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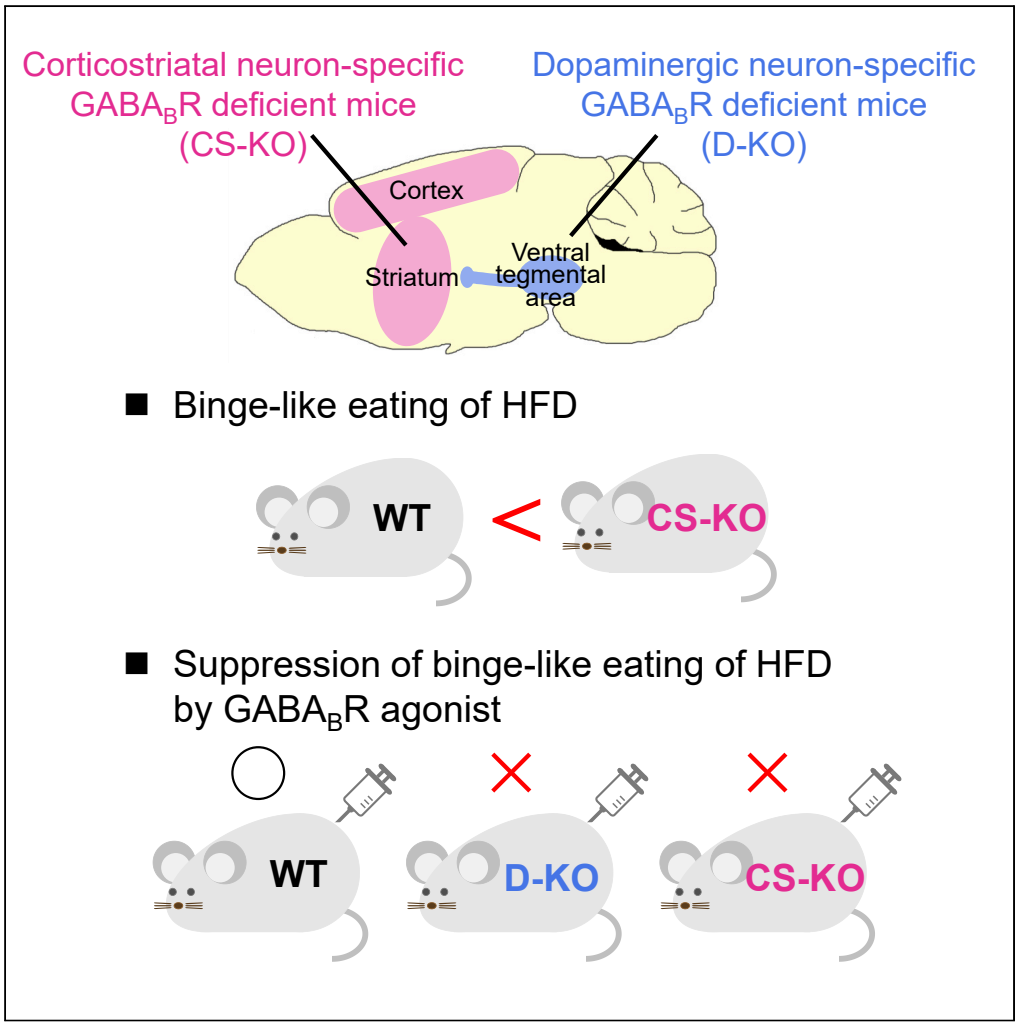

Taku Tsunekawa, Ryoichi Banno, Hiroshi Yaginuma, ..., Hidetaka Suga, Bernhard Bettler, Hiroshi Arima

ryouichi@med.nagoya-u.ac.jp (R.B.)
arima105@med.nagoya-u.ac.jp (H.A.)

HIGHLIGHTS

$GABA_B$R KO in corticostriatal neurons enhances binge-like feeding of HFD

Baclofen suppresses binge-like feeding of HFD via the mesolimbic system

$GABA_B$R signaling in mesolimbic system does not affect energy balance

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$GABA_B$ Receptor Signaling in the Mesolimbic System Suppresses Binge-like Consumption of a High-Fat Diet

Taku Tsunekawa,[1] Ryoichi Banno,[1,2,*] Hiroshi Yaginuma,[1] Keigo Taki,[1] Akira Mizoguchi,[1] Mariko Sugiyama,[1] Takeshi Onoue,[1] Hiroshi Takagi,[1] Daisuke Hagiwara,[1,3] Yoshihiro Ito,[1,4] Shintaro Iwama,[1] Motomitsu Goto,[1] Hidetaka Suga,[1] Bernhard Bettler,[5] and Hiroshi Arima[1,6,*]

SUMMARY

Binge eating could contribute to the development of obesity, and previous studies suggest that gamma-aminobutyric acid (GABA) type B receptor ($GABA_BR$) signaling is involved in the regulation of binge eating. Here, we show that time-restricted access to a high-fat diet (HFD) induces binge-like eating behavior in wild-type mice. HFD consumption during restricted time was significantly increased in corticostriatal neuron-specific $GABA_BR$-deficient mice compared with wild-type mice. Furthermore, the $GABA_BR$ agonist baclofen suppressed HFD intake during restricted time in wild-type mice but not in corticostriatal or dopaminergic neuron-specific $GABA_BR$-deficient mice. In contrast, there were no significant differences in food consumption among genotypes under *ad libitum* access to HFD. Thus, our data show that the mesolimbic system regulates food consumption under time-restricted but not *ad libitum* access to HFD and have identified a mechanism by which $GABA_BR$ signaling suppresses binge-like eating of HFD.

INTRODUCTION

Obesity has become a major health concern worldwide, as it is associated with the development of various diseases such as type 2 diabetes, cardiovascular diseases, cancer, and mood-related disorders (Finkelstein et al., 2009). Obesity is caused when energy intake overwhelms energy expenditure, and predisposing factors to obesity include excess in palatable and calorie-rich food intake such a high-fat diet (HFD) (O'Rahilly, 2009), as well as irregular eating such as binge eating (Kessler et al., 2016). Feeding behavior is controlled by both the homeostatic and reward systems (Waterson and Horvath, 2015). The latter is mainly composed of the mesolimbic system, in which dopaminergic neurons in the ventral tegmental area (VTA) project to the nucleus accumbens (NAc) and caudate putamen (CPu) in the striatum (Kenny, 2011).

Gamma-aminobutyric acid (GABA), the inhibitory neurotransmitter that has been implicated in the regulation of the mesolimbic system (Hayes et al., 2014), acts on two types of receptors: ionotropic $GABA_A$ and $GABA_C$, and metabotropic $GABA_B$ receptors ($GABA_BRs$) that are located both pre- and postsynaptically (Bettler et al., 2004; Gassmann and Bettler, 2012). Previous studies suggest that the $GABA_BR$ agonist baclofen is effective in reducing binge-like eating in rodents (Berner et al., 2009; Buda-levin et al., 2005; Czyzyk et al., 2010; Rao et al., 2008; Wojnicki et al., 2013; Wong et al., 2009) as well as binge eating in humans (De Beaurepaire et al., 2015; Broft et al., 2007; Corwin et al., 2012), although the site of action remains to be elucidated.

The time-restricted access to HFD causes binge-like eating behavior, as shown by increases in motivation to consume (Lardeux et al., 2013) and gradual increases in the consumption (Furlong et al., 2014; Valdivia et al., 2015). These behaviors are accompanied by the activation of neurons in both VTA and NAc (Valdivia et al., 2015) and increases in extracellular dopamine concentrations in the NAc (Liang et al., 2006; Naef et al., 2015; Sahr et al., 2008), suggesting that the time-restricted access to HFD is a good model to investigate the mechanisms underlying binge eating of HFD.

To investigate the role of $GABA_BRs$ in the mesolimbic system in binge eating, we generated mice that lack $GABA_BRs$ exclusively in dopaminergic or corticostriatal neurons and compared their binge-like eating behavior induced by the time-restricted access to HFD with wild-type (WT) littermate mice.

[1]Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan

[2]Research Center of Health, Physical Fitness and Sports, Nagoya University, Nagoya 464-8601, Japan

[3]Schaller Research Group on Neuropeptides, German Cancer Research Center (DKFZ), Heidelberg, Germany

[4]Department of CKD Initiatives/Nephrology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8560, Japan

[5]Department of Biomedicine, University of Basel, Basel 4056, Switzerland

[6]Lead Contact

*Correspondence: ryouichi@med.nagoya-u.ac.jp (R.B.), arima105@med.nagoya-u.ac.jp (H.A.)

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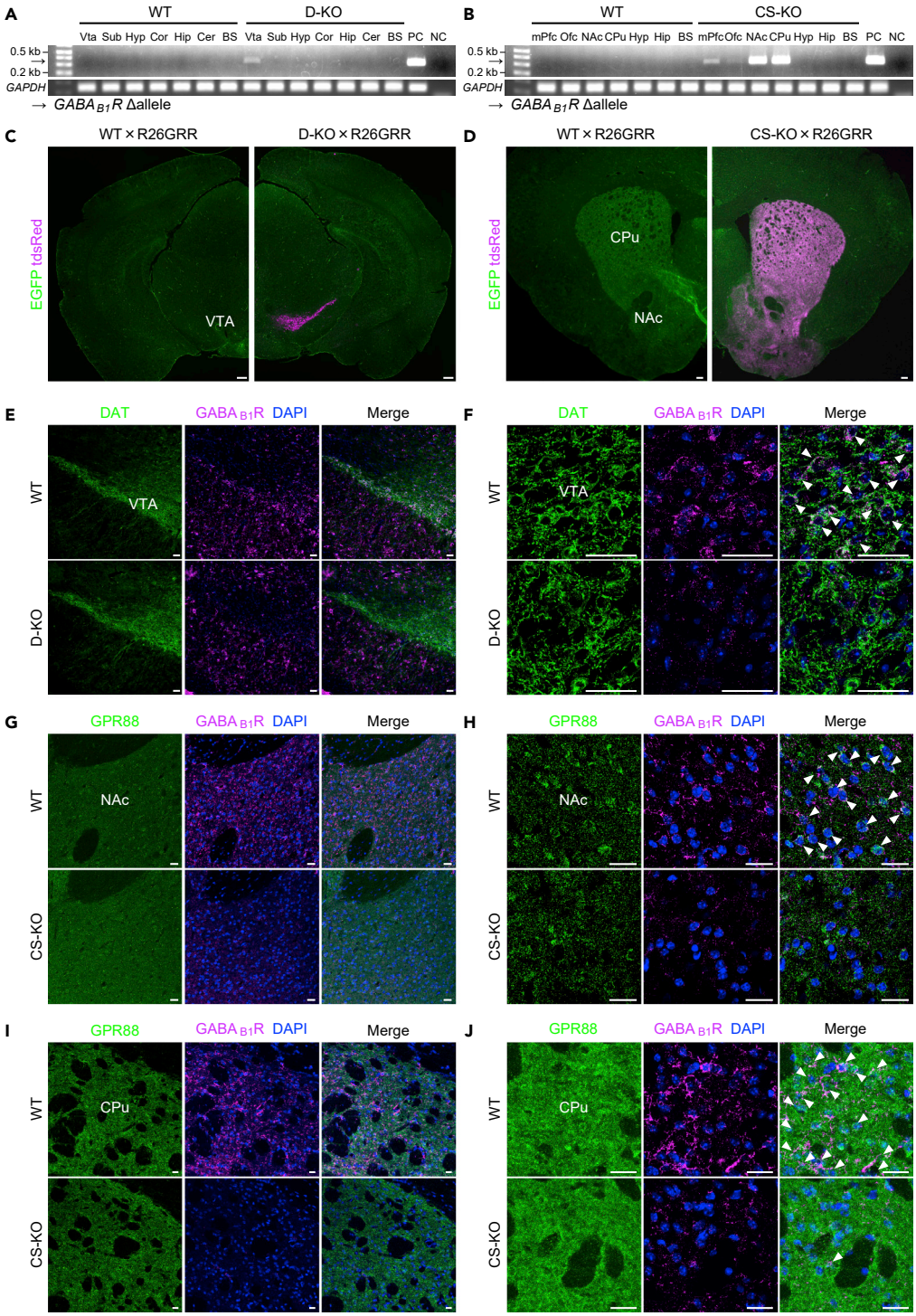

Figure 1. Generation of Dopaminergic Neuron-Specific and Corticostriatal Neuron-Specific $GABA_BR$ Deficient Mice

(A and B) Detection of deleted $GABA_{B1}R$ alleles (Δ) in $GABA_{B1}R^{lox511/lox511}$ *DAT-Cre* (D-KO) mice and $GABA_{B1}R^{lox511/lox511}$ *GPR88-Cre* (CS-KO) mice. DNA was extracted from different tissues, and deletion of the floxed allele was detected by PCR. Vta, ventral tegmental area; Sub, substantia nigra; Hyp, hypothalamus; Cor, cerebral cortex; Hip, hippocampus; Cer, cerebellum; BS, brain stem; mPfc, medial prefrontal cortex; Ofc, orbitofrontal cortex; NAc, nucleus accumbens; CPu, caudate putamen; PC, positive control; NC, negative control. PCR reaction with *GAPDH* was used as an internal control.

Figure 1. *Continued*

(C and D) Double-color imaging of EGFP (green) and tdsRed (magenta) fluorescence to assess *DAT-Cre* and *GPR88-Cre*. The VTA of $GABA_{B1}R^{lox511/lox511}$ *DAT-Cre R26GRR* mice as compared with that of $GABA_{B1}R^{+/+}$ *R26GRR* mice (C). The NAc and CPu of $GABA_{B1}R^{lox511/lox511}$ *GPR88-Cre R26GRR* mice as compared with those of $GABA_{B1}R^{+/+}$ *R26GRR* mice (D). Scale bar: 100 μm.

(E and F) The representative photographs showing the staining of DAT (green), $GABA_{B1}R$ (magenta), and DAPI (blue) in VTA in WT and D-KO mice. White arrow heads show colocalization of DAT and $GABA_{B1}R$. Scale bar: 40 μm.

(G–J) The representative photographs showing the staining of GPR88 (green), $GABA_{B1}R$ (magenta), and DAPI (blue) in NAc (G and H) and CPu (I and J) in WT and CS-KO mice. White arrow heads show colocalization of GPR88 and $GABA_{B1}R$. Scale bar: 20 μm. All data are from male mice.

See also Figure S1.

RESULTS

Generation of Dopaminergic Neuron-Specific and Corticostriatal Neuron-Specific $GABA_BR$-Deficient Mice

To generate dopaminergic neuron-specific $GABA_BR$ deficient (D-KO) mice, $GABA_{B1}R^{lox511/lox511}$ mice were crossed with dopamine transporter (DAT)-*Cre* (DAT-Cre) mice. Then, we crossed the $GABA_{B1}R^{+/lox511}$ *DAT-Cre* mice with $GABA_{B1}R^{lox511/lox511}$ or $GABA_{B1}R^{+/lox511}$ mice to yield $GABA_{B1}R^{lox511/lox511}$ *DAT-Cre* mice and littermate controls (hereafter termed WT mice). GPR88 is an orphan G-protein-coupled receptor that is highly expressed in striatal and cortical neurons (Hisatsune et al., 2013; Quintana et al., 2012). To generate corticostriatal neuron-specific $GABA_BR$-deficient (CS-KO) mice, $GABA_{B1}R^{lox511/lox511}$ mice were crossed with *GPR88-Cre* mice. Then, we crossed the $GABA_{B1}R^{+/lox511}$ *GPR88-Cre* mice with $GABA_{B1}R^{lox511/lox511}$ or $GABA_{B1}R^{+/lox511}$ mice to yield $GABA_{B1}R^{lox511/lox511}$ *GPR88-Cre* mice and WT mice. Deletion of the $GABA_{B1}$ receptor allele in D-KO mice was only detected in DNA extracts from the VTA (Figures 1A and S1A). Similarly, deletions of the $GABA_{B1}$ receptor allele in CS-KO mice were detected in DNA extracts from the NAc, CPu, medial prefrontal cortex (mPFC), and orbitofrontal cortex (OFC) (Figures 1B and S1B). In contrast, no recombined alleles were detected in WT mice (Figures 1A, 1B, S1A, and S1B). To visualize dopaminergic neuron-specific and corticostriatal neuron-specific Cre-mediated recombination, we crossed D-KO and CS-KO mice to *ROSA26* Cre-reporter knockin mice (hereafter termed R26GRR), in which green fluorescence changed to red fluorescence in Cre-recombined cells (Hasegawa et al., 2013). $GABA_{B1}R^{lox511/lox511}$ *DAT-Cre R26GRR* mice expressed tdsRed-positive cells in the VTA, and the tdsRed co-localized with DAT immunostaining (Figures 1C and S1C). Likewise, $GABA_{B1}R^{lox511/lox511}$ *GPR88-Cre R26GRR* mice expressed tdsRed-positive cells in NAc and CPu, and the tdsRed was co-localized with GPR88 immunostaining (Figures 1D and S1D). Immunostaining of both DAT and $GABA_BRs$ revealed that $GABA_BRs$ were expressed in the dopaminergic neurons of the VTA in WT mice, whereas $GABA_BRs$-expressing cells in the VTA were rarely detected in D-KO mice (Figures 1E and 1F). Similarly, immunostaining of both GPR88 and $GABA_BRs$ revealed that $GABA_BRs$ were expressed in the GPR88-positive neurons in NAc, CPu, mPFC, and OFC in WT but not in CS-KO mice (Figures 1G–1J and S1E–S1H).

Time-Restricted Access to HFD Gives Rise to Binge-like Eating

To examine the hedonic regulation of HFD intake, male WT mice were divided into three groups, "control group," "*ad libitum* HFD group," and "restricted HFD group" (Figures 2A and 2B). All mice were fed only a chow diet (CD) from conditioning day 1–5. Then, the mice in control group were given free access to both CD and HFD for 2 days, followed by access to only CD (Figures 2A and 2B). The mice in *ad libitum* HFD group could access both CD and HFD from the conditioning day 6 to the end of the experiments (Figure 2B). In the restricted HFD group, mice were given free access to both CD and HFD on conditioning days 6 and 7, followed by access to only CD from conditioning day 8–12. The mice were then given the access to HFD for 2 h (zeitgeber time 12 to 14), whereas they could always access CD from experimental day 1–6 (Figures 2A and 2B). The protocols of conditioning and experimental days are determined based on previous studies (Bake et al., 2014; Berner et al., 2008; Cao et al., 2014; Czyzyk et al., 2010; Johnson and Kenny, 2010; King et al., 2016) with some modifications.

The mice in the *ad libitum* HFD group showed increases in both body weight and daily food intake compared with restricted HFD and control groups (Figures 2C and 2D). The findings were supported by a group effect (F (2,15) = 9.580, $p = 0.002$, for body weight; F (2,15) = 8.479, $p = 0.003$, for food intake) and an interaction effect between time and group (F (34,255) = 9.311; $p < 0.001$, for body weight; F (34,255) = 5.309, $p < 0.001$, for food intake). In both control and restricted HFD groups, the food intake was decreased when the mice were returned to CD from HFD on conditioning day 8, as reported previously

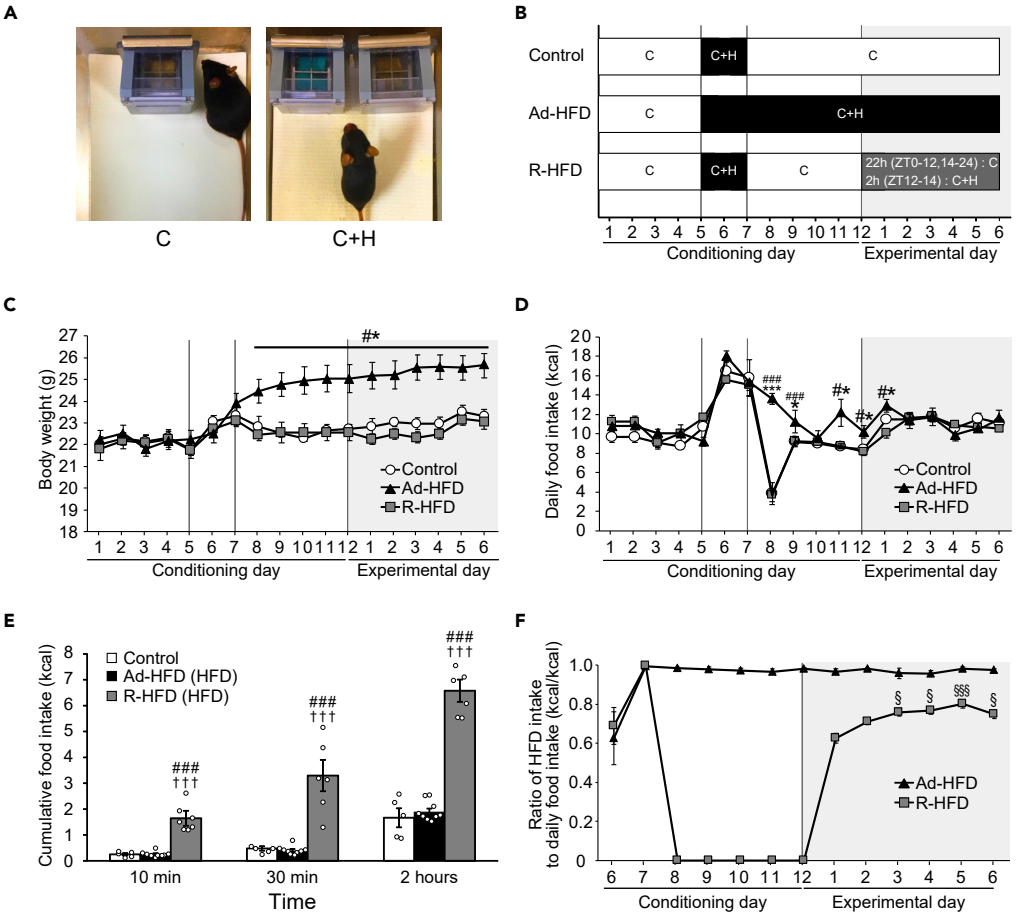

Figure 2. Time-Restricted Access to HFD Gives Rise to Binge-like Eating

(A) Photographs of chow feeding (termed as C) and CD and HFD feeding (termed as C + H).

(B) Protocol for the experiment. WT mice were divided into three groups, control group (Control), *ad libitum* HFD group (Ad-HFD), and restricted HFD group (R-HFD). The mice in R-HFD have *ad libitum* access to CD and HFD for 2 h (ZT 12–14) and only CD during the rest of the day (for 22 h) in experimental days.

(C) The mice in Ad-HFD showed increases in body weight compared with R-HFD and Control (group: $F(2,15) = 9.580$, $p = 0.002$; time: $F(17,255) = 25.412$, $p < 0.001$; group × time interaction: $F(34,255) = 9.311$, $p < 0.001$, $n = 6$ per group).

(D) The mice in Ad-HFD showed increases in daily food intake compared with R-HFD and Control (group: $F(2,15) = 8.479$, $p = 0.003$; time: $F(17,255) = 36.294$, $p < 0.001$; group × time interaction: $F(34,255) = 5.309$, $p < 0.001$, $n = 6$ per group).

(E) The mice in R-HFD consumed more calories during the restricted time (zeitgeber time 12 to 14) than mice in the other 2 groups (group: $F(2,16) = 80.096$, $p < 0.001$; time: $F(2,32) = 175.227$, $p < 0.001$; group × time interaction: $F(4,32) = 26.085$, $p < 0.001$, $n = 5$–9 per group).

(F) The ratios of HFD intake to daily calorie intake on experimental days 3–6 were significantly increased compared with that on experimental day 1 in R-HFD (time effect: $F(5,50) = 9.580$, $p < 0.001$, $n = 11$). The calorie intake in Ad-HFD group was almost from HFD, but not from CD, throughout experimental days ($n = 6$).

All values are mean ± SEM. Statistical analysis were performed using two-way ANOVA assessed by repeated measures (C–E) or one-way ANOVA assessed by repeated measures (F) followed by Bonferroni post hoc test. #$p < 0.05$, ###$p < 0.001$ versus Control; *$p < 0.05$, ***$p < 0.001$ versus R-HFD; †††$p < 0.001$ versus Ad-HFD; §$p < 0.05$, §§§$p < 0.001$ versus the ratio of HFD in R-HFD on experimental day 1. See also Table S1 for the details of statistics.

(Berner et al., 2008; Czyzyk et al., 2010). The mice in the restricted HFD group consumed only HFD during the restricted time (zeitgeber time 12 to 14), and they consumed more calories during this period than mice from the other two groups (Figure 2E). The mice in the restricted HFD group consumed about 60% of the daily calorie intake from HFD during the restricted time on experimental day 1, and consistent with previous studies (Johnson and Kenny, 2010; Valdivia et al., 2015), the ratios of HFD intake to daily calorie intake on experimental days 3–6 are significantly increased compared with that on experimental day 1 (time effect; $F(5,50) = 80.096$, $p < 0.001$, Figure 2F). On the other hand, calorie intake in the *ad libitum* HFD Ad-HFD group

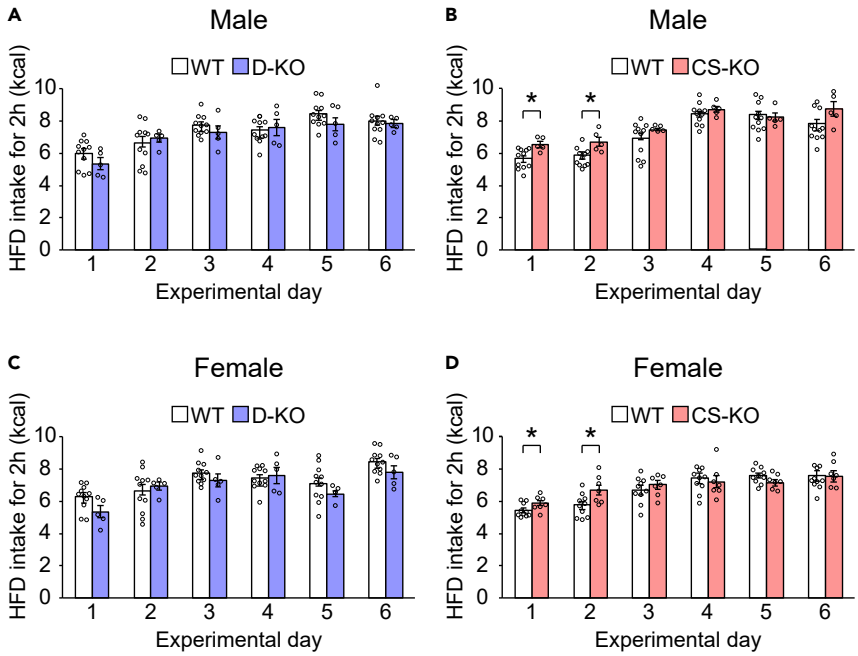

Figure 3. $GABA_B$ Receptor Signaling in Corticostriatal Neurons Suppresses HFD Consumption under Time-Restricted Access to HFD

(A and C) HFD intake of male (A) and female (C) *$GABA_{B1}R^{lox511/lox511}$ DAT-Cre* (D-KO) and *$GABA_{B1}R^{+/+}$* (WT) mice during 2 h (ZT 12–14) in R-HFD (male and female: WT, n = 11; D-KO, n = 5).

(B and D) HFD intake of male (B) and female (D) *$GABA_{B1}R^{lox511/lox511}$ GPR88-Cre* (CS-KO) and WT mice during 2 h (ZT 12–14) in R-HFD. The HFD intake during the 2 h was significantly increased in CS-KO compared with WT mice on experimental days 1 and 2 (male: genotype: $F(1,14) = 7.475$, $p = 0.003$; time: $F(5,70) = 31.215$, $p < 0.001$; genotype × time interaction: $F(5,70) = 1.074$, not significant, WT, n = 11; CS-KO, n = 5; female: genotype: $F(1,15) = 5.025$, $p = 0.041$; time: $F(5,75) = 20.126$, $p < 0.001$; genotype × time interaction: $F(5,75) = 0.811$, not significant, WT, n = 10; CS-KO, n = 7). All values are mean ± SEM. Statistical analyses were performed using two-way ANOVA assessed by repeated measures followed by Bonferroni post hoc test. *$p < 0.05$ versus WT. See also Table S2 for the details of statistics.

was almost from HFD, but not from CD, throughout experimental days (Figure 2F). These data suggest that time-restricted access to HFD gives rise to a pattern of binge-like eating of HFD.

$GABA_B$ Receptor Signaling in Corticostriatal Neurons Suppresses HFD Consumption under Time-Restricted Access to HFD

To clarify the role of $GABA_BR$ signaling in the hedonic regulation of HFD intake, we placed male D-KO and CS-KO mice on time-restricted HFD access. The HFD intake during the 2 h was significantly increased in male CS-KO, but not in D-KO, compared with WT mice in experimental days 1 and 2 (Figures 3A and 3B). This was supported by a genotype effect between WT and CS-KO mice ($F(1,14) = 7.475$, $p = 0.003$) and post hoc tests between WT and CS-KO mice on experimental days 1 ($F(1,14) = 7.661$, $p = 0.015$) and 2 ($F(1,14) = 6.603$, $p = 0.022$). On the other hand, the daily intake of CD was similar among groups (Figures S2A and S2B). We also found similar results in female mice (Figures 3C, 3D, S2C, and S2D). There were no differences in body weight among WT, D-KO, and CS-KO mice during these experiments (data not shown). These data suggest that endogenous $GABA_BR$ signaling in corticostriatal neurons, but not in dopaminergic neurons, suppresses HFD intake under time-restricted access to HFD.

Baclofen Suppresses HFD Consumption under Time-Restricted Access to HFD via $GABA_BR$ Signaling in Dopaminergic and Corticostriatal Neurons

To evaluate the effects of $GABA_BR$ agonists on the hedonic regulation of HFD intake, we injected male mice in the restricted HFD group with baclofen interperitoneally 30 min before the beginning of dark cycle (ZT 12) on experimental days 1 and 5. Baclofen at a dose of 3 μg/g body weight reduced HFD intake for 10 min, 30 min, and 2 h in male WT mice compared with vehicle on both days (treatment effect; day 1: *F*

(1,12) = 39.602, p < 0.001; day 5: $F(1,12) = 21.484$, p = 0.001), whereas it had no effect on the daily intake of CD (Figures 4A and 4D). In contrast, the effect of baclofen on HFD intake was absent in male D-KO (Figures 4B and 4E) and CS-KO mice (Figures 4C and 4F). Similar results were found in female mice on experimental day 5 (Figures 4G–4I). Baclofen at a dose of 0.3 μg/g body weight also suppressed HFD intake for 2 h compared with vehicle on day 5 in male WT mice but not in D-KO or CS-KO mice (Figures S3B–S3D). Baclofen had no effect on the locomotor activity in WT (Figure S3A), D-KO, and CS-KO mice (data not shown). Thus, baclofen suppresses HFD intake via $GABA_BR$ signaling in the mesolimbic system under time-restricted access to HFD.

There Were No Significant Differences in Energy Balance or the Effects of Baclofen on Feeding Behavior among Genotypes under *ad libitum* Access to HFD or CD

Finally, we examined the role of $GABA_BR$ signaling in the mesolimbic system in the regulation of food consumption and body weight under *ad libitum* access to HFD or CD. There were no significant differences in daily food intake, body weight, feed efficiency (Δ body weight/Δ food intake), or fat pad weight between WT and D-KO mice (Figures 5A–5H) or CS-KO mice on HFD (Figures 5I–5P) or CD (data not shown). There were no significant differences between genotypes in glucose metabolism estimated by fasted serum glucose, intraperitoneal glucose tolerance test, and insulin tolerance test (Figures S4A–S4F). The administration of baclofen (3 μg/g body weight) reduced daily HFD intake and body weight in WT mice, as reported previously (Sato et al., 2007) (Figures 5Q–5T), and it also reduced daily HFD intake and body weight in D-KO (Figures 5Q and 5R) and CS-KO mice (Figures 5S and 5T). These data suggest that the $GABA_BR$ signaling in the mesolimbic system does not affect the feeding behavior under *ad libitum* access to HFD.

DISCUSSION

In the present study, we generated dopaminergic neuron- and corticostriatal neuron-specific $GABA_BR$-deficient mice and demonstrated that HFD intake during the time-restricted access was significantly increased in corticostriatal neuron-specific KO mice compared with WT mice. Furthermore, the suppressing effect of baclofen on HFD intake during the restricted time observed in WT mice was absent in both KO mice. On the other hand, there were no significant differences in daily food consumption or body weight under *ad libitum* access to HFD among genotypes. Thus, our data indicate that the $GABA_BR$ signaling in the mesolimbic system suppresses food consumption under time-restricted access to HFD.

Consistent with previous studies (Berner et al., 2009; Buda-levin et al., 2005; Czyzyk et al., 2010; Johnson and Kenny, 2010; Rao et al., 2008; Valdivia et al., 2015; Wong et al., 2009), WT mice under the time-restricted access to HFD consumed a substantial amount of calories from the HFD, and baclofen suppressed the HFD intake in WT mice. Although the site of action of baclofen has not been investigated so far, we now clearly demonstrate that baclofen suppresses HFD intake via $GABA_BRs$ in dopaminergic and corticostriatal neurons. The effects of baclofen observed in the present study might be mediated via a decreased dopaminergic tone in the mesolimbic system, as it is reported that (1) dopaminergic neurons in the mesolimbic system receive GABAergic inputs from various neurons (Filip et al., 2015) and (2) GABA inhibits mesolimbic dopamine signaling in the VTA and striatum (Ferrario et al., 2016; Pierce and Kumaresan, 2006).

Our data also showed differences between CS-KO and D-KO mice: HFD intake under the restricted time was significantly increased in CS-KO, but not in D-KO, compared with WT mice. These data suggest that the suppressive effects of endogenous $GABA_BR$ signaling on hedonic HFD intake during the restricted time is more dominant in cortocostriatal than dopaminergic neurons. Because the striatal neurons receive neural projections from not only the VTA but also other areas such as hippocampus, amygdala, prefrontal cortex, and hypothalamus(Ferrario et al., 2016), it is possible that the lack of $GABA_BRs$ in the striatal neurons enhances the activity of these neurons. The detailed mechanisms by which the absence of $GABA_BRs$ in the corticostriatal neurons enhances hedonic consumption of HFD needs to be clarified in future experiments.

Our data clearly demonstrate that there are no significant differences in daily food consumption or body weight between WT, CS-KO, and D-KO mice under *ad libitum* access to HFD and that baclofen suppressed HFD intake in all genotypes. We previously reported that baclofen decreased orexigenic neuropeptide Y mRNA expression while increasing anorexic proopiomelanocortin mRNA expression in the hypothalamic arcuate nuclei, leading to reduced food intake and body weight in WT mice fed HFD (Sato et al., 2007). Furthermore, proopiomelanocortin neuron-specific $GABA_BR$ KO mice showed increased body weight under *ad libitum* access to HFD (Ito et al., 2013). Taken together with the data presented herein, it is

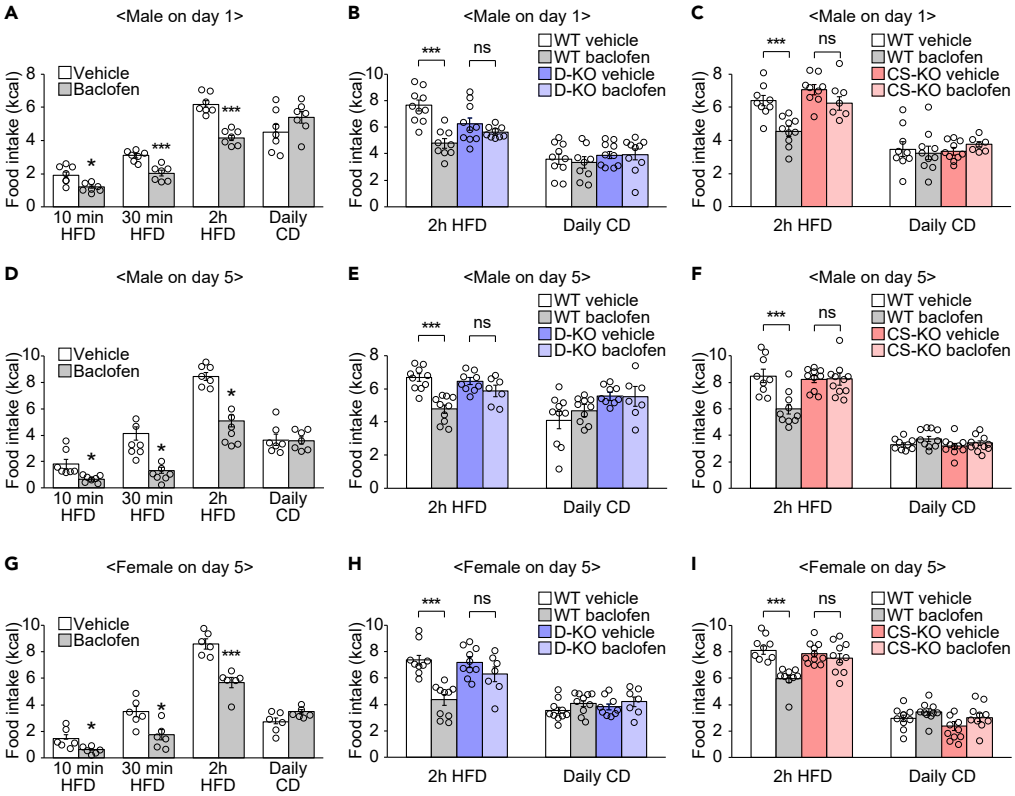

Figure 4. Baclofen Suppresses HFD Consumption under Time-Restricted Access to HFD via $GABA_BR$ Signaling in Dopaminergic and Corticostriatal Neurons

(A, D, and G) Intake of HFD during ZT12-14 and daily intake of CD in male (A and D) and female (G) $GABA_{B1}R^{+/+}$ (WT) mice in R-HFD under treatment of baclofen on experimental days 1 (A) and 5 (D and G). Baclofen at a dose of 3 μg/g body weight reduced HFD intake for 10 min, 30 min, and 2 h in male WT mice compared with vehicle on days 1 (treatment: $F(1,12) = 39.602$, $p < 0.001$; time: $F(2,24) = 626.053$, $p < 0.001$; treatment × time interaction: $F(2,24) = 20.949$, $p < 0.001$, n = 7 per group) and 5 (treatment: $F(1,12) = 21.484$, $p = 0.001$; time: $F(2,24) = 165.583$, $p < 0.001$; treatment × time interaction: $F(2,24) = 4.286$, $p = 0.026$, n = 7 per group) as well as in female WT mice on day 5 (treatment: $F(1,10) = 46.609$, $p < 0.001$; time: $F(2,20) = 167.084$, $p < 0.001$; treatment × time interaction: $F(2,20) = 4.465$, $p = 0.025$, n = 6 per group).

(B, E, and H) Intake of HFD during ZT12–14 and daily intake of CD in male (B and E) and female (H) $GABA_{B1}R^{lox511/lox511}$ *DAT-Cre* (D-KO) and WT mice in R-HFD under treatment of baclofen on experimental days 1 (B) and 5 (E and H). The inhibitory effect of baclofen on HFD intake was absent in male D-KO on days 1 (treatment: $F(1,35) = 22.669$, $p < 0.001$; genotype: $F(1,35) = 0.568$, not significant; treatment × genotype interaction: $F(1,35) = 9.658$, $p = 0.004$, n = 9–10 per group) and 5 (treatment: $F(1,32) = 23.803$, $p < 0.001$; genotype: $F(1,32) = 2.969$, not significant; treatment × genotype interaction: $F(1,32) = 6.546$, $p = 0.015$, n = 7–10 per group) as well as in female D-KO mice on day 5 (treatment: $F(1,32) = 20.867$, $p < 0.001$; genotype: $F(1,32) = 4.326$, $p = 0.025$; treatment × genotype interaction: $F(1,32) = 6.026$, $p = 0.02$, n = 7–10 per group).

(C, F, and I) Intake of HFD during ZT12–14 and daily intake of CD in male (C and F) and female (I) $GABA_{B1}R^{lox511/lox511}$ *GPR88-Cre* (CS-KO) and WT mice in R-HFD under treatment of baclofen on experimental days 1 (C) and 5 (F and I). The inhibitory effect of baclofen on HFD intake was absent in male CS-KO on days 1 (treatment: $F(1,31) = 16.937$, $p < 0.001$; genotype: $F(1,31) = 12.671$, $p < 0.001$; treatment × genotype interaction: $F(1,31) = 4.503$, $p = 0.021$, n = 7–10 per group) and 5 (treatment: $F(1,36) = 9.661$, $p = 0.004$; genotype: $F(1,36) = 6.320$, $p = 0.017$; treatment × genotype interaction: $F(1,36) = 9.770$, $p = 0.003$, n = 9–11 per group) as well as in female CS-KO on day 5 (treatment: $F(1,38) = 18.808$, $p < 0.001$; genotype: $F(1,38) = 5.077$, $p = 0.03$; treatment × genotype interaction: $F(1,38) = 10.005$, $p = 0.003$, n = 9–11 per group).

All values are mean ± SEM. Statistical analysis were performed using two-way ANOVA assessed by repeated measures (A, D, and G) or two-way factorial ANOVA (B, C, E, F, H, and I) followed by Bonferroni post hoc test. *$p < 0.05$, ***$p < 0.001$ versus vehicle in WT. Ns, not significant. See also Table S3 for the details of statistics.

suggested that $GABA_BR$ signaling in the mesolimbic system regulates hedonic food consumption, whereas that in hypothalamic neurons plays an important role in homeostatic regulation of energy balance. These results contrast with previous studies showing that food intake and body weight are increased in mice with

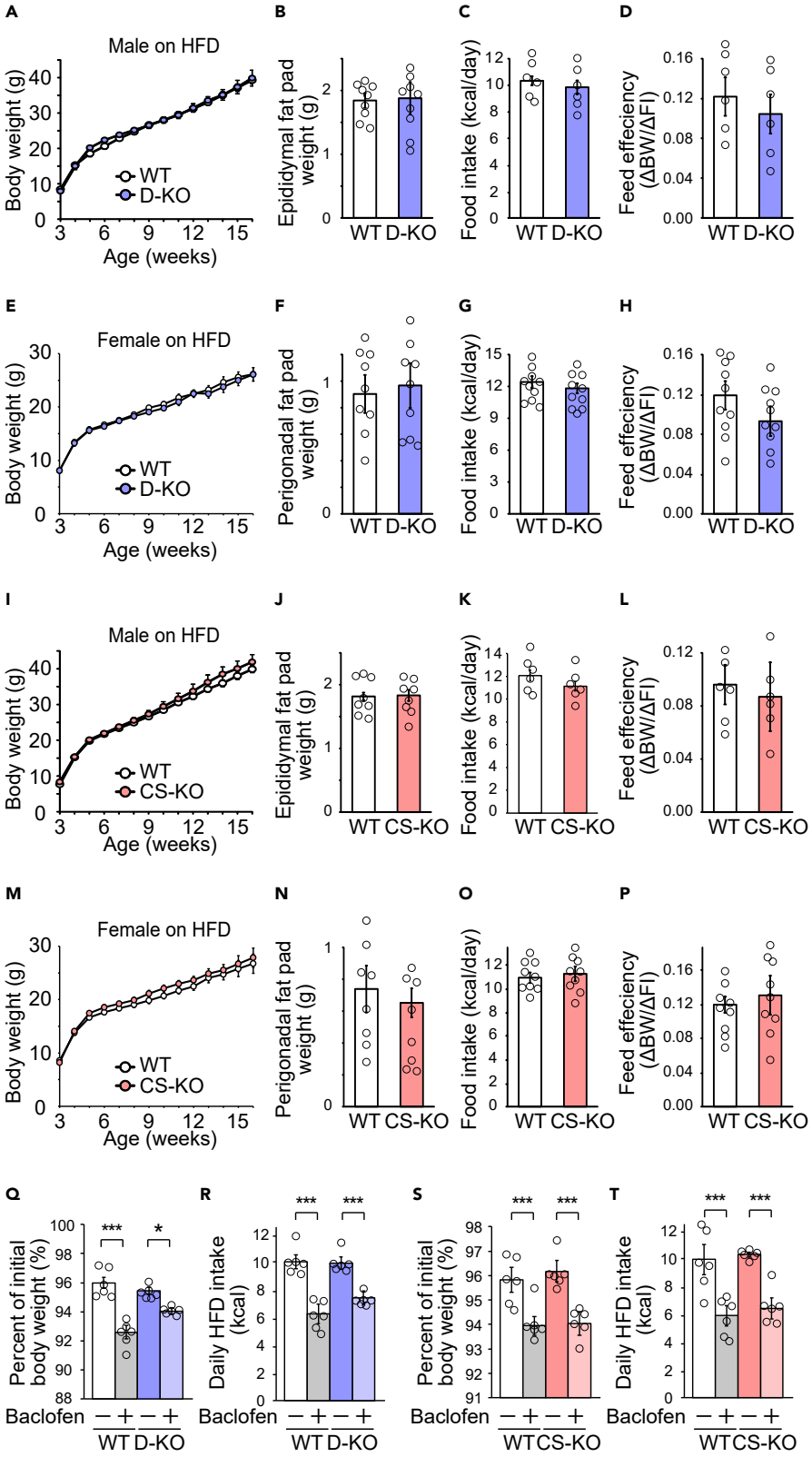
A
Male on HFD
Body weight (g)
Age (weeks)
WT
D-KO
B
Epididymal fat pad weight (g)
WT D-KO
C
Food intake (kcal/day)
WT D-KO
D
Feed effeciency (ΔBW/ΔFI)
WT D-KO
E
Female on HFD
Body weight (g)
Age (weeks)
WT
D-KO
F
Perigonadal fat pad weight (g)
WT D-KO
G
Food intake (kcal/day)
WT D-KO
H
Feed effeciency (ΔBW/ΔFI)
WT D-KO
I
Male on HFD
Body weight (g)
Age (weeks)
WT
CS-KO
J
Epididymal fat pad weight (g)
WT CS-KO
K
Food intake (kcal/day)
WT CS-KO
L
Feed effeciency (ΔBW/ΔFI)
WT CS-KO
M
Female on HFD
Body weight (g)
Age (weeks)
WT
CS-KO
N
Perigonadal fat pad weight (g)
WT CS-KO
O
Food intake (kcal/day)
WT CS-KO
P
Feed effeciency (ΔBW/ΔFI)
WT CS-KO
Q
Percent of initial body weight (%)
***
*
Baclofen
WT D-KO
R
Daily HFD intake (kcal)
***
***
Baclofen
WT D-KO
S
Percent of initial body weight (%)
***
***
Baclofen
WT CS-KO
T
Daily HFD intake (kcal)
***
***
Baclofen
WT CS-KO

Figure 5. There Were No Significant Differences in Energy Balance or the Effects of Baclofen on Feeding Behavior among Genotypes under *ad libitum* Access to HFD

(A, E, I, and M) Body weight of male (A and I) and female (E and M) $GABA_{B1}R^{lox511/lox511}$ *DAT-Cre* (D-KO) (A and E), $GABA_{B1}R^{lox511/lox511}$ *GPR88-Cre* (CS-KO) (I and M) and $GABA_{B1}R^{+/+}$ (WT) (A, E, I, and M) mice under *ad libitum* access to HFD (n = 8–10 per group).

(B, F, J, and N) Epididymal (B and J) and perigonadal (F and N) fat pad weight of male D-KO (B and F), CS-KO (J and N), and WT (B, F, J, and N) mice at the age of 16 weeks (n = 8–9 per group).

(C, G, K, and O) Daily food intake of male (C and K) and female (G and O) D-KO (C and G), CS-KO (K and O), and WT (C, G, K, and O) mice at the age of 8 weeks (n = 6–10 per group).

(D, H, L, and P) Feed efficiency of male (D and L) and female (H and P) D-KO (D and H), CS-KO (L and P) and WT (D, H, L, and P) mice at the age of 8 weeks (n = 6–10 per group).

(Q–T) Body weight changes (Q and S) and daily food intake (R and T) of male D-KO (Q and R), CS-KO (S and T), and WT (Q–T) mice treated with baclofen (3 μg/g body weight every 6 h) or vehicle for 2 days under *ad libitum* access to HFD (Q: treatment: $F(1,20) = 15.961$, $p < 0.001$; genotype: $F(1,20) = 3.289$, not significant; treatment × genotype interaction: $F(1,20) = 2.932$, not significant; R: treatment: $F(1,20) = 79.515$, $p < 0.001$; genotype: $F(1,20) = 2.411$, not significant; treatment × genotype interaction: $F(1,20) = 3.147$, not significant; S: treatment: $F(1,20) = 40.887$, $p < 0.001$; genotype: $F(1,20) = 0.497$, not significant; treatment × genotype interaction: $F(1,20) = 0.157$, not significant; T: treatment: $F(1,20) = 44.684$, $p < 0.001$; genotype: $F(1,20) = 0.575$, not significant; treatment × genotype interaction: $F(1,20) = 0.026$, not significant, n = 6 per group).

BW, body weight; FI, food intake. All values are mean ± SEM. Statistical analysis were performed using two-way ANOVA assessed by repeated measures (A, E, I, and M), unpaired t test (B–D, F–H, J–L, and N–P) or two-way factorial ANOVA (Q–T) followed by Bonferroni post hoc test. *$p < 0.05$, ***$p < 0.001$ versus vehicle in the same genotype. See also Table S4 for the details of statistics.

a genetic lack of insulin or leptin receptors in the mesolimbic system (Brönneke et al., 2011; Georgescu et al., 2006) and further highlight a role of the $GABA_BR$ signal in the mesolimbic system in binge-like eating of HFD.

As shown in Figure 2F, intake of HFD during 2 h under the time-restricted access increased only in the first 2 days. These results are consistent with previous studies in which the duration of time-restricted HFD was set for 30 days or longer (Bake et al., 2014; Berner et al., 2008; Johnson and Kenny, 2010; King et al., 2016) and suggest that, although time-restricted access to HFD induces binge-like eating behavior, HFD intake reaches a plateau in the first few days. The finding that CD consumption was decreased when the mice were returned to CD from HFD on day 8 (Figure 2D) is also consistent with previous studies (Berner et al., 2008; Corwin et al., 1998; Czyzyk et al., 2010; King et al., 2016). A possible interpretation is that mice subjected to the time-restricted access to HFD learned to wait for HFD.

In conclusion, our data show that the mesolimbic system regulates binge-like eating of HFD and provide a mechanism by which the $GABA_BR$ signaling suppresses palatable food consumption.

Limitations of the Study

GPR88-positive neurons include not only striatal neurons but also mPFC and OFC. Indeed, we showed that $GABA_BRs$ were knocked out in mPFC and OFC in CS-KO mice. As these areas have a crucial role in decision making (Rangel et al., 2008) and have been implicated in reward-guided behavior (Miller and Cohen, 2001), we cannot exclude the possibility that phenotypes observed in CS-KO mice were due to deficiency of $GABA_BRs$ in mPFC or OFC. Furthermore, both dopamine D1 and D2 receptors are expressed in GPR88-positive neurons (Massart et al., 2009); it remains to be established which receptor is critical for $GABA_BR$ signaling to suppress binge-eating behavior.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.09.032.

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AUTHOR CONTRIBUTIONS

T.T., R.B., B.B., and H.A. designed the study and wrote the manuscript. T.T., H.Y., K.T., A.M., M.S., T.O., H.T., D.H., Y.I., S.I., M.G., and H.S. performed experiments.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

$GABA_B$ Receptor Signaling in the Mesolimbic System Suppresses Binge-like Consumption of a High-Fat Diet

Taku Tsunekawa, Ryoichi Banno, Hiroshi Yaginuma, Keigo Taki, Akira Mizoguchi, Mariko Sugiyama, Takeshi Onoue, Hiroshi Takagi, Daisuke Hagiwara, Yoshihiro Ito, Shintaro Iwama, Motomitsu Goto, Hidetaka Suga, Bernhard Bettler, and Hiroshi Arima

Supplemental Figures

Figure S1

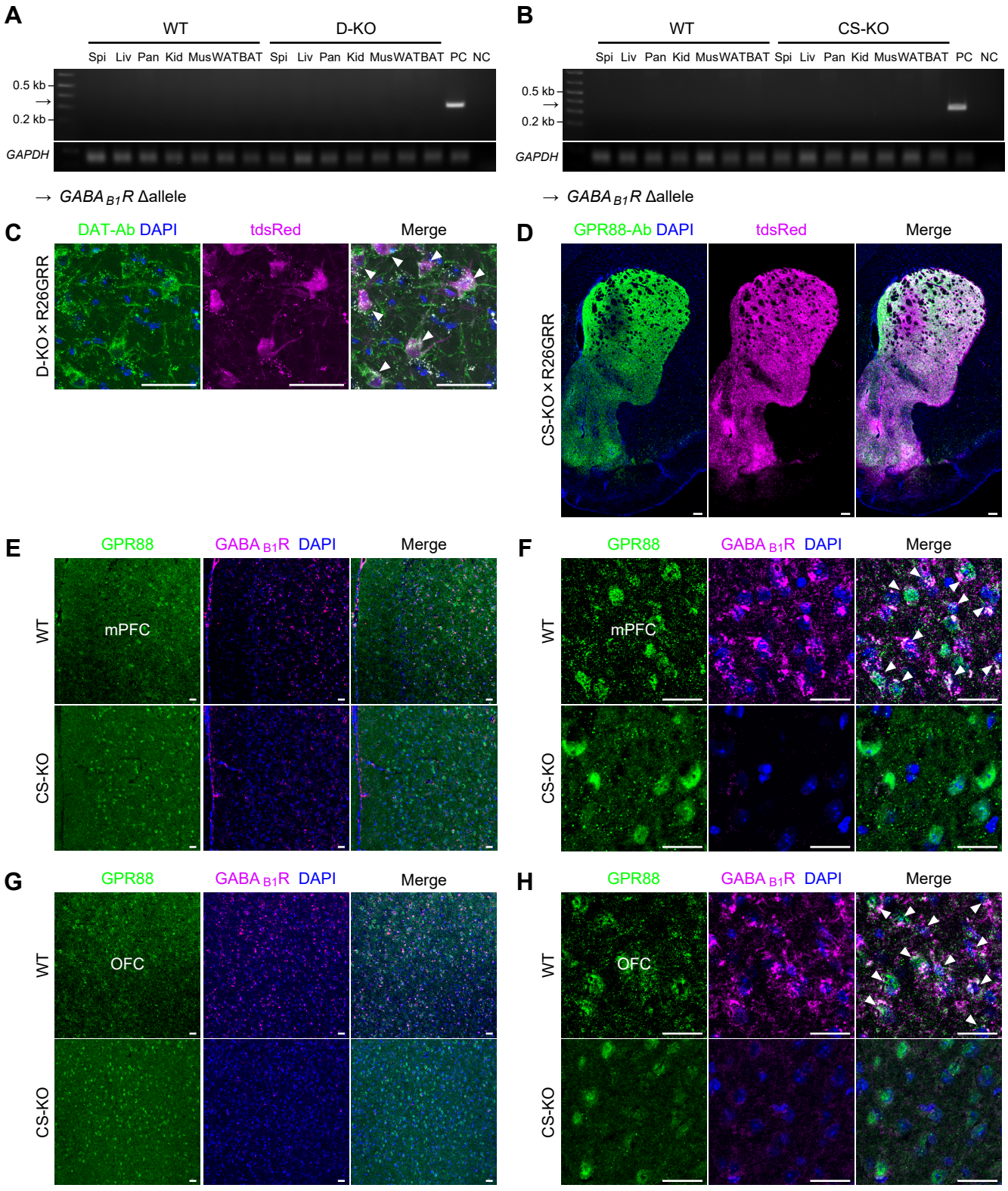

Figure S1. Generation of dopaminergic neuron-specific and corticostriatal neuron-specific $GABA_BR$ deficient mice, related to Figure 1

(A and B) Detection of deletion of *$GABA_{B1}R$* alleles (Δ) in *$GABA_{B1}R^{lox511/lox511}$ DAT-Cre* (D-KO) and *$GABA_{B1}R^{lox511/lox511}$ GPR88-Cre* (CS-KO) mice. DNA was extracted from different tissues, and deletion of the floxed allele was detected by PCR. Spi, spine; Liv, liver; Pan, pancreas; Kid, kidney; Mus, muscle; WAT, white adipose tissue; BAT, brown adipose tissue; PC, positive control; NC, negative control. PCR reaction with *GAPDH* was used as an internal control. (C)The representative photographs showing the staining of DAT (green), tdsRed (magenta) and DAPI (blue) in the VTA of *$GABA_{B1}R^{lox511/lox511}$ DAT-Cre R26GRR* mice. White arrow heads show colocalization of DAT and tdsRed. Scale bar: 50 μm. (D) The representative photographs showing the staining of GPR88 (green), tdsRed (magenta) and DAPI (blue) in the NAc and CPu of *$GABA_{B1}R^{lox511/lox511}$ GPR88-Cre R26GRR* mice. Scale bar: 100 μm. (E-H) The representative photographs showing the staining of GPR88 (green), $GABA_{B1}R$ (magenta) and DAPI (blue) in mPFC (E and F) and OFC (G and H) in WT and CS-KO mice. White arrow heads show colocalization of GPR88 and $GABA_{B1}R$. Scale bar: 20 μm. All data are from male mice.

Figure S2

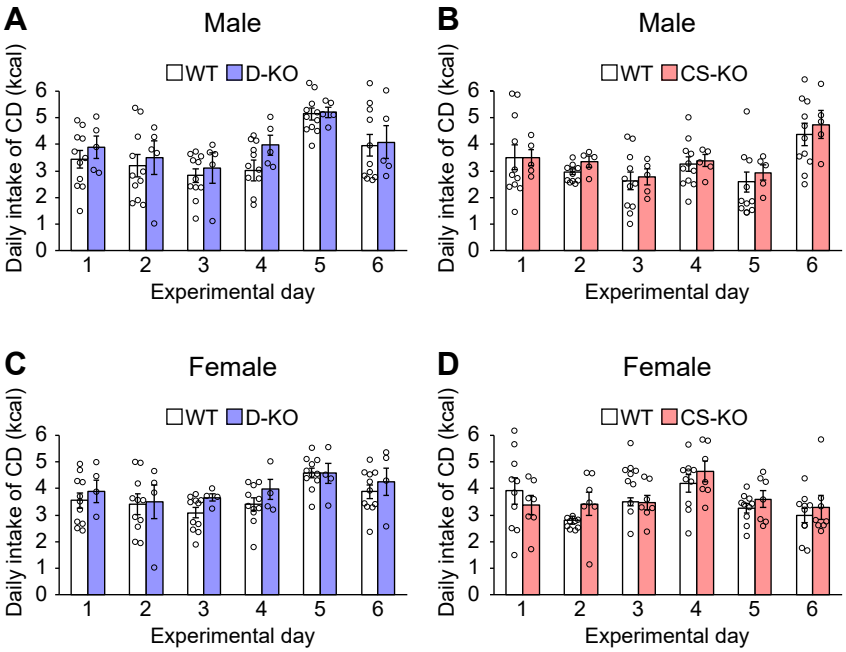

Figure S2. GABA$_B$ receptor signaling in corticostriatal neurons suppresses HFD consumption under time-restricted access to HFD, related to Figure 3

Daily CD intake in male (A, B) and female (C, D) D-KO (A, C), CS-KO (B, D) and WT (A, B, C, D) mice in R-HFD (male: WT, n=11; D-KO, n=5; CS-KO, n=5 ; female: WT, n=10-11;D-KO, n=4; CS-KO, n=7). All values are mean ± SEM. Statistical analysis were performed using two-way ANOVAassessed by repeated measures. See also Table S5 for the details of statistics

Figure S3

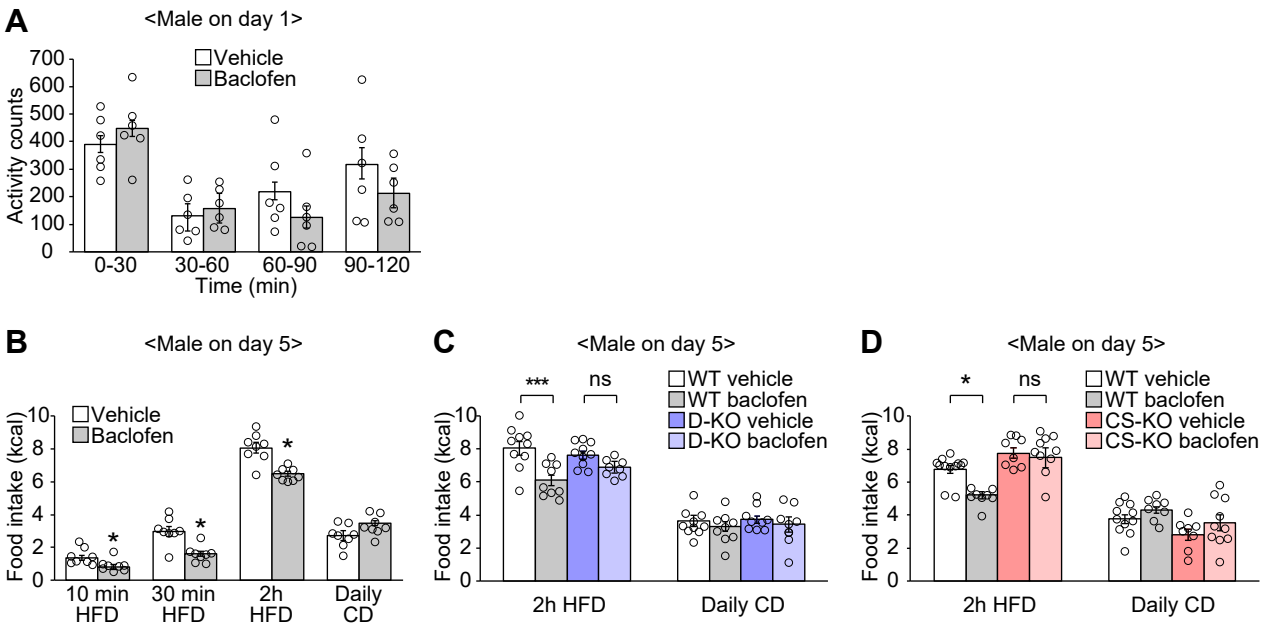

Figure S3. Baclofen suppresses HFD consumption under time-restricted access to HFD via GABA$_B$R signaling in dopaminergic and corticostriatal neurons, related to Figure 4

(A) Locomotor activity during ZT12-14 in male WT mice in R-HFD under treatment of 3 μg/g body weight baclofen on experimental day 1 (n=6 per group). (B) HFD intake during ZT12-14 and daily CD intake in male WT mice in R-HFD under treatment of 0.3 μg/g body weight baclofen on experimental day 5. Baclofen reduced HFD intake for 10 min, 30 min and 2 hours in male WT mice compared to vehicle (treatment: F (1,14) = 21.153, $P < 0.001$; time: F (2,28) = 881.763, $P < 0.001$; treatment × time interaction: F (2,28) = 6.043, $P = 0.007$, n=8 per group). (C, D) Intake of HFD during ZT12-14 and daily intake of CD in male D-KO (C), CS-KO (D) and WT (C, D) in R-HFD under treatment of 0.3 μg/g body weight baclofen on experimental day 5. The inhibitory effect of baclofen on HFD intake was absent in D-KO (treatment: F (1,33) = 18.611, $P <$ 0.001; genotype: F (1,33) = 0.330, not significant; treatment × genotype interaction: F (1,33) = 3.826, $P = 0.049$, n=8-10 per group) and CS-KO (treatment: F (1,33) = 5.655, $P < 0.001$; genotype: F (1,33) = 17.915, $P < 0.001$; treatment × genotype interaction: F (1,33) = 3.772, $P = 0.023$, n=8-11 per group). All values are mean ± SEM. Ns: not significant. Statistical analysis were performed using two-way ANOVAassessed by repeated measures (A, B) or two-way factorial ANOVA (C, D) followed by Bonferroni post-hoc test. * $P < 0.05$, *** $P < 0.001$ versus vehicle in WT. See also the Table S6 for the details of statistics

Figure S4

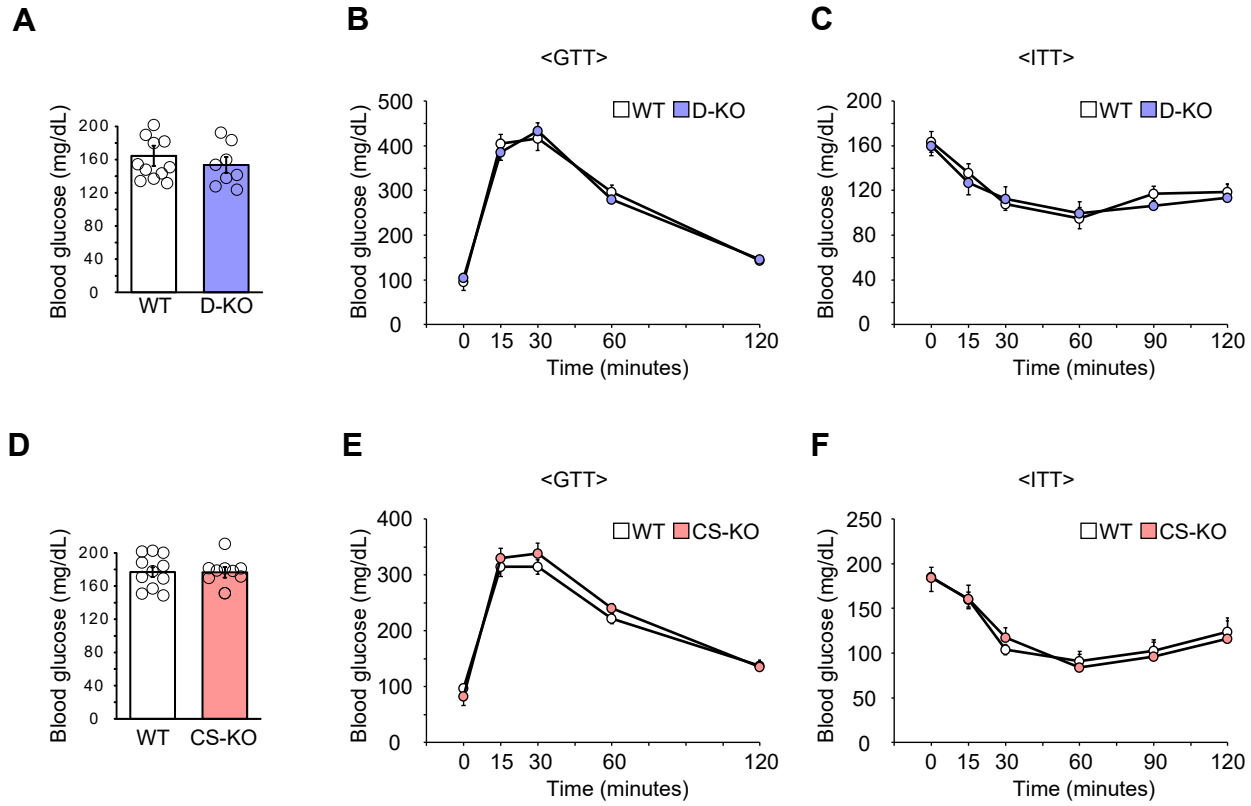

Figure S4. There were no significant differences in energy balance or the effects of baclofen on feeding behavior among genotypes under *ad libitum* access to HFD, related to Figure 5

(A, D) Blood glucose of D-KO (A), CS-KO (D) and WT (A, D) under *ad libitum* access to HFD (n=8-11 per group). (B, E) Glucose tolerance test (GTT) in D-KO (B), CS-KO (E) and WT (B, E) mice under *ad libitum* access to HFD (n=8-10 per group). (C, F) Insulin tolerance test (ITT) in D-KO (C), CS-KO (F) and WT (C, F) under *ad libitum* access to HFD (n=8-11 per group). All values are mean ± SEM. Statistical analyses were performed using two-way ANOVA with repeated measures (B, C, E, F) or unpaired *t*-test (A, D). See also Table S7 for the details of statistics

Supplemental tables

Table S1. The details of statistics related to Figure 2.

Two-way ANOVA assessed by repeated measures

	Time			Group			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure 2C	$F(17,255) = 25.412$	$P < 0.001$	0.66	$F(2,15) = 9.580$	$P = 0.002$	0.51	$F(34,255) = 9.311$	$P < 0.001$	0.66
Figure 2D	$F(17,255) = 36.294$	$P < 0.001$	0.77	$F(2,15) = 8.479$	$P = 0.003$	0.51	$F(34,255) = 5.309$	$P < 0.001$	0.50
Figure 2E	$F(2,32) = 175.227$	$P < 0.001$	0.92	$F(2,16) = 80.096$	$P < 0.001$	0.91	$F(4,32) = 26.085$	$P < 0.001$	0.77

Bonferroni post-hoc test

	Control versus Ad-HFD				Control versus R-HFD				Ad-HFD versus R-HFD			
	F value	*P* value	*r*	95% CI	*F* value	*P* value	*r*	95% CI	*F* value	*P* value	*r*	95% CI
Figure 2C												
Conditioning day 8	$F(2,15) = 7.923$	$P = 0.010$	0.68	-2.897 - -0.370	$F(2,15) = 7.923$	*Ns*	0.07	-1.297 - 1.230	$F(2,15) = 7.923$	$P = 0.012$	0.67	0.337 - 2.863
Conditioning day 9	$F(2,15) = 13.102$	$P = 0.001$	0.80	-4.258 - -1.208	$F(2,15) = 13.102$	*Ns*	0.52	-2.058 - 0.992	$F(2,15) = 13.102$	$P = 0.004$	0.71	0.675 - 3.725
Conditioning day 10	$F(2,15) = 13.662$	$P = 0.001$	0.81	-4.088 - -1.212	$F(2,15) = 13.662$	*Ns*	0.60	-2.005 - 0.872	$F(2,15) = 13.662$	$P = 0.004$	0.67	0.645 - 3.522
Conditioning day 11	$F(2,15) = 9.986$	$P = 0.002$	0.75	-3.922 - -0.845	$F(2,15) = 9.986$	*Ns*	0.46	-1.939 - 1.139	$F(2,15) = 9.986$	$P = 0.010$	0.75	0.445 - 3.512
Conditioning day 12	$F(2,15) = 9.860$	$P = 0.009$	0.73	-4.040 - -0.560	$F(2,15) = 9.860$	*Ns*	0.21	-1.406 - 2.073	$F(2,15) = 9.860$	$P = 0.003$	0.80	0.894 - 4.373
Experimental day 1	$F(2,15) = 13.576$	$P = 0.007$	0.73	-3.906 - -0.594	$F(2,15) = 13.576$	*Ns*	0.56	-0.806 - 2.506	$F(2,15) = 13.576$	$P < 0.001$	0.80	1.444 - 4.756
Experimental day 2	$F(2,15) = 13.027$	$P = 0.010$	0.74	-3.759 - -0.475	$F(2,15) = 13.027$	*Ns*	0.52	-0.725 - 2.559	$F(2,15) = 13.027$	$P < 0.001$	0.83	1.391 - 4.675
Experimental day 3	$F(2,15) = 16.199$	$P = 0.002$	0.80	-4.283 - -0.950	$F(2,15) = 16.199$	*Ns*	0.41	-0.933 - 2.400	$F(2,15) = 16.199$	$P < 0.001$	0.81	1.683 - 5.017
Experimental day 4	$F(2,15) = 14.104$	$P = 0.003$	0.80	-4.267 - -0.867	$F(2,15) = 14.104$	*Ns*	0.33	-1.117 - 2.283	$F(2,15) = 14.104$	$P < 0.001$	0.72	1.450 - 4.850
Experimental day 5	$F(2,15) = 8.070$	$P = 0.027$	0.70	-3.665 - -0.201	$F(2,15) = 8.070$	*Ns*	0.29	-1.215 - 2.249	$F(2,15) = 8.070$	$P = 0.005$	0.79	0.718 - 4.182
Experimental day 6	$F(2,15) = 12.896$	$P = 0.003$	0.78	-4.372 - -0.928	$F(2,15) = 12.896$	*Ns*	0.19	-1.422 - 2.022	$F(2,15) = 12.896$	$P = 0.001$	0.76	1.228 - 4.672
Figure 2D												
Conditioning day 8	$F(2,15) = 57.372$	$P < 0.001$	0.92	-13.567 - -6.172	$F(2,15) = 57.372$	*Ns*	0.09	-3.744 - 3.941	$F(2,15) = 57.372$	$P < 0.001$	0.93	6.500 - 13.437
Conditioning day 9	$F(2,15) = 6.095$	$P = 0.024$	0.63	-4.457 - 0.105	$F(2,15) = 6.095$	*Ns*	0.07	-2.539 - 2.201	$F(2,15) = 6.095$	$P = 0.049$	0.61	0.132 - 4.147
Conditioning day 11	$F(2,15) = 17.287$	$P = 0.003$	0.83	-5.840 - -1.240	$F(2,15) = 17.287$	*Ns*	0.25	-2.475 - 2.306	$F(2,15) = 17.287$	$P = 0.002$	0.77	1.298 - 5.613
Conditioning day 12	$F(2,15) = 5.861$	$P = 0.028$	0.59	-3.840 - 0.041	$F(2,15) = 5.861$	*Ns*	0.05	-2.316 - 1.976	$F(2,15) = 5.861$	$P = 0.004$	0.82	6.500 - 13.440
Experimental day 1	$F(2,15) = 3.915$	$P = 0.045$	0.57	-4.134 - 0.296	$F(2,15) = 3.915$	*Ns*	0.01	-1.168 - 4.474	$F(2,15) = 3.915$	$P = 0.017$	0.65	0.526 - 5.619
Figure 2E												
10 min	$F(2,16) = 36.581$	*Ns*	0.01	-0.457 - 0.451	$F(2,16) = 36.581$	$P < 0.001$	0.9	-1.875 - -0.845	$F(2,16) = 36.581$	$P < 0.001$	0.88	-1.811 - -0.902
30 min	$F(2,16) = 31.016$	*Ns*	0.23	-0.785 - 0.989	$F(2,16) = 31.016$	$P < 0.001$	0.87	-3.381 - -1.368	$F(2,16) = 31.016$	$P < 0.001$	0.83	-3.364 - -5.856
2 hours	$F(2,16) = 82.215$	*Ns*	0.19	-1.236 - 0.802	$F(2,16) = 82.215$	$P < 0.001$	0.97	-5.856 - -3.545	$F(2,16) = 82.215$	$P < 0.001$	0.95	-5.502 - -3.465

One-way ANOVA assessed by repeated measures

	Time		
	F value	*P* value	η^2
Figure2F R-HFD	$F(5,50) = 9.580$	$P < 0.001$	0.49

Bonferroni post-hoc test

	F value	*P* value	*r*	95% CI
Figure 2F				
Day1 versus day2	$F(5,50) = 9.580$	*Ns*	0.52	-0.188 - 0.22
Day1 versus day3	$F(5,50) = 9.580$	$P = 0.022$	0.69	-0.248 - -0.016
Day1 versus day4	$F(5,50) = 9.580$	$P = 0.034$	0.72	-0.271 - -0.008
Day1 versus day5	$F(5,50) = 9.580$	$P < 0.0001$	0.78	-0.239 - -0.107
Day1 versus day6	$F(5,50) = 9.580$	$P = 0.005$	0.64	-0.209 - -0.035

CI = confidence interval, ns = not significant.

Table S2. The details of statistics related to Figure 3.

Two-way ANOVA assessed by repeated measures

	Time			Genotype			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure 3A	$F(5,70) = 14.505$	$P < 0.001$	0.51	$F(1,14) = 1.034$	*Ns*	0.07	$F(5,70) = 0.761$	*Ns*	0.05
Figure 3B	$F(5,70) = 31.215$	$P < 0.001$	0.69	$F(1,14) = 7.475$	$P = 0.003$	0.35	$F(5,70) = 1.074$	*Ns*	0.07
Figure 3C	$F(5,70) = 11.835$	$P < 0.001$	0.46	$F(1,14) = 2.233$	*Ns*	0.14	$F(5,70) = 1.080$	*Ns*	0.07
Figure 3D	$F(5,75) = 20.126$	$P < 0.001$	0.57	$F(1,15) = 5.025$	$P = 0.041$	0.25	$F(5,75) = 0.811$	*Ns*	0.05

Bonferroni post-hoc test

	WT versus CS-KO			
	F value	*P* value	*r*	95% CI
Figure 3B				
Experimental day1	$F(1,14) = 7.661$	$P = 0.015$	0.60	-1.567 - -0.199
Experimental day2	$F(1,14) = 6.603$	$P = 0.022$	0.57	-1.439 - -0.130
Figure 3D				
Experimental day1	$F(1,15) = 4.923$	$P = 0.042$	0.50	-0.873 - -0.018
Experimental day2	$F(1,15) = 5.935$	$P = 0.028$	0.53	-1.725 - -0.115

CI = confidence interval, ns = not significant.

Table S3. The details of statistics related to Figure 4.

Two-way ANOVA assessed by repeated measures

	Treatment			Time			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure 4A	$F(1,12) = 39.602$	$P < 0.001$	0.77	$F(2,24) = 626.053$	$P < 0.001$	0.98	$F(2,24) = 20.949$	$P < 0.001$	0.64
Figure 4D	$F(1,12) = 21.484$	$P = 0.001$	0.64	$F(2,24) = 165.583$	$P < 0.001$	0.93	$F(2,24) = 4.286$	$P = 0.026$	0.26
Figure 4G	$F(1,10) = 46.609$	$P < 0.001$	0.82	$F(2,20) = 167.084$	$P < 0.001$	0.94	$F(2,20) = 4.465$	$P = 0.025$	0.31

Bonferroni post-hoc test

	WT-vehicle versus WT-baclofen			
	F value	*P* value	*r*	95% CI
Figure 4A 10 min	$F(1,12) = 10.736$	$P = 0.007$	0.69	0.232 - 1.152
30 min	$F(1,12) = 25.068$	$P < 0.001$	0.82	0.601 - 1.527
2 hours	$F(1,12) = 54.790$	$P < 0.001$	0.91	1.419 - 2.603
Figure 4D 10 min	$F(1,12) = 9.671$	$P = 0.009$	0.67	0.342 - 1.945
30 min	$F(1,12) = 15.519$	$P = 0.002$	0.75	0.951 - 3.304
2 hours	$F(1,12) = 20.445$	$P = 0.001$	0.79	1.260 - 3.604
Figure 4G 10 min	$F(1,10) = 8.053$	$P = 0.018$	0.67	0.176 - 1.464
30 min	$F(1,10) = 9.732$	$P = 0.011$	0.70	0.490 - 2.940
2 hours	$F(1,10) = 24.883$	$P < 0.001$	0.86	1.656 - 4.104

Two-way factorial ANOVA

	Treatment			Genotype			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure 4B HFD	$F(1,35) = 22.669$	$P < 0.001$	0.34	$F(1,35) = 0.568$	*Ns*	0.01	$F(1,35) = 9.658$	$P = 0.004$	0.14
CD	$F(1,35) = 0.070$	*Ns*	0	$F(1,35) = 1.546$	*Ns*	0.04	$F(1,35) = 0.145$	*Ns*	0
Figure 4C HFD	$F(1,31) = 16.937$	$P < 0.001$	0.25	$F(1,31) = 12.671$	$P < 0.001$	0.19	$F(1,31) = 4.503$	$P = 0.021$	0.04
CD	$F(1,31) = 0.058$	*Ns*	0	$F(1,31) = 0.303$	*Ns*	0.01	$F(1,31) = 0.848$	*Ns*	0.03
Figure 4E HFD	$F(1,32) = 23.803$	$P < 0.001$	0.34	$F(1,32) = 2.969$	*Ns*	0.04	$F(1,32) = 6.546$	$P = 0.015$	0.10
CD	$F(1,32) = 0.410$	*Ns*	0.01	$F(1,32) = 6.779$	$P = 0.014$	0.17	$F(1,32) = 0.441$	*Ns*	0.01
Figure 4F HFD	$F(1,36) = 9.661$	$P = 0.004$	0.16	$F(1,36) = 6.320$	$P = 0.017$	0.10	$F(1,36) = 9.770$	$P = 0.003$	0.16
CD	$F(1,36) = 0.084$	*Ns*	0.08	$F(1,36) = 0.319$	*Ns*	0.03	$F(1,36) = 0.311$	*Ns*	0.01
Figure 4H HFD	$F(1,32) = 20.867$	$P < 0.001$	0.31	$F(1,32) = 4.326$	$P = 0.025$	0.07	$F(1,32) = 6.026$	$P = 0.02$	0.09
CD	$F(1,32) = 2.832$	*Ns*	0.08	$F(1,32) = 0.637$	*Ns*	0.02	$F(1,32) = 0.052$	*Ns*	0
Figure 4I HFD	$F(1,38) = 18.808$	$P < 0.001$	0.27	$F(1,38) = 5.077$	$P = 0.03$	0.07	$F(1,38) = 10.005$	$P = 0.003$	0.14
CD	$F(1,35) = 4.047$	*Ns*	0.10	$F(1,35) = 3.002$	*Ns*	0.07	$F(1,35) = 0.105$	*Ns*	0

Bonferroni post-hoc test

	WT-vehicle versus WT-baclofen				KO-vehicle versus KO-baclofen			
	F value	*P* value	*r*	95% CI	*F* value	*P* value	*r*	95% CI
Figure 4B HFD	$F(1,35) = 30.145$	$P < 0.001$	0.79	1.799 - 3.911	$F(1,35) = 1.405$	*Ns*	0.29	-0.428 - 1.628
Figure 4C HFD	$F(1,31) = 17.878$	$P < 0.001$	0.72	0.969 - 2.776	$F(1,31) = 2.730$	*Ns*	0.41	-0.158 - 1.823
Figure 4E HFD	$F(1,32) = 31.389$	$P < 0.001$	0.80	1.214 - 2.600	$F(1,32) = 2.406$	*Ns*	0.38	0.186 - 1.376
Figure 4F HFD	$F(1,36) = 18.501$	$P < 0.001$	0.71	1.324 - 3.687	$F(1,36) < 0.001$	*Ns*	0.03	-1.117 - 1.131
Figure 4H HFD	$F(1,32) = 27.988$	$P < 0.001$	0.79	1.852 - 4.171	$F(1,32) = 1.996$	*Ns*	0.35	-0.400 - 2.213
Figure 4I HFD	$F(1,38) = 28.125$	$P < 0.001$	0.78	1.339 - 2.992	$F(1,38) = 0.689$	*Ns*	0.19	-0.488 - 1.166
	WT-vehicle versus KO-vehicle				WT-baclofen versus KO-baclofen			
	F value	*P* value	*r*	95% CI	*F* value	*P* value	*r*	95% CI
Figure 4B HFD	$F(1,35) = 7.661$	$P = 0.009$	0.47	0.373 - 2.429	$F(1,35) = 2.698$	*Ns*	0.51	-1.910 - 0.202
Figure 4C HFD	$F(1,31) = 2.046$	*Ns*	0.36	-1.577 - 0.277	$F(1,31) = 12.656$	$P = 0.001$	0.66	-2.659 - -0.721
Figure 4E HFD	$F(1,32) = 0.375$	*Ns*	0.17	-0.498 - 0.927	$F(1,32) = 8.567$	$P = 0.006$	0.56	-1.862 - -0.334
Figure 4F HFD	$F(1,36) = 0.178$	*Ns*	0.11	-0.936 - 1.427	$F(1,36) = 16.744$	$P < 0.001$	0.66	-3.391 - -1.143
Figure 4H HFD	$F(1,32) = 0.076$	*Ns*	0.07	-1.030 - 1.352	$F(1,32) = 9.610$	$P = 0.004$	0.60	-3.222 - -0.667
Figure 4I HFD	$F(1,38) = 0.435$	*Ns*	0.15	-0.544 - 1.069	$F(1,38) = 14.002$	$P = 0.001$	0.64	-2.410 - -0.718

CI = confidence interval, ns = not significant.

Table S4. The details of statistics related to Figure 5.

Two-way ANOVA assessed by repeated measures

	Time			Genotype			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure 5A	$F(13,208) = 561.677$	$P < 0.001$	0.97	$F(1,16) = 0.379$	*Ns*	0.02	$F(13,208) = 0.864$	*Ns*	0.05
Figure 5E	$F(13,234) = 265.533$	$P < 0.001$	0.94	$F(1,18) = 0.229$	*Ns*	0.01	$F(13,234) = 0.336$	*Ns*	0.02
Figure 5I	$F(13,182) = 376.879$	$P < 0.001$	0.96	$F(1,14) = 0.041$	*Ns*	0	$F(13,182) = 0.180$	*Ns*	0.01
Figure 5M	$F(13,182) = 177.169$	$P < 0.001$	0.93	$F(1,14) = 1.435$	*Ns*	0.09	$F(13,182) = 1.027$	*Ns*	0.07

Two-way factorial ANOVA

	Treatment			Genotype			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure 5Q	$F(1,20) = 15.961$	$P < 0.001$	0.7	$F(1,20) = 3.289$	*Ns*	0.03	$F(1,20) = 2.932$	*Ns*	0.12
Figure 5R	$F(1,20) = 79.515$	$P < 0.001$	0.76	$F(1,20) = 2.411$	*Ns*	0.02	$F(1,20) = 3.147$	*Ns*	0.03
Figure 5S	$F(1,20) = 40.887$	$P < 0.001$	0.67	$F(1,20) = 0.497$	*Ns*	0.01	$F(1,20) = 0.157$	*Ns*	0
Figure 5T	$F(1,20) = 44.684$	$P < 0.001$	0.69	$F(1,20) = 0.575$	*Ns*	0.01	$F(1,20) = 0.026$	*Ns*	0

Bonferroni post-hoc test

	WT-vehicle versus WT-baclofen				KO-vehicle versus KO-baclofen			
	F value	*P* value	*r*	95% CI	*F* value	*P* value	*r*	95% CI
Figure 5Q	$F(1,20) = 92.682$	$P < 0.001$	0.92	1.337 - 2.075	$F(1,20) = 15.892$	$P = 0.001$	0.93	0.337 - 1.075
Figure 5R	$F(1,20) = 57.150$	$P < 0.001$	0.89	2.719 - 4.293	$F(1,20) = 25.512$	$P < 0.001$	0.91	1.473 - 3.546
Figure 5S	$F(1,20) = 17.985$	$P < 0.001$	0.76	0.479 - 1.406	$F(1,20) = 23.059$	$P < 0.001$	0.89	0.604 - 1.531
Figure 5T	$F(1,20) = 23.429$	$P < 0.001$	0.77	2.307 - 5.802	$F(1,20) = 21.281$	$P < 0.001$	0.93	2.117 - 5.612

CI = confidence interval, ns = not significant.

Table S5. The details of statistics related to Figure S2.

Two-way ANOVA assessed by repeated measures

	Time			Genotype			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure S2A	$F(5,70) = 7.013$	$P < 0.001$	0.33	$F(1,14) = 1.433$	*Ns*	0.09	$F(5,70) = 0.323$	*Ns*	0.02
Figure S2B	$F(5,70) = 10.048$	$P < 0.001$	0.42	$F(1,14) = 0.408$	*Ns*	0.03	$F(5,70) = 0.219$	*Ns*	0.02
Figure S2C	$F(5,65) = 2.647$	$P = 0.031$	0.17	$F(1,13) = 2.065$	*Ns*	0.14	$F(5,65) = 0.330$	*Ns*	0.03
Figure S2D	$F(5,75) = 4.272$	$P = 0.002$	0.22	$F(1,15) = 0.838$	*Ns*	0.05	$F(5,75) = 0.811$	*Ns*	0.05

Ns = not significant.

Table S6. The details of statistics related to Figure S3.

Two-way ANOVA assessed by repeated measures

	Treatment			Time			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure S3A	$F(1,10) = 0.455$	*Ns*	0.04	$F(3,30) = 10.408$	$P < 0.001$	0.51	$F(3,30) = 1.161$	*Ns*	0.10
Figure S3B	$F(1,14) = 21.153$	$P < 0.001$	0.60	$F(2,28) = 881.763$	$P < 0.001$	0.98	$F(2,28) = 6.043$	$P = 0.007$	0.30

Bonferroni post-hoc test

	WT-vehicle versus WT-baclofen			
	F value	*P* value	*r*	95% CI
Figure S3B 10 min	$F(1,14) = 6.190$	$P = 0.026$	0.55	0.075 - 1.013
30 min	$F(1,14) = 16.796$	$P = 0.001$	0.74	0.652 - 2.085
2 hours	$F(1,14) = 19.097$	$P = 0.001$	0.76	0.796 - 2.329

Two-way ANOVA assessed by repeated measures

	Treatment			Genotype			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure S3C HFD	$F(1,33) = 18.611$	$P < 0.001$	0.33	$F(1,33) = 0.330$	*Ns*	0.01	$F(1,33) = 3.826$	$P = 0.049$	0.07
CD	$F(1,33) = 0.927$	*Ns*	0.03	$F(1,33) = 0.100$	*Ns*	0	$F(1,33) = 0.003$	*Ns*	0
Figure S3D HFD	$F(1,33) = 5.655$	$P < 0.001$	0.10	$F(1,33) = 17.915$	$P < 0.001$	0.31	$F(1,33) = 3.772$	$P = 0.023$	0.05
CD	$F(1,33) = 3.135$	*Ns*	0.08	$F(1,33) = 5.864$	$P = 0.021$	0.14	$F(1,33) = 0.050$	*Ns*	0

Bonferroni post-hoc test

	WT-vehicle versus WT-baclofen				KO-vehicle versus KO-baclofen			
	F value	*P* value	*r*	95% CI	*F* value	*P* value	*r*	95% CI
Figure S3C HFD	$F(1,33) = 20.304$	$P < 0.001$	0.67	1.068 - 2.825	$F(1,35) = 2.694$	*Ns*	0.50	-0.175 - 1.639
Figure S3D HFD	$F(1,33) = 8.344$	$P = 0.007$	0.75	0.467 - 2.689	$F(1,33) = 0.249$	*Ns*	0.10	-0.856 - 1.413
	WT-vehicle versus KO-vehicle				WT-baclofen versus KO-baclofen			
	F value	*P* value	*r*	95% CI	*F* value	*P* value	*r*	95% CI
Figure S3C HFD	$F(1,33) = 1.041$	*Ns*	0.21	-0.426 - 1.284	$F(1,33) = 2.957$	*Ns*	0.47	-1.715 - 0.144
Figure S3D HFD	$F(1,33) = 3.144$	*Ns*	0.50	-2.080 - 0.143	$F(1,33) = 16.546$	$P < 0.001$	0.64	-3.402 - -1.134

CI = confidence interval, ns = not significant.

Table S7. The details of statistics related to Figure S4.

Two-way ANOVA assessed by repeated measures

	Time			Genotype			Interaction		
	F value	*P* value	η_p^2	*F* value	*P* value	η_p^2	*F value*	*P* value	η_p^2
Figure S4B	$F(4,64) = 187.947$	$P < 0.001$	0.92	$F(1,16) = 0.006$	*Ns*	0	$F(4,64) = 0.554$	*Ns*	0.03
Figure S4C	$F(5,85) = 87.299$	$P < 0.001$	0.84	$F(1,17) = 0.749$	*Ns*	0.04	$F(5,85) = 1.609$	*Ns*	0.09
Figure S4E	$F(4,64) = 179.038$	$P < 0.001$	0.92	$F(1,16) = 0.353$	*Ns*	0.02	$F(4,64) = 0.473$	*Ns*	0.03
Figure S4F	$F(5,80) = 79.812$	$P < 0.001$	0.83	$F(1,16) = 1.461$	*Ns*	0.08	$F(5,80) = 0.369$	*Ns*	0.02

Ns = not significant.

Transparent Methods

Mice

All animal procedures were approved by the Animal Care and Use Committee of Nagoya University Graduate School of Medicine and performed in accordance with the institutional guidelines that conform to the National Institutes of Health animal care guidelines. Mice were maintained on a 12 h light/12 h dark cycle in a temperature-controlled barrier facility, with free access to water and food. Age-matched littermates were used for all experiments.

Mice with DAT-specific and GPR88-sepecific deletion of *$GABA_{B1}$*

$GABA_{B1}R^{lox511/lox511}$ mice were generated previously(Haller et al., 2004). *DAT-Cre* transgene mice (RRID: IMSR_JAX:006660) express functional Cre-recombinase only in dopaminergic neurons(Turiault et al., 2007), and *GPR88-Cre* transgene (RRID: IMSR_RBRC10287) mice express functional Cre-recombinase mainly in medium spiny neurons and a small population of parvalbumin-positive interneurons in the caudate-putamen and nucleus accumbens(Hisatsune et al., 2013). ROSA26 Cre-reporter knock-in C57BL/6N mice (RRID: IMSR_RBRC04874), which exhibit green emission before and red after Cre mediated recombination(Hasegawa et al., 2013). *GPR88-Cre* transgene mice and ROSA26 Cre-reporter knock-in C57BL/6N mice were provided by RIKEN BRC through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan. DNA was extracted from a tail from each experimental mouse at the age of 10 days. DNA was subjected to genotyping analyses by PCR with KOD FX DNA polymerase (Toyobo, Osaka, Japan) and the oligonucleotide primers. The PCR was performed with SimpliAmp™ Thermal Cycler (The Applied Biosystems™, CA, USA). The condition was 5 min at 95℃ followed by 30 cycles at 95℃ for 30 sec, 56℃ for 20 sec and 72℃ for 60 sec with a 7 min final extension. Primer sequences used for genotyping of *$GABA_{B1}R^{lox511/lox511}$*, *DAT-Cre* and *GPR88-Cre* mice were as follows: *$GABA_{B1}R$* forward, 5’-TGGGGTGTGTCCTACATGCAGCGGACGG; reverse, 5’-GCTCTTCACCTTTCAACCCAGCCTCAGGC AGGC; *DAT-Cre* forward, 5’-TGGCTGTTGGTGTAAAGTGG; reverse, 5’-GGACAGGGACATGGTTGACT [to detect wild-type (WT) gene] or 5’- CCAAAAGACGGCAATATGGT (to detect transgene); *GPR88-Cre* forward, 5’- ACC TGATGGACATGTTCAGGGATCG; reverse, 5’-TCCGGTTATTCAACTTGCACCATGC. *R26GRR* mice were genotyped using the following *R26GRR* primers: forward, 5’-AAAGTCGCTCTGAGTTGTTAT; reverse, 5’-CTTGTACAGCTCGTCCATGCCGAG. Primer sequences used for the

occurrence of a spurious germline deletion were as follows: *GABA$_{B1}$R* Δ/Δ forward, 5'-ATCTCTTCCTTGGCT GGGTCTTTGCTTCGCTCG; reverse, 5'-GGGTTATTGAATATGATCGGAATTCCTCGACT; *GAPDH* (for an internal control) forward, 5'-AACGACCCCTTCATTGAC; reverse, 5'-TCCACGACATACTCAGCAC. All *GABA$_{B1}$R$^{lox511/lox511}$* mice, *DAT-Cre* and *GPR88-Cre* mice were backcrossed more than 10 generations onto a C57BL/6J background.

Isolating DNA from tissues for detection of recombination of floxed alleles

Tissues (VTA, substantia nigra, hypothalamus, cerebral cortex, hippocampus, cerebellum, brain stem, mPFC, OFC, NAc, CPu, spine, liver, pancreas, kidney, muscle, white adipose tissue, brown adipose tissue) of male mice at the age of 8 weeks were digested by 50 mM NaOH for 10 minutes at 95°C, and 1 M Tris-HCl (pH 8.0) was added to the digestion. Samples were centrifuged for 10 minutes at 12,000 × *g*, and supernatants were transferred to a fresh tube. Then DNA was subjected to genotyping analyses by PCR as described above.

Body composition and food intake

At weaning (3 weeks old), mice were placed on diets of either CD (CE-2, CLEA Japan, Tokyo, Japan; 24.9% protein, 4.6% fat and 70.5% carbohydrate) or HFD (Test Diet 58Y1, PMI Nutrition International, KS, USA; 18.3% protein, 60.9% fat, and 20.1% carbohydrate). The composition of fats in the HFD was as follows: 39.2% total saturated fatty acids, 40.1% total monounsaturated fatty acids, 13.5% linoleic acid, 1.1% linolenic acid, 0.2% arachidonic acid and 1.1% omega-3 fatty acids. Body weight was monitored until the age of 16 weeks. Measurement of epididymal or perigonadal fat pad weight was performed at the age of 16 weeks in the beginning of the light cycle (between 09:00 and 10:00 a.m.) when mice were in the fed state. Food intake in both CD and HFD was assessed by multifeeders (Shinfactory, Fukuoka, Japan) at the age of 8 weeks. Feed efficiency was calculated as grams of body weight gained per grams of food consumed over a 3-day period.

Assessment of feeding behavior under time-restricted access to HFD

As shown Figure 2B, mice (8 weeks of age) were divided into three groups. Food intake of both CD and HFD were assessed by multifeeders (Shinfactory, Fukuoka, Japan). We conducted interperitoneally injection of $GABA_B$ agonist baclofen (Sigma-Aldrich, MO,

USA; 3 μg/g body weight or 0.3 μg/g body weight) or vehicle (saline) 30 minutes before the beginning of dark cycle (ZT 12).

Locomotor activity under during time-restricted access to HFD

Male mice in R-HFD were acclimated to the test cage for 22h on conditioning day 12. Locomotor activity was measured simultaneously by infrared beam interruption (Model MK-5000RQ/02; Muromachi Kikai, Tokyo, Japan) during ZT12-14 on experimental day 1 and reported as average counts per 30 minutes.

Effect of baclofen on *ad libitum* HFD feeding

Male mice (10-12 weeks of age) housed individually were fed HFD for 6 weeks. Thereafter, weight-matched male mice were injected intraperitoneally with baclofen (3 μg/g body weight) or vehicle (saline) every 6 hours (ZT 0, 6, 12, 18) for 2 days, and mean daily food intake and body weight were measured.

Glucose tolerance test (GTT) and insulin tolerance test (ITT)

GTT and ITT were performed in male mice (10-12 weeks of age) fed HFD for 4 weeks. GTTs were performed on fasted (12 h) mice. Animals were injected intraperitoneally with D-glucose (20% solution; 1 mg/g of body weight), and blood glucose values were determined at 0, 15, 30, 60 and 120 min postinjection. ITTs were performed on fasted (6 h) animals. Blood glucose values were measured immediately before and at 15, 30, 60, 90 and 120 min after intraperitoneal injection of the insulin (Humulin R; Eli Lilly Japan, Kobe, Japan). The insulin dose was 0.6 mU/g body weight. Blood glucose was assayed in tail blood using a glucometer (Medi-safe mini; Terumo, Tokyo, Japan). Measurements were taken after the onset of the light cycle (between ZT 0 and ZT 2).

Brain collection for immunohistochemistry

Male mice (9 weeks of age) were deeply anesthetized and transcardially perfused with a cold fixative containing 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) pH 7.4, between ZT 0 and ZT 1 in the fed state. After fixation, brains were removed and immersed in the same fixative for 2 h at 4°C. The brains were kept in PBS containing 10-20% sucrose at 4°C for cryoprotection. They were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Tokyo, Japan) and stored at -80°C until sectioning. Brains were cut into 20-μm sections on a cryostat at -20°C, thawed and mounted on Superfrost Plus microscope slides (Matsunami, Tokyo, Japan), and stored at -80°C until immunohistochemistry.

Immunohistochemistry

The frozen sections were washed with PBS, 0.3% Triton X-100 in PBS (15min) and 50 mM glycine (15 min) followed by blocking with a mixture of 3% bovine serum albumin (Wako, Osaka, Japan) in PBS for 1 h at room temperature. Next, the sections were incubated with anti-DAT antibody (1:500; Merck Millipore, Darmstadt, Germany, RRID:AB_2190413), anti-GPR88 (1:750; Laboratory for Developmental Neurobiology, RIKEN Brain Science Institute, Japan) or anti-$GABA_{B1}$ receptor (1:500; abcam, Cambridge, UK, RRID:AB_941703) overnight at 4°C. The sections were then treated with Alexa Fluor 488-conjugated anti-rabbit IgG secondary antibody (1:500; Invitrogen, CA, USA, RRID:AB_221544), Alexa Fluor 488-conjugated anti-rat IgG secondary antibody (1:1000; Invitrogen CA, USA, RRID:AB_2722511), Alexa Fluor 594-conjugated anti-mouse IgG secondary antibody (1:500; Invitrogen CA, USA, RRID:AB_2650601) for 1 h at room temperature. After washing in 1×PBS, sections were placed on slides, air dried, and cover slipped with Vectashield (Vector Labs, CA, USA). All fluorescently stained sections were examined with either a confocal laser microscope (TiEA1R; NIKON INSTECH, Tokyo, Japan) or a fluorescence microscope (BZ-9000, Keyence, Japan, RRID:SCR_015486), and viewed using NIS-Elements software (NIKON INSTECH, Tokyo, Japan, RRID:SCR_014329).

Statistical analysis

The statistical significance of the differences between groups was analyzed by either unpaired *t*-test, one-way ANOVA with repeated measures, two-way factorial ANOVA or two-way ANOVA assessed by repeated measures followed by Bonferroni post-hoc test by using SPSS Statistics 25 (IBM, NY, USA, RRID:SCR_002865). Results are expressed as means ± SEM, and differences were considered significant at $P < 0.05$. The detail of statistical analyses is shown in Table S1 to S7.

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