L
M
*

Figure S3

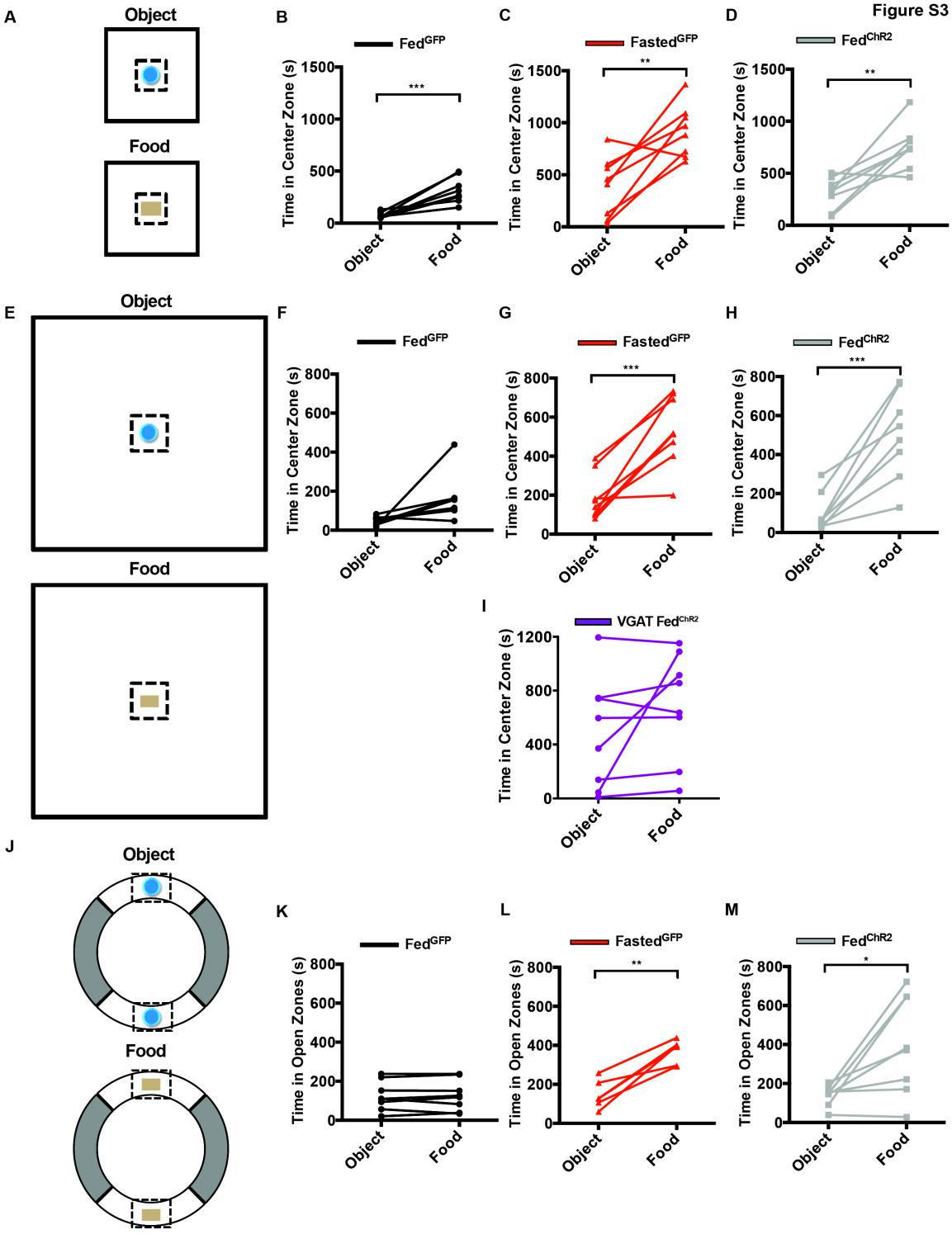
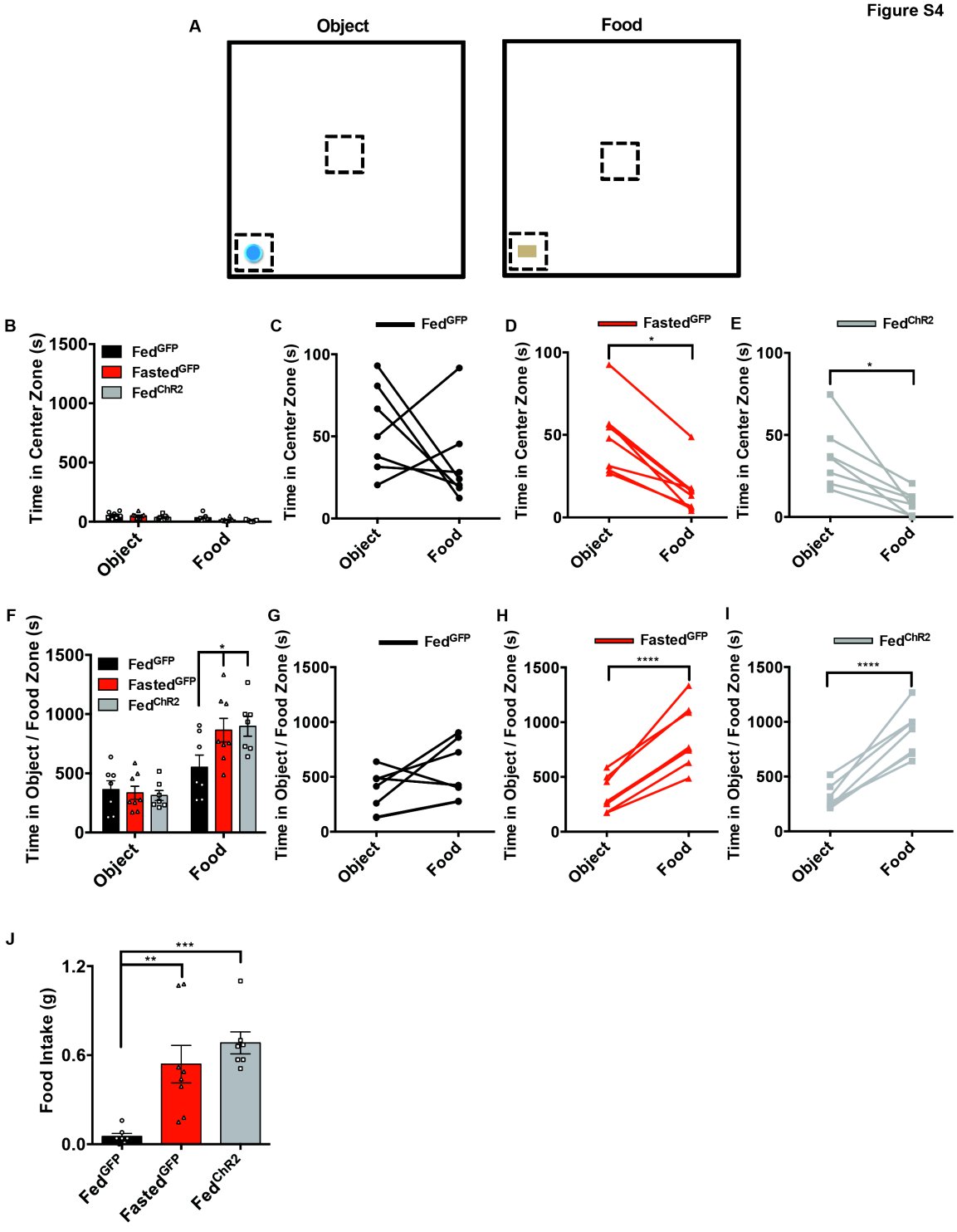

A
Object
Food
B
Fed^GFP
Time in Center Zone (s)
1500
1000
500
0
***
Object
Food
C
Fasted^GFP
**
D
Fed^ChR2
**
E
F
G
H
800
600
400
200
I
VGAT Fed^ChR2
1200
J
K
Time in Open Zones (s)
L
M
*

Figure S4


A
Object
Food
B
Time in Center Zone (s)
1500
1000
500
0
Fed^GFP
Fasted^GFP
Fed^ChR2
Object
Food
C
Fed^GFP
Time in Center Zone (s)
100
50
0
Object
Food
D
Fasted^GFP
*
Time in Center Zone (s)
100
50
0
Object
Food
E
Fed^ChR2
*
Time in Center Zone (s)
100
50
0
Object
Food
F
Time in Object / Food Zone (s)
1500
1000
500
0
Fed^GFP
Fasted^GFP
Fed^ChR2
*
Object
Food
G
Fed^GFP
Time in Object / Food Zone (s)
1500
1000
500
0
Object
Food
H
Fasted^GFP
****
Time in Object / Food Zone (s)
1500
1000
500
0
Object
Food
I
Fed^ChR2
****
Time in Object / Food Zone (s)
1500
1000
500
0
Object
Food
J
***
**
Food Intake (g)
1.2
0.6
0.0
Fed^GFP
Fasted^GFP
Fed^ChR2

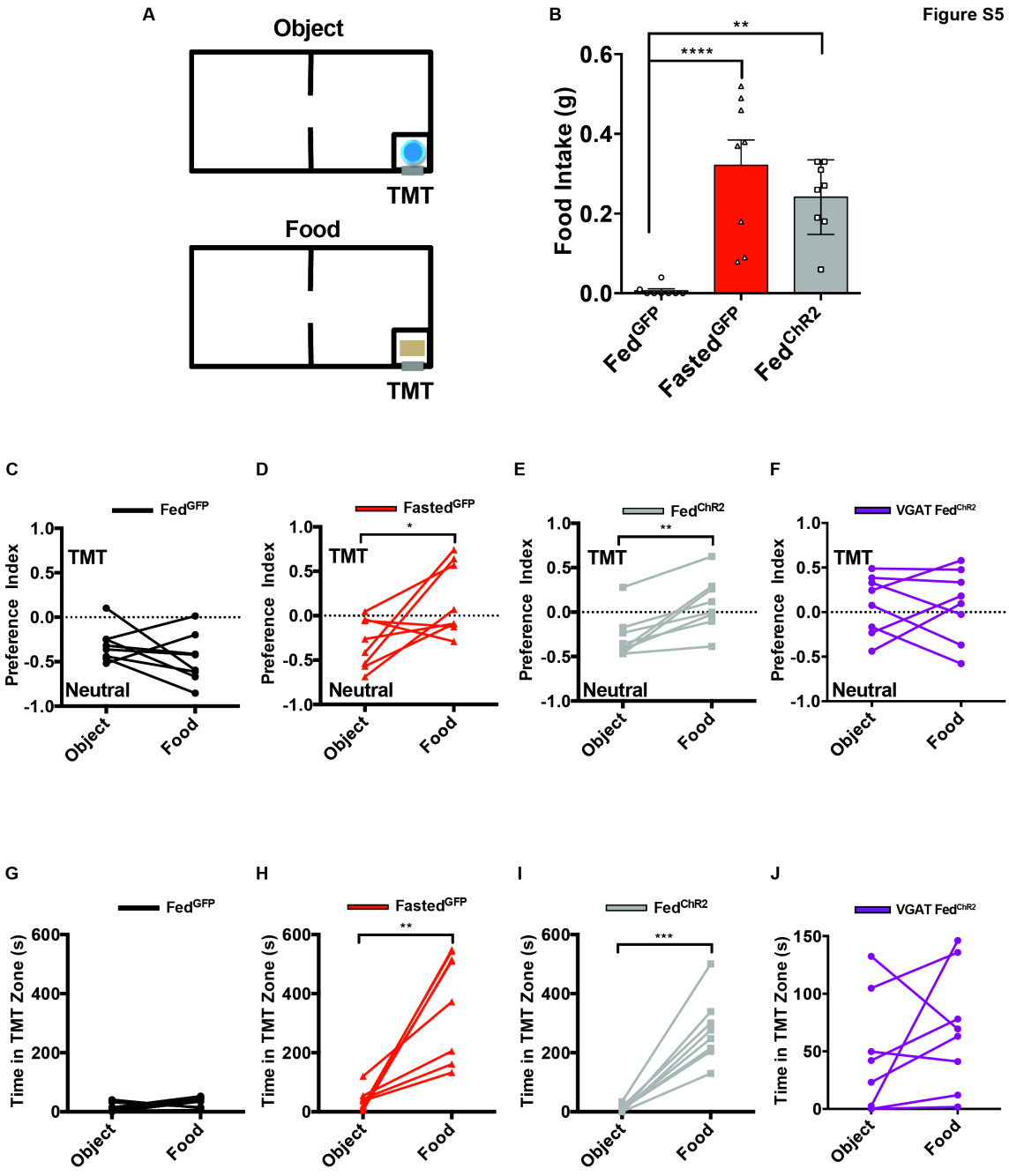

A
Object
TMT
Food
TMT
B
Figure S5
Food Intake (g)
0.0
0.2
0.4
0.6
**
****
Fed^GFP
Fasted^GFP
Fed^ChR2
C
D
E
F
Fed^GFP
Fasted^GFP
Fed^ChR2
VGAT Fed^ChR2
Preference Index
1.0
0.5
0.0
-0.5
-1.0
TMT
Neutral
Object
Food
*
**
G
H
I
J
Time in TMT Zone (s)
600
400
200
0
150
100
50
***

Figure S6

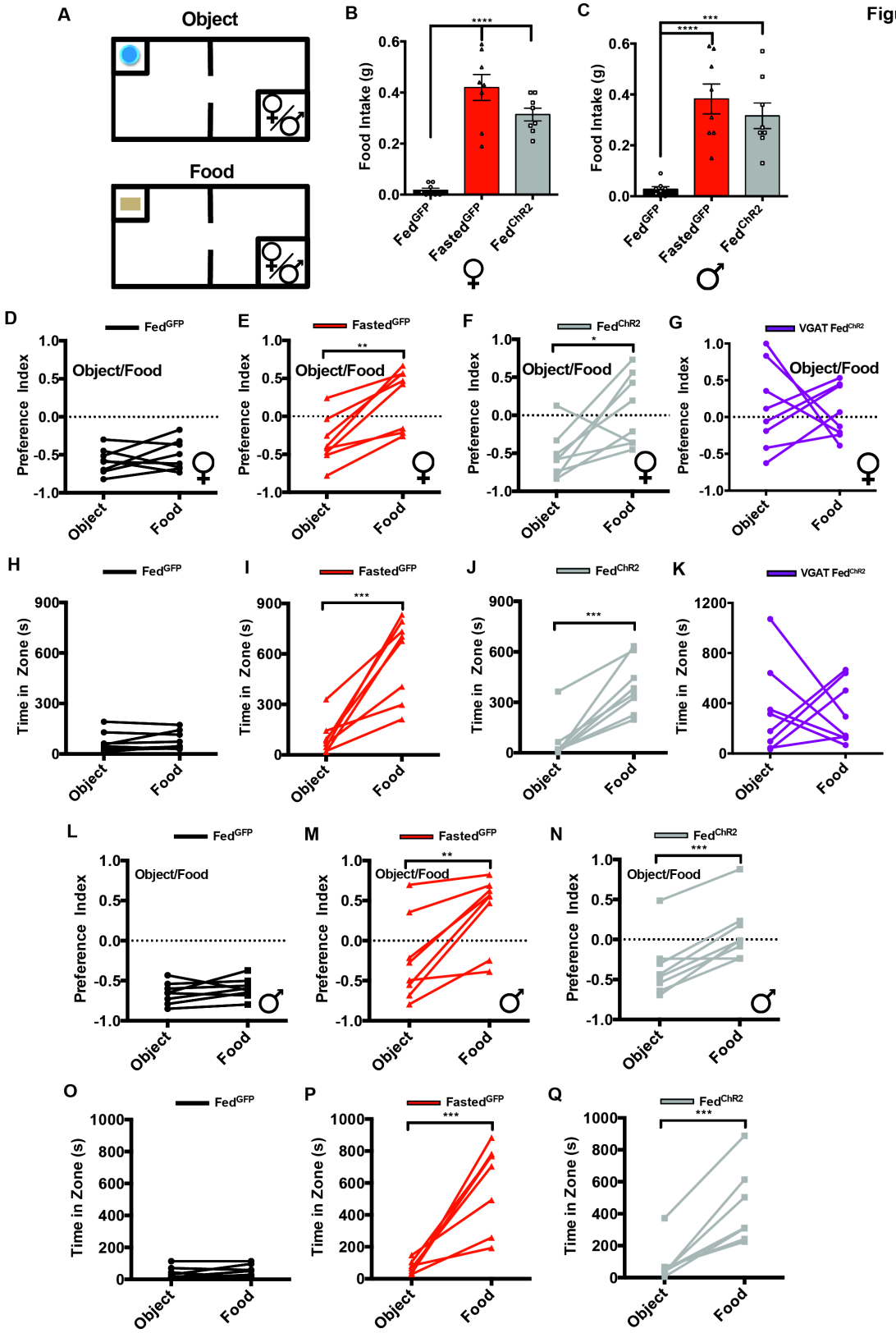

A
Object
Food
B
Food Intake (g)
Fed^GFP
Fasted^GFP
Fed^ChR2
C
Food Intake (g)
D
Fed^GFP
Object/Food
Preference Index
E
Fasted^GFP
F
Fed^ChR2
G
VGAT Fed^ChR2
H
Time in Zone (s)
I
J
K
L
M
N
O
P
Q
Object
Food

Figure S7

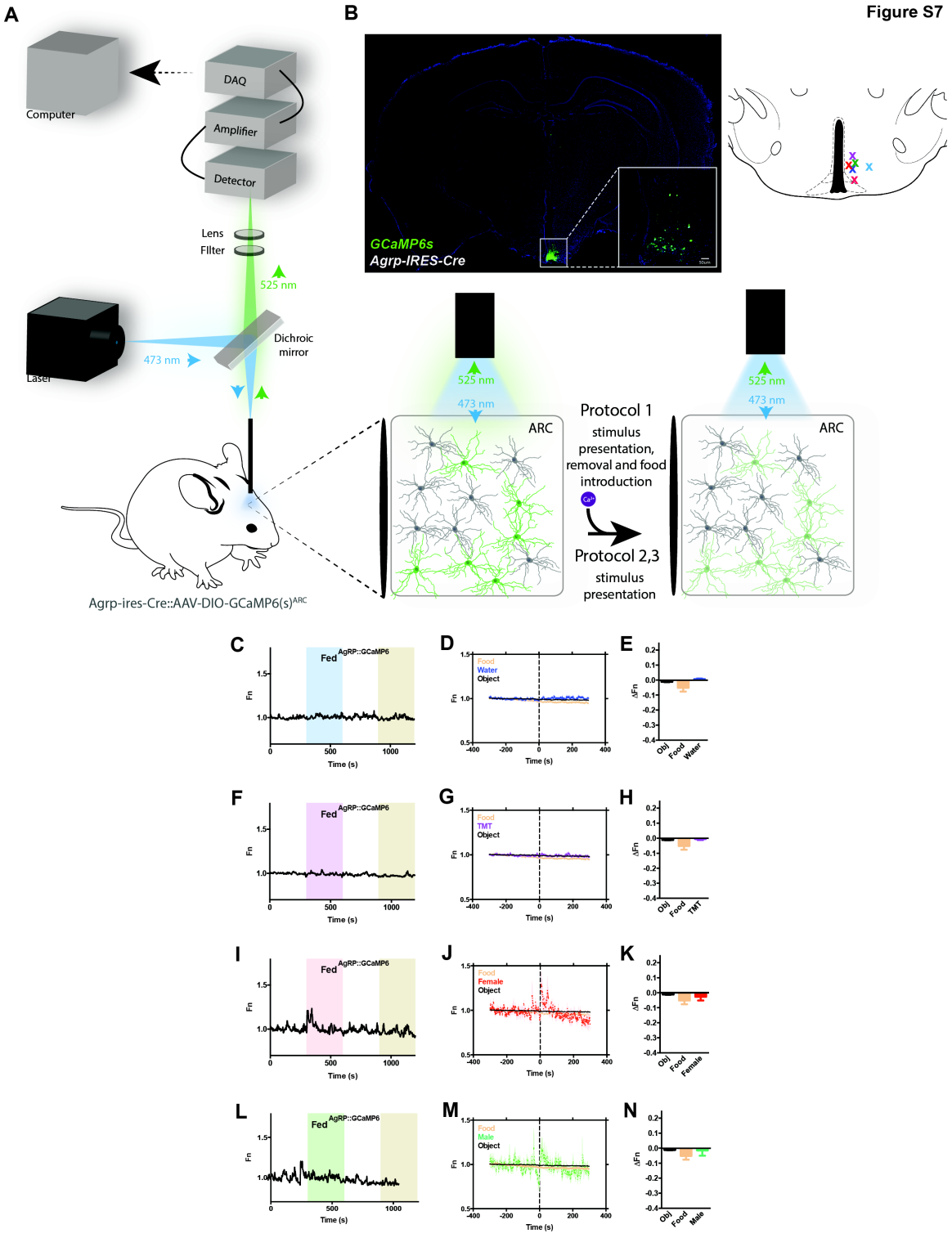

A
B
Computer
DAQ
Amplifier
Detector
Lens
FIlter
525 nm
Laser
473 nm
Dichroic
mirror
GCaMP6s
Agrp-IRES-Cre
525 nm
473 nm
ARC
Protocol 1
stimulus
presentation,
removal and food
introduction
Protocol 2,3
stimulus
presentation
Agrp-ires-Cre::AAV-DIO-GCaMP6(s)ARC
C
D
E
F
G
H
I
J
K
L
M
N
Fed
AgRP::GCaMP6
Time (s)
Food
Water
Object
TMT
Female
Male
Obj
Food
Water
TMT
Female
Male

Figure S1. Related to Figure 1
(A) Schematic of viral payload delivery and optical fiber placement in *Agrp-IRES-Cre* mice. (B) Physiological or ARC^{AgRP}-mediated hunger significantly enhanced homecage food intake compared to sated controls. (C) ChR2-tdTomato transduction ARC^{AgRP} cell counts. (D) All groups of mice consumed comparable amounts of grain, sucrose and saccharin pellets during 3 consecutive nights of exposure in the absence of blue light photoactivation. (E) Schematic of viral payload delivery and optical fiber placement in *Vgat-IRES-Cre* mice. (F) LH^{VGAT}-mediated hunger significantly enhanced homecage consumption of grain, sucrose and saccharin pellets in sated animals compared to the same mice in the absence of blue light photoactivation. (G-H) Schematic of empty or food pellet-baited 16-well hole-board assay, respectively. Error bars represent mean +/- SEM. $^{*}p < 0.05$. $^{**}p < 0.01$,$^{***}p < 0.001$,$^{****}p < 0.0001$.

Figure S2. Related to Figure 2
(A) Schematic of PhenoTyper® homecage where animals have free access to water and object/food. Within-subject analyses of water intake in (B,F,J) *ad lib* water condition (object versus food), (C,G,K) water restricted condition (object versus food), (D,H,L) object condition (*ad lib* water versus water restricted) and (E,I,M) food condition (*ad lib* water versus water restricted). Error bars represent mean +/- SEM. $^{*}p < 0.05$. $^{**}p < 0.01$,$^{***}p < 0.001$,$^{****}p < 0.0001$.

Figure S3. Related to Figure 3
(A) Schematic of open field apparatus with object or food placed in the center. (B-D) Within-subject analyses of time spent in the center zone (object versus food). (E) Schematic of big open field apparatus with object or food placed in the center. (F-I) Within-subject analyses of time spent in the center zone (object versus food). (J) Schematic of elevated zero maze apparatus with object or food placed in the center of the open arms. (K-M) Within-subject analyses of time spent in the center zones of the open arms (object versus food). Error bars represent mean +/- SEM. $^{*}p < 0.05$. $^{**}p < 0.01$,$^{***}p < 0.001$,$^{****}p < 0.0001$.

Figure S4. Related to Figure 3
(A) Schematic of big open field apparatus with object or food placed in the corner. (B) Physiological or ARC^{AgRP}-mediated hunger failed to enhance big open field center zone duration time in either the object or food condition, compared to sated controls. (C-E) Within-subject analyses of time spent in the center zone (object versus food). (F) Physiological or ARC^{AgRP}-mediated hunger significantly enhanced big open field corner zone duration time in the food condition, but failed to do so in the object condition, compared to sated controls. (G-I) Within-subject analyses of time spent in the corner zone (object versus food). (J) Increased corner zone duration time during the food condition was related to levels of food intake. Error bars represent mean +/- SEM. $^{*}p < 0.05$. $^{**}p < 0.01$,$^{***}p < 0.001$,$^{****}p < 0.0001$.

Figure S5. Related to Figure 4
(A) Schematic of two-chamber TMT fear apparatus with object or food placed in the corner designated the TMT zone. (B) Increased TMT zone duration time during the food condition was related to levels of food intake. Within-subject analyses of (C-F) chamber preference and (G-J) TMT zone duration (object versus food). Error bars represent mean +/- SEM. $^{*}p < 0.05$. $^{**}p < 0.01$,$^{***}p < 0.001$,$^{****}p < 0.0001$.

Figure S6. Related to Figure 5
(A) Schematic of two-chamber social interaction apparatus with object or food placed in the one corner and receptive female or juvenile male placed in the opposite corner. (B-C) Increased object/food zone duration time during the food condition was related to levels of food intake in both the female and male assays, respectively. Within-subject analyses of (D-G, L-N) chamber preference and (H-K, O-Q) object/food zone duration (object versus food) in the female and male assays, respectively. Error bars represent mean +/- SEM. $^{*}p < 0.05$. $^{**}p < 0.01$,$^{***}p < 0.001$,$^{****}p < 0.0001$.

Figure S7. Related to Figure 6
(A) Schematic of the fiber photometry setup and outline of recording protocols. (B) Representative image of ARC^{AgRP} GCaMP6s expression and fiber placement. (C,F,I,L) Normalized representative *in vivo* calcium imaging traces showing GCaMP fluorescent signal fluctuations through the presentation of baseline→stimuli→removal of stimuli→food in sated mice. (D,G,J,M) Plots showing calcium signal changes between object, stimuli (water, TMT, female, or juvenile male), and food. (E,H,K,N) Calcium

levels were not affected upon the presentation of object, stimuli (water, TMT, female, or juvenile male), or food.

Experimental Procedures

Animals

Agrp-ires-Cre(Tong et al., 2008), Slc32a1(VGAT)-ires-Cre(Vong et al., 2011), and *Ai32(RCL-ChR2(H134R/EYFP)*(Madisen et al., 2010) mice were generated and maintained as previously described. All mice are on a mixed background. All animal care and experimental procedures were approved by the National Institute of Health Animal Care and Use Committee. Mice were housed at 22–24 °C with a 12 h light:12 h dark cycle with standard mouse chow (Teklad F6 Rodent Diet 8664; 4.05 kcal g^{-1}, 3.3 kcal g^{-1} metabolizable energy, 12.5% kcal from fat; Harlan Teklad) and water provided *ad libitum*, unless otherwise stated. All diets were provided as pellets. For all behavioral studies male mice between 8-14 weeks were used.

Brain tissue preparation

Mice were terminally anesthetised with chloral hydrate (Sigma Aldrich) and transcardially perfused with phosphate-buffered saline (PBS) followed by 10% neutral buffered formalin (Fisher Scientific). Brains were extracted, cryoprotected in 20% sucrose, and sectioned coronally on a freezing sliding microtome (Leica Biosystems) at 50 μm and collected in four equal series.

Neuron counting

ARC^{AgRP} neurons with red fluorescence (AAV10-FLEX-ChR2(H134R)-tdTomato) were counted manually using Zen, image software. Abercrombie's correction was applied to the neuron counts (slice thickness, 50 μm; AGRP neuron diameter, 14 ± 1 μm; correction factor, 0.78).(Abercrombie, 1946; Aponte et al., 2011) In the text, neuron counts were rounded to the nearest hundred.

Viral injections

Stereotaxic injections were performed as previously described(Krashes et al., 2011). Mice were anaesthetised with isoflurane and placed into a stereotaxic apparatus (Stoelting Just for Mice). For postoperative care, mice were injected intraperitoneally with meloxicam (0.5 mg per kg). After exposing the skull via small incision, a small hole was drilled for injection. A pulled-glass pipette with 20–40 mm tip diameter was inserted into the brain and virus was injected by an air pressure system. A micromanipulator (Grass Technologies, Model S48 Stimulator) was used to control injection speed at 25 nl min^{-1} and the pipette was withdrawn 5 min after injection. For *in vivo* optogenetic experiments, AAV5-EF1α-DIO-EYFP (University of North Carolina Vector Core; titer 1.4×10^{12} genome copies per ml) or AAV10-CAG-FLEX-ChR2(H134R)-tdTomato (University of Pennsylvania School of Medicine; titer 1.3×10^{13} genome copies per ml) were injected into the ARC (200-300 nl, bregma: AP: –1.44 mm, DV: −5.70 mm, ML: +/−0.25 mm) or LH (200-300 nl, bregma: AP: –1.3 mm, DV: −5.30 mm, ML: +/−0.9 mm). For *in vivo* photometry experiments, AAV1.Syn.Flex.GCaMP6s (University of Pennsylvania School of Medicine; titer 1.1×10^{12} genome copies per ml) was injected into the ARC (500 nl, bregma: AP: –1.40 mm, DV: −5.80/-5.90 mm, ML: +/−0.30 mm)

Optic fiber implantation

For optogenetic experiments, optical fibers (200 μm diameter core; BFH37-200 Multimode; NA 0.37; Thor Labs) were implanted unilaterally over the ARC (bregma: AP: −1.50 mm, DV: −5.50 mm, ML: −0.20 mm) or bilaterally over the LH (bregma: AP: –1.30 mm, DV: −4.80 mm, ML: +/−1.00 mm). Fibers were fixed to the skull using C&B Metabond Quick Cement and dental acrylic and mice were allowed 2 weeks for recovery before acclimatization investigator handling (12 h light/dark cycle starting at 6am) for 1 week before experiments.

Photostimulation protocol

Fiber optic cables (1.5 m long, 200 mm diameter; Doric Lenses) were firmly attached to the implanted fiber optic cannulae with zirconia sleeves (Doric Lenses) and coupled to lasers via a fiber-optic rotary joint (Doric Lenses). During photostimulation experiments, light pulse trains (10-ms pulses of 20 Hz; 2 second on, 2 seconds off; 5-ms pulses of 20Hz continuously, for ARC^{AgRP} and LH^{VGAT} respectively) were

programmed using a waveform generator (PCGU100; Valleman Instruments or Arduino electronics platform) that provided input to a blue light laser (473 nm; Laserglow). We adjusted the light power of the laser such that the light power exiting the fiber optic cable was 10-15 mW using an online light transmission calculator for brain tissue http://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php. We estimated the light power at the ARC and LH at 4.99 and 5.24 mW/mm^2, respectively. Note that this is probably a high estimation because some light was probably lost at the interface between the fiber optic cable and the implanted fiber optic cannula. After the completion of photostimulation experiments, mice were perfused and the approximate locations of fiber tips were identified based on the coordinates of Franklin and Paxinos.(Franklin, 2008)

Home cage food intake screening studies

Food intake studies on chow were performed as previously described(Garfield et al., 2015). All animals were singly housed for at least 2.5 weeks following surgery and handled for 10 consecutive days before the assay to reduce stress response. Studies were conducted in a home-cage environment near the beginning of the light cycle (9am) with *ad libitum* food access. A screening trial consisted of assessing food intake from *Agrp-ires-Cre* mice (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) after they received 1 hr photostimulation. $Fasted^{GFP}$ and fed^{ChR2} mice that failed to consume >.40g were excluded from analysis to ensure hungry animals would eat at physiological levels.

Home cage caloric vs. non-caloric food pellet studies

Grain (20 mg, Test Diets) and sucrose (20 mg, Test Diets) pellets were used to examine caloric intake and 1% saccharin pellets (20 mg, Test Diets) were used to examine non-caloric consumption. During the exposure phase, *Agrp-ires-Cre* mice housed with Carefresh bedding (white), to ensure quantification accuracy, were presented with 5 grain, sucrose or 1% saccharin pellets in a small metal dish for 3 consecutive dark cycle phases. Each animal was exposed to each type of pellet for 3 consecutive days to both eliminate neophobia and ensure consumption. During the testing phase, *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 1 hr near the beginning of the light cycle (9am) in the presence of 50 pellets (grain, sucrose or 1% saccharin) and food intake was assessed. Each animal was tested for consumption of each type of pellet on different days under identical conditions.

Hole-board assay

All *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 10 minutes in the homecage in the absence of food before being placed in the behavioral apparatus. The hole-board arena (Stoelting) measured 40x40x35cm and consisted of 16 total wells (4x4; 12 on the periphery and 4 centrally). Animals were run in two distinct conditions, each lasting 20 min while under photostimulation. In the non-baited condition, no food was present in the chamber and the total number of head-dips was assessed using 3 independent cameras positions and scored manually. In the baited condition, each well was baited with a grain pellet (20 mg, Test Diets) and the total number and quantity of food intake was measured.

Y-maze assay

All parts of the experiment were conducted in a Y-maze apparatus (Stoelting; arms 5x35cm with 10cm walls). The Y-maze was surrounded by patterned cues on curtains placed in each direction, as well as cued color and shape on the internal arms adjacent to the "choice point." All animals received a 15-minute habituation period in the maze before the start of the experiment. One arm was deemed the "start arm", where animals would begin each trial. The other two "choice" arms contained 15-mL conical tube caps toward the distal end, which were either empty or contained a single grain pellet (20 mg, Test Diets); importantly mice were acclimated to the pellets and caps beforehand to prevent neophobia. Prior to conditioning, all *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were placed in the "start arm" and scored for distribution into one of the two "choice" arms in the absence of any food reward. All animals were then food restricted and maintained at 85% body weight to motivate exploration of the maze and increase salience of a food reward. During conditioning, each animal received 2 rounds of a forced-choice trial toward the unpaired arm (empty cap) and a forced-choice trial toward the paired arm (baited cap) daily for 5 consecutive days for a total of 10 sets of conditioning trials. Importantly, for each set of trials, animals were first restricted to the start arm for 10 seconds; after this timeout period the animals began the forced-choice trial that concluded as soon as the animal's head crossed into the cap (and

subsequently consumed the pellet in the food-paired arm). In between each trial, the maze was cleaned of any excrement and odorant cues. After the final training trial, a post-conditioning test was conducted to assess learning of the task. Each animal was confined to the start arm for 10 seconds, before a free choice trial with access to both the unpaired arm (empty cap) and paired arm (baited cap). Importantly, a food pellet was attached to the outside of the unpaired arm of the maze to control for the smell of food guiding performance on this task. The trial ended as soon as the animal's center-point crossed fully into one of the arms, signifying a choice to enter one of the two arms and the distribution was recorded. Animals were then returned to their home cages and subdivided into their respective groups. On test day, all animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) received a 10-minute photostimulation period before a free-choice trial with access to both arms, both of which were devoid of food (empty caps). Again, the trial ended as soon as the animal's center-point crossed fully into one of the arms, signifying a choice to enter one of the two arms and the distribution was recorded. All trials were recorded on video using EthoVision XT 9.

Home cage water intake studies

All *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were housed and acclimated in Phenotyper instrumented observation cages (Noldus). Water intake was assessed via lickometers that accurately detect any contact (change in capacitance between the spout and the metal plate attached to an electronic box) made with the spout of the water bottle. All mice were assessed for the total number of spout interactions in four different conditions; in the presence of a non-food object or food under *ad libitum* water access preceding the experiment and identical parameters under 24 hr water restriction preceding the experiment. All *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 1 hr near the beginning of the light cycle (9am) and spout interactions and food intake (if present) was measured.

Open field assays

All *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 10 minutes in the homecage in the absence of food before being placed in the behavioral apparatus. The open field arenas (Stoelting) measured 40x40x35cm and 100x100x35cm. Each animal was run in two distinct conditions, each lasting 300 min while under photostimulation. In the non-food object condition, a 15-mL conical tube cap was placed in the center of the chamber, while in the food condition, a ~4-gram chow pellet was placed in the center of the chamber. Both the cap and food were secured to the apparatus using adhesive putty. Animals were pre-exposed to both cap and chow pellets to limit neophobic responses. Importantly, a crossover design was implemented to control for secondary exposure to the assay. A square sector (14x14cm and 25x25cm, for small and big open field assays, respectively) was designated the object/food zone in the center of the chamber. For the corner big open field, the designated object/food zone was randomly in one corner of the assay while the center zone remained the same. All trials were recorded on video using EthoVision XT 9.

Elevated zero maze assay

All *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 10 minutes in the homecage in the absence of food before being placed in the behavioral apparatus. The elevated zero maze apparatus (Stoelting) had the following measurements: 50cm diameter, 5cm lane width 15cm wall height and 40cm leg height. Each animal was run in two distinct conditions, each lasting 20 min while under photostimulation. In the non-food object condition, two 15-mL conical tube caps were placed in the center of the open arms, while in the food condition, two ~4-gram chow pellets were placed in the center of the open arms. Both the cap and food were secured to the apparatus using adhesive putty. Animals were pre-exposed to both cap and chow pellets to limit neophobic responses. Importantly, a crossover design was implemented to control for secondary exposure to the assay. Square sectors were designated the object/food zone in the center of the open arms. All trials were recorded on video using EthoVision XT 9.

TMT fear assay

The two-chambered apparatus measured 50x26x30cm with a partition equally separating the sides that allowed mice to move freely between the two chambers. To assess the effects of TMT on wildtype mice, 10uL of water or TMT (Contech Enterprises Inc.) was added to a 2x2cm piece of filter paper and placed on a corner wall of the apparatus. A square sector (9.25x9.25cm) adjacent to the filter paper was designated the water/TMT zone. For experimental studies, all *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 10 minutes in the homecage in the absence of food before being placed in

the behavioral apparatus. 10uL of TMT was added to a 2x2cm piece of filter paper and placed on a corner wall of the apparatus. A square sector (9.25x9.25cm) adjacent to the filter paper was designated the TMT zone. Each animal was run in two distinct conditions, each lasting 20 min while under photostimulation. In the non-food object condition, a 15-mL conical tube cap was placed in the TMT zone, while in the food condition, a ~4-gram chow pellet was placed in the TMT zone. Both the cap and food were secured to the apparatus using adhesive putty. Animals were pre-exposed to both cap and chow pellets to limit neophobic responses. Importantly, a crossover design was implemented to control for secondary exposure to the assay. The experiments were performed in a sound attenuation chamber with an outward-blowing fan on the side of the odor to limit diffusion across the apparatus. All trials were recorded on video using EthoVision XT 9.

Social interaction assay

All *Agrp-ires-Cre* animals (fed^{GFP}, $fasted^{GFP}$ and fed^{ChR2} groups) were photostimulated for 10 minutes in the homecage in the absence of food before being placed in the behavioral apparatus. The two-chambered apparatus measured 50x26x30cm with a partition equally separating the sides that allowed mice to move freely between the two chambers. A receptive female (aged >2 months) or juvenile male (aged <2 months) was isolated in a 3D-printed social interaction cage (11x11x12) located in one corner of the apparatus. Each animal was run in two distinct conditions, each lasting 20 min while under photostimulation. In the non-food object condition, a 15-mL conical tube cap was placed in the opposite corner of the apparatus from the social interaction cage, while in the food condition, a ~4-gram chow pellet was placed in the opposite corner of the apparatus from the social interaction cage. Both the cap and food were secured to the apparatus using adhesive putty. A square sector (9.25cmx9.25cm) was designated the object/food zone in this corner of the apparatus. Animals were pre-exposed to both cap and chow pellets to limit neophobic responses. Importantly, a crossover design was implemented to control for secondary exposure to the assay. The experiments were performed in a sound attenuation chamber. All trials were recorded on video using EthoVision XT 9.

In vivo fiber photometry

Unilateral optic-fiber cannulas (fiber: core=400μm; NA=0.48;M3 thread titanium receptacle; Doric Lenses Inc) were implanted in the ARC of each experimental mouse. Behavioral testing started 4 weeks later to allow for viral expression and recovery from surgery. Mice were then allowed to adapt to the experimental cages and the fiber patch cord for at least 3 days prior to experiments (core 400uM; NA 0.53; M3 connector; Doric Lenses Inc). Continuous blue LED at 465nm served as a light source (~20uW, Doric Lenses Inc.) delivered through optic fibers connected to a rotary joint (FRJ 1X1, Doric Lenses Inc.) to allow for movement. GCaMP calcium GFP signals were collected through the same fibers via a dichroic port (FMC4 port, Doric Lenses Inc) into a femtowatt silicon photoreceiver (2151, Newport). Digital signals were then amplified and collected through Plexon softwares OmniPlex. Synchronized multi-angled videos were recorded via Cineplex for time-locked data analysis in NeuroExplorer. A separate cohort of GFP control animals was used to validate the absence of potential movement artifacts.

In the fasted condition, food was taken away from the animals 30 min before their dark cycle and the animals remained food deprived until experiments started 2 hours into their light cycle. Experiments in the fed condition were conducted at the same time as fasted condition but animals had *ad lib* access to food prior to recordings. Conspecifics were fasted overnight and introduced in grid enclosure social isolation cages. Photometry data was normalized against the average of first half of the baseline recording.

Statistical analysis

Statistical analyses were performed using Prism 6.0 (GraphPad) software. In all statistical tests normal distribution and equal variance was established. The data presented met the assumptions of the statistical test employed. Exclusion criteria for experimental fed^{ChR2} animals were <0.40 grams of food intake during the screening period. This criterion was established prior to data collection. N-numbers represent final number of animals used per assay.

Figure 1

A) 2-way ANOVA indicates a main effect of pellet type, $F(2, 56) = 89.72$, $P < 0.0001$, main effect of group, $F(2, 28) = 72.55$, $P < 0.0001$, and an interaction of pellet type and group, $F(4, 56) = 18.02$, $P < 0.0001$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=15).
(B) One-way ANOVA indicates significant differences across groups, $F(2, 21) = 13.53$, $P = 0.0002$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(C) One-way ANOVA indicates significant differences across groups, $F(2, 21) = 73.89$, $P < 0.0001$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(E) Binomial distribution; Fed^{GFP} (n=16), P (two-tailed) > 0.9999. $Fasted^{GFP}$ (n=16), P (two-tailed) = 0.4545. Fed^{ChR2} (n=16), P (two-tailed) = 0.8036.
(F) Binomial distribution; Fed^{GFP} (n=16), P (two-tailed) = 0.0213. $Fasted^{GFP}$ (n=16), P (two-tailed) = 0.0042. Fed^{ChR2} (n=16), P (two-tailed) = 0.0042.
(G) Binomial distribution; Fed^{GFP} (n=16), P (two-tailed) = 0.8036. $Fasted^{GFP}$ (n=16), P (two-tailed) = 0.0042. Fed^{ChR2} (n=16), P (two-tailed) < 0.0001.

Figure 2
(A) 2-way ANOVA indicates a main effect of trial condition, $F(1, 29) = 35.02$, $P < 0.0001$, main effect of group, $F(2, 29) = 10.40$, $P = 0.0004$, and an interaction of trial condition and group, $F(2, 29) = 8.710$, $P = 0.0011$. Fed^{GFP} (n=10), $fasted^{GFP}$ (n=11), fed^{ChR2} (n=11).
(B) Correlation Pearson, R squared = 0.4152, P (two-tailed) < 0.0001, Number of XY Pairs = 32
(C) 2-way ANOVA indicates a main effect of trial condition, $F(1, 29) = 26.00$, $P < 0.0001$, no main effect of group, $F(2, 29) = 0.3608$, $P = 0.7002$, and an interaction of trial condition and group, $F(2, 29) = 8.622$, $P = 0.0012$. Fed^{GFP} (n=10), $fasted^{GFP}$ (n=11), fed^{ChR2} (n=11).
(D) Correlation Pearson, R squared = 0.1755, P (two-tailed) = 0.0170, Number of XY Pairs = 32
(E) 2-way ANOVA indicates no main effect of trial condition, $F(1, 30) = 1.966$, $P = 0.1711$, a main effect of group, $F(2, 30) = 27.82$, $P < 0.0001$, and an interaction of trial condition and group, $F(2, 30) = 4.695$, $P = 0.0168$. Fed^{GFP} (n=10), $fasted^{GFP}$ (n=11), fed^{ChR2} (n=11).
(F) Paired t test, $P < 0.0001$, Fed^{GFP} (n=10)

Figure 3
(B) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 51.52$, $P < 0.0001$, main effect of group, $F(2, 21) = 23.79$, $P < 0.0001$, but not an interaction of trial condition and group, $F(2, 21) = 2.287$, $P = 0.1263$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(C) One-way ANOVA indicates significant differences across groups, $F(2, 21) = 20.53$, $P < 0.0001$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(E) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 65.72$, $P < 0.0001$, main effect of group, $F(2, 21) = 10.98$, $P = 0.0005$, and an interaction of trial condition and group, $F(2, 21) = 6.393$, $P = 0.0068$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(F) One-way ANOVA indicates significant differences across groups, $F(2, 21) = 9.938$, $P = 0.0009$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(H) 2-way ANOVA indicates a main effect of trial condition, $F(1, 19) = 23.73$, $P = 0.0001$, main effect of group, $F(2, 19) = 5.038$, $P = 0.0176$, and an interaction of trial condition and group, $F(2, 19) = 6.335$, $P = 0.0078$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=6), fed^{ChR2} (n=8).
(I) One-way ANOVA indicates significant differences across groups, $F(2, 21) = 11.40$, $P = 0.0004$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=6), fed^{ChR2} (n=8).

Figure 4
(B) Paired t test, $P = 0.0202$, wt (n=6)
(C) Paired t test, $P = 0.0063$, wt (n=6)
(E) Paired t test, $P = 0.0027$ (unpaired chamber), $P = 0.0019$ (paired chamber), $P = 0.0073$ (zone), wt (n=6)
(G) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 9.128$, $P = 0.0065$, main effect of group, $F(2, 21) = 5.420$, $P = 0.0126$, and an interaction of trial condition and group, $F(2, 21) = 6.419$, $P = 0.0067$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(H) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 60.64$, $P < 0.0001$, main effect of group, $F(2, 21) = 18.99$, $P < 0.0001$, and an interaction of trial condition and group, $F(2, 21) = 13.15$, $P = 0.0002$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).

Figure 5
(B) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 25.84$, $P < 0.0001$, main effect of group, $F(2, 21) = 7.952$, $P = 0.0027$, and an interaction of trial condition and group, $F(2, 21) = 4.285$, $P = 0.0275$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(C) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 66.30$, $P < 0.0001$, main effect of group, $F(2, 21) = 11.47$, $P = 0.0004$, and an interaction of trial condition and group, $F(2, 21) = 16.67$, $P < 0.0001$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(E) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 43.88$, $P < 0.0001$, main effect of group, $F(2, 21) = 9.362$, $P = 0.0012$, and an interaction of trial condition and group, $F(2, 21) = 8.736$, $P = 0.0017$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(F) 2-way ANOVA indicates a main effect of trial condition, $F(1, 21) = 63.87$, $P < 0.0001$, main effect of group, $F(2, 21) = 12.29$, $P = 0.0003$, and an interaction of trial condition and group, $F(2, 21) = 16.97$, $P < 0.0001$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).

Figure 6
One-way ANOVA analysis was conducted with pairing and multiple comparisons. Values compared were 5min averages from each condition between object, food and
(C) water: $F(1.035,4.140)=8.367$, $p=0.0423$.
(F) TMT: $F(1.069,4.276)=9.721$, $p=0.0318$.
(I) female: $F(1.164,4.658)=13.32$, $p=0.0155$.
(L) male: $F(1.561,6.242)=9.766$, $p=0.0143$.
(O) water: Paired t test, $P = 0.0652$.
(R) TMT: Paired t test, $P = 0.0788$.
(U) female: Paired t test, $P = 0.8520$.
(X) male: Paired t test, $P = 0.0690$.

Figure 7
One-way ANOVA analysis was conducted with pairing and multiple comparisons. Values compared were area under the curve from t=1-20 seconds between the first interaction with object, female, and male in the
(C) fasted state: $F(1.295, 5.179)=6.1$, $p=0.0506$.
(F) fed state: $F(1.052, 5.261)=2.383$, $p=0.1811$.
(H) Between the first interactions of the 4 exposures to the empty cage: $F(1.66,8.298)=0.8824$, $p=0.4299$.
Between the first interactions of the 4 exposures to the female: $F(1.383,6.915)=1.722$, $p=0.2407$.

Figure S1
(B) One-way ANOVA indicates significant differences across groups, $F(2, 165) = 281.2$, $P < 0.0001$. Fed^{GFP} (n=56), $fasted^{GFP}$ (n=56), fed^{ChR2} (n=56).
(D) 2-way ANOVA indicates a main effect of pellet type, $F(2, 56) = 3.730$, $P = 0.0301$, but no main effect of group, $F(2, 28) = 0.002161$, $P = 0.9978$, and no interaction between pellet type and group, $F(4, 56) = 0.002161$, $P > 0.9999$. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=15).
(F) 2-way ANOVA indicates a main effect of pellet type, $F(2, 27) = 30.37$, $P < 0.0001$, main effect of group, $F(1, 27) = 247.5$, $P < 0.0001$, and an interaction of pellet type and group, $F(2, 27) = 11.59$, $P = 0.0002$. VGAT Fed^{ChR2} – no stim (n=10), VGAT Fed^{ChR2} –stim (n=10).

Figure S2
(B) Paired t test, $P = 0.5804$, Fed^{GFP} (n=10)
(C) Paired t test, $P = 0.5039$, Fed^{GFP} (n=10)
(D) Paired t test, $P < 0.0001$, Fed^{GFP} (n=10)
(E) Paired t test, $P < 0.0001$, Fed^{GFP} (n=10)
(F) Paired t test, $P = 0.0014$, $Fasted^{GFP}$ (n=11)
(G) Paired t test, $P = 0.0014$, $Fasted^{GFP}$ (n=11)
(H) Paired t test, $P = 0.0002$, $Fasted^{GFP}$ (n=11)
(I) Paired t test, $P = 0.3005$, $Fasted^{GFP}$ (n=11)
(J) Paired t test, $P = 0.0016$, Fed^{ChR2} (n=11)
(K) Paired t test, $P = 0.0020$, Fed^{ChR2} (n=11)
(L) Paired t test, $P = 0.0354$, Fed^{ChR2} (n=11)

(M) Paired t test, P = 0.1393, Fed^{ChR2} (n=11)

Figure S3
(B) Paired t test, P = 0.0010, Fed^{GFP} (n=8)
(C) Paired t test, P = 0.0045, $Fasted^{GFP}$ (n=8)
(D) Paired t test, P = 0.0031, Fed^{ChR2} (n=8)
(F) Paired t test, P = 0.0534, Fed^{GFP} (n=8)
(G) Paired t test, P = 0.0008, $Fasted^{GFP}$ (n=8)
(H) Paired t test, P = 0.0008, Fed^{ChR2} (n=8)
(I) Paired t test, P = 0.1766, VGAT Fed^{ChR2} (n=8)
(K) Paired t test, P = 0.7324, Fed^{GFP} (n=8)
(L) Paired t test, P = 0.0017, $Fasted^{GFP}$ (n=6)
(M) Paired t test, P = 0.0189, Fed^{ChR2} (n=8)

Figure S4
(B) 2-way ANOVA indicates a main effect of trial condition, F (1, 19) = 20.44, P = 0.0002, main effect of group, F (2, 19) = 3.622, P = 0.0465, but not an interaction of trial condition and group, F (2, 19) = 0.4243, P = 0.6603. Fed^{GFP} (n=7), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=7).
(C) Paired t test, 0.2772, Fed^{GFP} (n=7)
(D) Paired t test, 0.0002, $Fasted^{GFP}$ (n=8)
(E) Paired t test, 0.0078, Fed^{ChR2} (n=7)
(F) 2-way ANOVA indicates a main effect of trial condition, F (1, 19) = 85.97, P < 0.0001, interaction of trial condition and group, F (2, 19) = 6.773, P = 0.0060 and no main effect of group, F (2, 19) = 1.445, P = 0.2605. Fed^{GFP} (n=7), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=7).
(G) Paired t test, 0.1369, Fed^{GFP} (n=7)
(H) Paired t test, < 0.0001, $Fasted^{GFP}$ (n=8)
(I) Paired t test, 0.0002, Fed^{ChR2} (n=7)
(J) One-way ANOVA indicates significant differences across groups, F (2, 19) = 12.86, P = 0.0003. Fed^{GFP} (n=7), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=7).

Figure S5
(B) One-way ANOVA indicates significant differences across groups, F (2, 21) = 15.69, P < 0.0001. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(C) Paired t test, P = 0.2417, Fed^{GFP} (n=8)
(D) Paired t test, P = 0.0331, $Fasted^{GFP}$ (n=8)
(E) Paired t test, P = 0.0022, Fed^{ChR2} (n=8)
(F) Paired t test, P = 0.9940, VGAT Fed^{ChR2} (n=8)
(G) Paired t test, P = 0.1128, Fed^{GFP} (n=8)
(H) Paired t test, P = 0.0020, $Fasted^{GFP}$ (n=8)
(I) Paired t test, P = 0.0002, Fed^{ChR2} (n=8).
(J) Paired t test, P = 0.2826, VGAT Fed^{ChR2} (n=8)

Figure S6
(B) One-way ANOVA indicates significant differences across groups, F (2, 21) = 39.91, P < 0.0001. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(C) One-way ANOVA indicates significant differences across groups, F (2, 21) = 17.45, P < 0.0001. Fed^{GFP} (n=8), $fasted^{GFP}$ (n=8), fed^{ChR2} (n=8).
(D) Paired t test, P = 0.3620, Fed^{GFP} (n=8)
(E) Paired t test, P = 0.0010, $Fasted^{GFP}$ (n=8)
(F) Paired t test, P = 0.0237, Fed^{ChR2} (n=8)
(G) Paired t test, P = 0.8325, VGAT Fed^{ChR2} (n=8)
(H) Paired t test, P = 0.3408, Fed^{GFP} (n=8)
(I) Paired t test, P = 0.0008, $Fasted^{GFP}$ (n=8)
(J) Paired t test, P = 0.0004, Fed^{ChR2} (n=8).
(K) Paired t test, P = 0.9112, VGAT Fed^{ChR2} (n=8)
(L) Paired t test, P = 0.2621, Fed^{GFP} (n=8)

(M) Paired t test, P = 0.0036, Fasted^{GFP} (n=8)
(N) Paired t test, P = 0.0005, Fed^{ChR2} (n=8)
(O) Paired t test, P = 0.2905, Fed^{GFP} (n=8)
(P) Paired t test, P = 0.0008, Fasted^{GFP} (n=8)
(Q) Paired t test, P = 0.0005, Fed^{ChR2} (n=8).

Figure S7
One-way ANOVA analysis was conducted with pairing and multiple comparisons. Values compared were 5min averages from each condition between object, food and
(E) water: F (1.043,5.214)=4.239, p=0.0917.
(H) TMT: F (1.1,5.502)=3.244, p=0.1255.
(K) female: F (1.941,9.705)=1.142, p=0.3568.
(N) male: F (1.761,8.806)=0.9505, p=0.4113.

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