Adaptive Control of Dorsal Raphe by 5-HT4 in the Prefrontal Cortex Prevents Persistent Hypophagia following Stress

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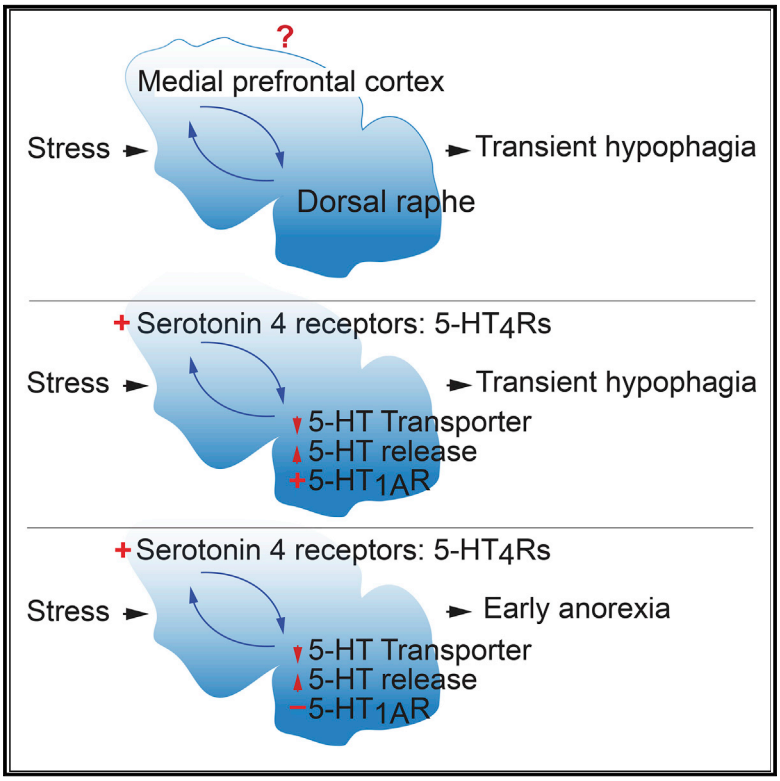
Graphical Abstract



Highlights

- mPFC-5-HT$_4$Rs are causally linked to hypophagia following stress
- mPFC-5-HT$_4$Rs mediate stress-induced changes in DR-5-HT parameters
- mPFC-5-HT$_4$Rs are counterbalanced by DR-5-HT$_{1A}$ to prevent early anorexia
- Hypophagia due to stress does not occur in early stages of development

Authors

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In Brief

Jean et al. report causal relationships between serotonin 4 receptors and stress-induced hypophagia, attributable to specific neural signals of depression resistance in the dorsal raphe nucleus, which protect from early anorexia.

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Adaptive Control of Dorsal Raphe by 5-HT4 in the Prefrontal Cortex Prevents Persistent Hypophagia following Stress

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SUMMARY

Transient reduced food intake (hypophagia) following high stress could have beneficial effects on longevity, but paradoxically, hypophagia can persist and become anorexia-like behavior. The neural underpinnings of stress-induced hypophagia and the mechanisms by which the brain prevents the transition from transient to persistent hypophagia remain undetermined. In this study, we report the involvement of a network governing goal-directed behavior (decision). This network consists of the ascending serotonergic inputs from the dorsal raphe nucleus (DR) to the medial prefrontal cortex (mPFC). Specifically, adult restoration of serotonin 4 receptor (5-HT_4R) expression in the mPFC rescues hypophagia and specific molecular changes related to depression resistance in the DR (5-HT release elevation, 5-HT_{1A} receptor, and 5-HT transporter reductions) of stressed 5-HT_4R knockout mice. The adult mPFC-5-HT_4R knockdown mimics the null phenotypes. When mPFC-5-HT_4Rs are overexpressed and DR-5-HT1ARs are blocked in the DR, hypophagia following stress persists, suggesting an antidepressant action of early anorexia.

INTRODUCTION

In the face of environmental changes, behavioral disturbances often correlate with deregulations of neural circuits. Exploring these correlations in simpler transgenic animal models makes possible the study of molecular and behavioral phenotypes in isolation and has revealed the conservation of specific molecular mechanisms in humans (Bevilacqua et al., 2010; reviewed in Donaldson and Hen, 2015).

Food intake is an evolutionarily conserved behavior across all species and involves numerous biological systems including the phylogenetically old serotonergic system. In mammals, the serotonergic neuronal cell bodies assemble in the raphe nuclei (reviewed in Azmitia, 1999). Among nine nuclei, the dorsal and median raphe nuclei (DR, MR) send axons to the whole forebrain (reviewed in Azmitia, 1999). In particular, the serotonergic axons in the cerebral cortex mainly arise from the DR (Figure S1). Serotonin (5-hydroxytryptamine: 5-HT) binds 18 G protein coupled receptors (5-HTRs), more often located at 100 μm (volume transmission) (Descarries et al., 1975) than at 20 nm (synaptic transmission) from the site of 5-HT release. The preponderant 5-HT volume transmission extends the ubiquitous distribution of the 5-HT system, supporting its multiple functions.

The 5-HT system commonly mediates reduction in food intake (i.e., hypophagia) (reviewed in Compan et al., 2015). Stimulating Gi-coupled 5-HT_{1A} receptors in the DR (DR-5-HT_{1A}R) reduces the firing activity of DR 5-HT neurons (Figure S1), mediating hyperphagia (reviewed in Compan, 2013). Most studies describe the occurrence of hypophagia following stimulation of the 5-HT_{1B} and 5-HT_{2C} receptors in the hypothalamus (5-HT_{1B}R, 5-HT_{2C}R) (reviewed in Compan et al., 2015), whereas 5-HT_{1A}R and 5-HT_{2B}R can exceptionally serve to enhance feeding (Yadav et al., 2009). The serotonergic system can also mediate motivation for food in food-deprived mice, mediating anorexia-like behavior through the activation of addictive signaling (cAMP/PKA/CART: cocaine- and amphetamine-regulated transcript) under the control of 5-HT_4Rs in the nucleus accumbens (NAc), a critical structure in the brain's reward system (Jean et al., 2007, 2012).

Humans who had recovered from one of the symptoms of anorexia nervosa, i.e., from persistent food restriction (called anorexia), show elevated activity of DR-5-HT_{1A}R (Bailer et al., 2007). In contrast, 5-HT depletion and compensatory high levels of NAc-5-HT_4Rs in both rats and humans are seen in obesity (Compan et al., 1996; Haahr et al., 2012; Ratner et al., 2012).

Food intake then depends on the activity of the 5-HT system and both are influenced by external stressors (reviewed in Compan, 2013; Hardaway et al., 2015); however, whether changes in food intake and the activity of the 5-HT system in response to external stress are causally related or correlated remains undetermined. One of the animal models most

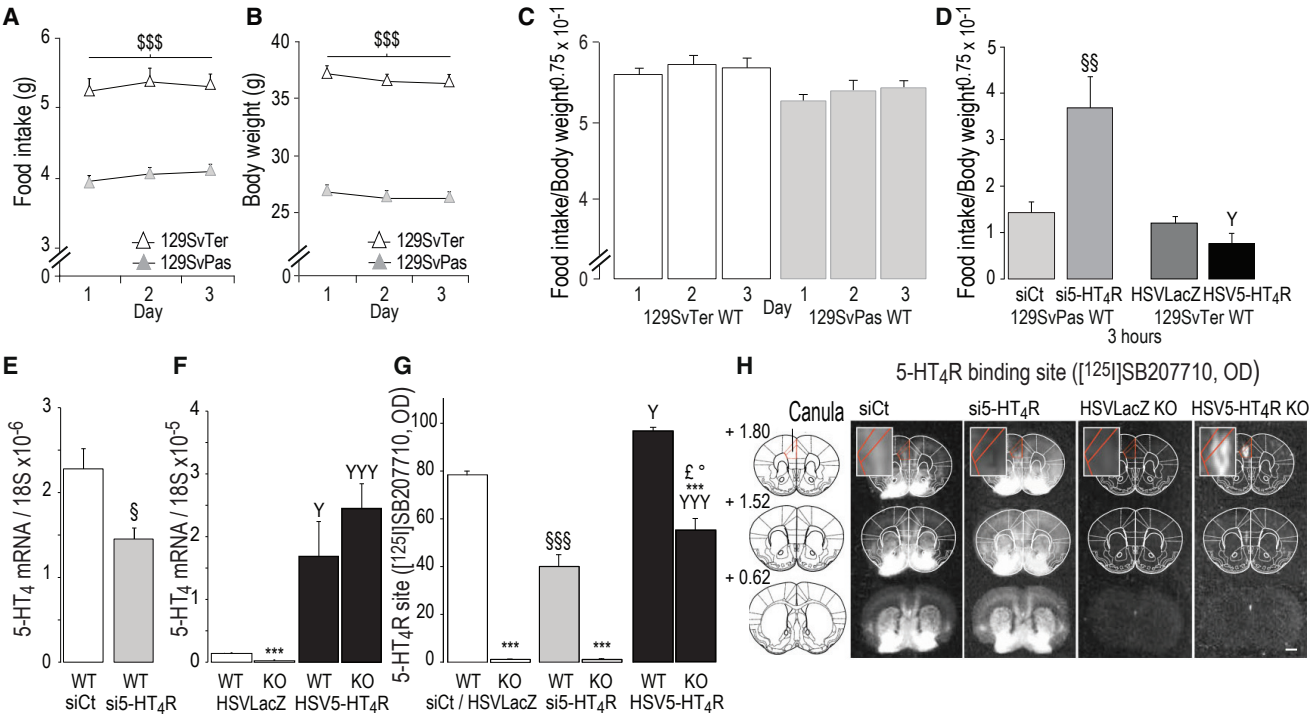

Figure 1. The mPFC-5-HT$_4$Rs Exert Negative Control on Food Intake under Basal Conditions

(A–D) Daily food intake (A), body weight (B), food intake/active tissue (body weight$^{0.75}$, C and D). (D) Food intake/active tissue 3 hr post-treatment.

(E and F) mPFC-5-HT$_4$R mRNA 5 and 77 hr after respective infusion into the mPFC of (1) si5-HT$_4$R in 129SvPas WT (E), and (2) HSV5-HT$_4$R in 129SvTer mice of both genotypes (F).

(G) 5-HT$_4$R binding sites (optical density [OD] evaluated on rectangular surface 80 μm^2).

(H) Labeling of the [^{125}I]SB207710 (5-HT$_4$R antagonist) visualized from autoradiographs in frontal brain sections (scale bar, 1 mm).

$^{\$\$\$}p < 0.0001$, differences between 129SvPas and 129SvTer; $^{***}p < 0.001$, between genotypes; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$, and $^{\S\S\S}p < 0.001$ compared with siCt; and $^{Y}p < 0.05$, $^{YYY}p < 0.001$ with HSVLacZ; $^{£}p < 0.05$ compared to siCt-WT; and $^{\circ}p < 0.05$ compared to si5-HT$_4$R-WT following ANOVA: two-way repeated-measured (A–C) n = 30–60, $F(1,88) = 88.27$, $p < 0.0001$; one-way (D) n = 10–16, $F(3,71) = 12.32$, $p < 0.0001$; (E) n = 6–9, $F(1,13) = 4.81$, $p < 0.05$; two-way (F) n = 4–5, HSV5-HT$_4$R in 129SvTer mice of both genotypes, treatment $F(1,14) = 40.11$, $p = 0.0001$; (G) n = 10–20, genotype × treatment $F(5,88) = 86.1$, $p < 0.0001$. n = number of mice/group.

employed to identify the neural basis of hypophagia owing to stress is forced immobilization, called restraint stress (reviewed in Laurent et al., 2012). Mice can neither escape nor learn how to escape from the stressor. This stressor is out of their control (uncontrollable stress) (Amat et al., 2005) and enhances 5-HT transmission (reviewed in Laurent et al., 2012). Restrained mice treated with 8-OH-DPAT (Dourish et al., 1987), a 5-HT$_{1A}$R/5-HT$_7$R agonist, or lacking 5-HT$_4$Rs exhibit attenuated hypophagia (Compan et al., 2004).

The cerebral location of the mediation of stress-induced hypophagia by 5-HT$_4$Rs is unknown. The location of 5-HT$_4$Rs in brain is conserved in humans, with the highest levels in the NAc and the lowest in the cerebral cortex (Bonaventure et al., 2000; Compan et al., 1996). The 5-HT$_4$Rs serve to activate the DR-5-HT cells, not from the DR (they are absent) but from the ventral part of the medial prefrontal cortex (mPFC) (Figure S1). In this study, we tested whether the 5-HT$_4$Rs in the mPFC (mPFC-5-HT$_4$Rs) are necessary and sufficient to mediate hypophagia following stress. The experimental procedures employed here were utilized in our earlier studies (Compan et al., 2004; Jean et al., 2007, 2012). Notably, the expression of 5-HT$_4$Rs was rescued in 5-HT$_4$R knockout (KO) mice by transferring the *Htr4* gene (*Herpès simplex* virus: HSV5-HT$_4$R transduced mice) into the mPFC. In parallel, knockdown of the mPFC-5-HT$_4$Rs was induced by small interference RNA (si5-HT$_4$R). These latter experiments required wild-type (WT) mice only and were conducted in WT mice from a 129SvPas similar genetic background in order to reserve 129SvTer WT mice as controls for corresponding 129SvTer 5-HT$_4$R KO mice (Supplemental Experimental Procedures).

RESULTS

No Differences between Food Intake per Active Tissue in 129SvTer and 129SvPas WT Mice

Food intake and body weight were lower in 129SvPas than in 129SvTer WT mice (Figures 1A and 1B), but both strains ate a similar amount of food per active tissue (body weight$^{0.75}$) (West et al., 1997) when untreated (Figure 1C) or infused with control treatments (siCt, HSVLacZ) into the mPFC under basal conditions (Figure 1D).

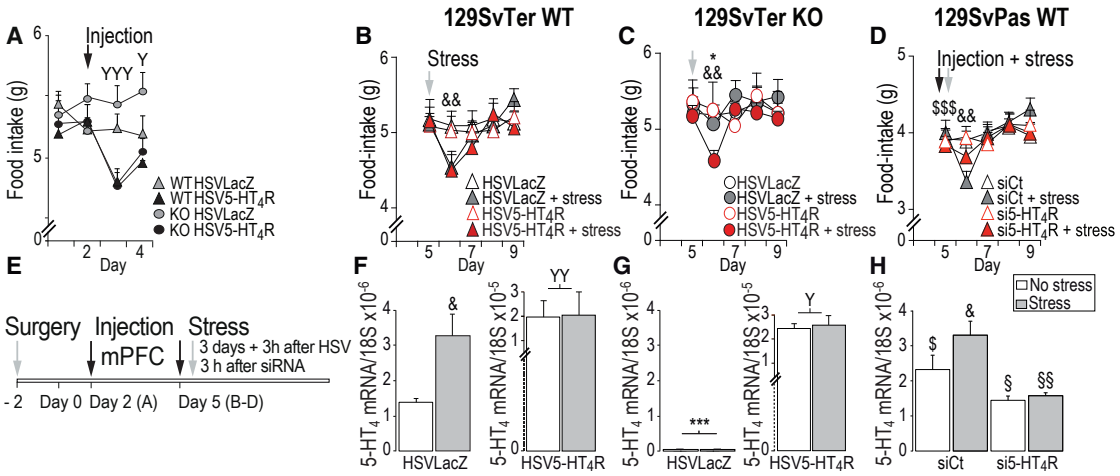

Figure 2. Causal Relationship between the 5-HT$_4$Rs in the mPFC and Hypophagia following Stress

(A–D) Daily food intake for 21 hr before stress (A), and after stress in 129SvTer WT (B), 129 SvTer KO (C) and 129SvPas WT (D) mice.

(E) Experimental design.

(F–H) mPFC-5-HT$_4$R mRNA immediately after stress in 129SvTer WT (F), 129 SvTer KO (G), and 129SvPas WT (H) mice.

$^{\$}p < 0.05$, $^{\$\$\$}p < 0.0001$, and $^{*}p < 0.05$, $^{***}p < 0.0001$ differences between 129SvPas and 129SvTer, and genotypes, respectively; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ compared with siCt, and $^{Y}p < 0.05$, $^{YY}p < 0.01$, and $^{YYY}p < 0.001$ compared with HSVLacZ; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ compared to unstressed, following ANOVA: two-way repeated-measured (A) n = 14–17, treatment $F(1,58) = 4.51$, $p < 0.05$; time $F(4,232) = 12.74$, $p < 0.0001$; time × treatment $F(4,232) = 6.08$, $p < 0.05$; and time × genotype × treatment $F(4,232) = 2.30$, $p < 0.05$; (B and C) n = 5–10, time $F(4,168) = 11.6$, $p < 0.0001$; time × stress $F(4,168) = 7.07$, $p < 0.0001$; time × stress × genotype $F(4,168) = 2.5$, $p < 0.05$; time × stress × treatment $F(4,168) = 2.6$, $p < 0.05$; (D) n = 13–16, time × stress × treatment $F(4, 224) = 3$, $p < 0.05$; stress $F(4, 224) = 6.3$, $p < 0.0001$; time $F(4, 224) = 16.9$, $p < 0.0001$; treatment $F(4, 224) = 2.7$, $p < 0.05$, and (D) additional two-way repeated-measures revealed differences in basal food intake between 129SvPas and 129SvTer WT mice, $F(1,80) = 45$, $p < 0.0001$; two-way (F and G) n = 3–5, treatment $F(1,28) = 15.4$, $p < 0.001$; (H) n = 6–7, treatment $F(1,23) = 25.6$, $p < 0.0001$, stress $F(1,23) = 5.3$, $p < 0.0001$; stress × treatment $F(1,23) = 3.1$, $p = 0.05$; one-way (F) stress in HSVLacZ-transduced WT mice $F(1,8) = 8.8$, $p < 0.05$; (B and C) one-way ANOVA day 6 stress $F(1,40) = 12.5$, $p < 0.01$; genotype × treatment × stress $F(1,40) = 4.0$, $p < 0.05$. n = number of mice/group.

See also Figure S2.

Knockdown of 5-HT$_4$Rs in the mPFC Induces Overeating under Basal Conditions

Injecting si5-HT$_4$R into the mPFC induced overeating at 3 hr post-infusion compared with that following injection with control (siCt, Figure 1D). si5-HT$_4$R reduced the levels of both mRNA (38%, Figure 1E) and binding site (49%, Figures 1G and 1H) of the mPFC-5-HT$_4$Rs at 5 hr compared with control treatment. No modifications in food intake or in the mRNA levels of mPFC-5-HT$_4$Rs were detected 24 hr after treatments in the mPFC compared with control treatment (means ± SEM, food intake: siCt 4.19 ± 0.16 g, si5-HT$_4$R 4.01 ± 0.19 g; 5-HT$_4$R mRNA/18S × 10^{-6}: siCt 2.28 ± 0.31, si5-HT$_4$R 2.10 ± 0.15).

Overexpression of 5-HT$_4$Rs in the mPFC Triggers Hypophagia under Basal Conditions

HSV5-HT$_4$R-transduced WT mice ate less from 3 hr (Figure 1D) until 48 hr (Figure 2A: days 3–4) than did control mice (HSV-LacZ) and consumed the same amount of food 72 hr (Figure 2B: day 5) post-injection compared with control (HSVLacZ) under basal conditions, as seen in HSV5-HT$_4$R-transduced 5-HT$_4$R KO mice at 48 and 72 hr (Figures 2A and 2B).

HSV5-HT$_4$R-transduced mice of both genotypes displayed increases in the mRNA and binding site levels of mPFC-5-HT$_4$Rs 77 hr post-injection compared with those in controls (Figures 1F and 1G: WT 23%, 5-HT$_4$R KO 783%). The levels of mPFC-5-HT$_4$Rs were lower in the HSV5-HT$_4$R-transduced mutants than in control siCt/HSVLacZ-treated WT mice because the examined tissue in the mutant mice was deprived of 5-HT$_4$Rs (Figures 1G, −29%, and 1H); however, the levels of mPFC-5-HT$_4$Rs were higher in HSV5-HT$_4$R-transduced mutants than in si5-HT$_4$R-treated WT mice (39%, Figure 1G).

The mPFC-5-HT$_4$Rs Mediate Hypophagia following Stress

We next tested whether there was a causal relationship between the mPFC-5-HT$_4$Rs and hypophagia following stress. Unstressed 129SvTer WT mice still ate more than unstressed 129SvPas WT mice (Figures 2B and 2D). Stress induced a transient hypophagia in WT mice of both strains but not in HSVLacZ-transduced 5-HT$_4$R KO mice compared with controls (Figures 2B–2D). The elective rescue of the mPFC-5-HT$_4$Rs in 5-HT$_4$R KO mice restored stress-induced hypophagia (Figure 2C). Hypophagia was not enhanced in stressed WT mice with mPFC-5-HT$_4$R overexpression (Figure 2B), suggesting adaptive changes and/or a ceiling effect.

The mRNA levels of mPFC-5-HT$_4$Rs in HSVLacZ-transduced 129SvTer WT mice were lower than in unstressed siCt-treated 129SvPas WT mice (Figures 2F and 2H), consistent with their differences in basal food intake (Figures 2B and 2D). Similarly, stress-induced hypophagia also provoked increases in the mRNA levels of mPFC-5-HT$_4$Rs in WT compared with those in unstressed WT animals in both strains (Figures 2F and 2H).

In contrast, stress failed to provoke hypophagia and to increase the mRNA levels of the mPFC-5-HT_4Rs following injection of si5-HT_4R into the mPFC in 129SvPas WT mice compared with the levels in controls (Figures 2D and 2H). The 5-HT_4R knockdown in other sites (cingulate cortex area 2, NAc) failed to alter stress-induced hypophagia compared with that in controls (e.g., NAc: Figures S2A and S2B). According to these results, the mRNA levels of NAc-5-HT_4Rs were reduced in stressed WT mice in a mPFC-5-HT_4R-dependent manner, because the reduction was attenuated in mice with mPFC-5-HT_4R knockdown (Figure S2C).

Finally, injection into the mPFC of an agonist (BIMU8) or antagonist (RS39604) of the 5-HT_4Rs mimicked the feeding responses induced by the knockdown and overexpression of the mPFC-5-HT_4Rs under basal and stressful conditions (Figures S2D and S2E). Additionally, stimulation of the mPFC-5-HT_4Rs attenuated the weakness of motor reactivity to novelty (Figure S2F), another classic behavioral response to restraint stress (Kennett et al., 1987). When the mPFC-5-HT_4Rs were brought to a standstill, both unstressed and stressed mice were less active (Figure S2F). Thus, "moving less and eating more" involves the blockade of the mPFC-5-HT_4Rs, extending and reinforcing the results of earlier studies (Compan et al., 2004; Jean et al., 2007, 2012). Therefore, locomotion and feeding can independently segregate under the influence of mPFC-5-HT_4Rs.

The mPFC-5-HT_4Rs Control DR-5-HT Responses to Stress

We next set out to explore the mechanisms whereby mPFC-5-HT_4Rs mediate hypophagia following stress.

The abnormal resistance to stress-induced hypophagia in 5-HT_4R KO mice was not supported by a general maladaptive response to stress (Figures S2G and S2H). We therefore focused on the DR-5-HT system. Because we were not able to record the DR-5-HT neurons firing in freely moving stressed mice, we evaluated the mRNA levels of *Fos*, a marker of neural activity, in the raphe nuclei (DR/MR). The mRNA levels of *Fos* were increased in stressed mice, although to a lesser extent in stressed mutants (Figure 3A) and si5-HT_4R-mPFC-treated WT mice than in WT mice themselves (Figure S3). Rescuing mPFC-5-HT_4Rs in mutant mice restored the stress-induced increases in the mRNA levels of DR/MR-*Fos* (Figure 3A). We then conducted analyses in freely moving mice and detected increases in the levels of extracellular DR-5-HT in stressed WT but not in 5-HT_4R KO mice (Figure 3B). The use of si5-HT_4R was excluded because it must be injected 3 hr before stress, which overlapped with the required 2 hr needed to achieve a steady state with the use of the dialysis probe (Supplemental Procedures). However, stressed HSV5-HT_4R-transduced 5-HT_4R KO mice mimicked the response of stressed WT mice (Figure 3B).

Nonetheless, stressed mice of both genotypes displayed an increase in the 5-HT turnover index at the end of stress; stressed mutants displayed this increase to a greater extent than did WT mice (Table S1). The levels of extracellular DR-5-HT in 5-HT_4R KO mice could potentially be higher, but are likely more rapidly diminished by higher uptake than in controls (Conductier et al., 2006). Accordingly, the mRNA levels of the 5-HT transporter (SERT) in 5-HT_4R KO mice remained high in the stressful compared to the basal conditions, whereas the levels were reduced after stress in the DR/MR of WT mice compared to those in unstressed mice (Figure 3D). Rescuing the mPFC-5-HT_4R expression in 5-HT_4R KO mice partly restored the adequate response (reduced levels) to stress (Figure 3D). Similar changes in SERT binding site concentration have also been observed in these experimental conditions (not illustrated).

Early Anorexia in Stressed Mice with mPFC-5-HT_4R Overexpression and DR-5-HT_{1A}R Blockade

There were no changes in the mRNA levels of DR-5-HT_{1A}R in either group of mice, although stress induced decreases in the levels of receptor binding sites (Figures 3E and 3F). In unstressed HSV5-HT_4R-transduced mice of both genotypes, the levels of DR-5-HT_{1A}R were increased; the increase was more prominent in 5-HT_4R KO than in WT mice (Figure 3F). Rescuing mPFC-5-HT_4R in mutant mice partly restored the response (reduced levels) to stress compared with that of the controls (Figure 3F).

Reduced DR-5-HT_{1A}R levels following stress can favor DR-5-HT cell hyperactivity and hypophagia. Following homeostasis, stimulation of DR-5-HT_{1A}Rs should bring the activity of DR-5-HT cells to baseline, cutting the duration of hypophagia. We blocked DR-5-HT_{1A}Rs with the antagonist WAY100635 the day after stress and observed persistent hypophagia in stressed mice with mPFC-5-HT_4R overexpression, whereas neither the DR-5-HT_{1A}R blockade nor 5-HT_4R overexpression alone maintained the duration of hypophagia (Figures 4A and 4B). Stressed mice displayed body weight loss at 24 hr, whereas only mice treated with mPFC-HSV5-HT_4R and DR-5-HT_{1A}R antagonist exhibited a loss for 96 hr compared to the body weight of the controls (Figures 4C and 4D).

DISCUSSION

The present study describes causal relationships between a molecular network and stress-dependent food intake. Stress triggers an elevation in DR-5-HT release along with a reduction in DR-SERT and 5-HT_{1A}R levels upon the control of the mPFC-5-HT_4Rs, causing a transient hypophagia (Movie S1). The identified molecular network may protect the brain from early anorexia-like behavior, because blocking DR-5-HT_{1A}Rs with overexpression of mPFC-5-HT_4Rs mediates persistent hypophagia following stress.

The mPFC-5-HT_4R is shown here to be necessary and sufficient for stress-induced hypophagia because their restitution in 5-HT_4R KO adult mice restores this phenotype, excluding their contribution in other brain areas and developmental processes. The restored brains also regain the ability to accumulate 5-HT and reduce the levels of DR-SERT/5-HT_{1A}R after stress, which are needed for stress-induced hypophagia. These neural events are not implemented during earlier developmental stages, but adapt rapidly, with high flexibility, in response to uncontrollable stress. Thus, a maladaptive feeding response to uncontrollable stress can be restored in adult animals.

Among these events, there exists the potential for positive mPFC-5-HT_4R control of DR-5-HT release, in agreement with the mPFC-5-HT_4R positive feedback effect (Figure S1).

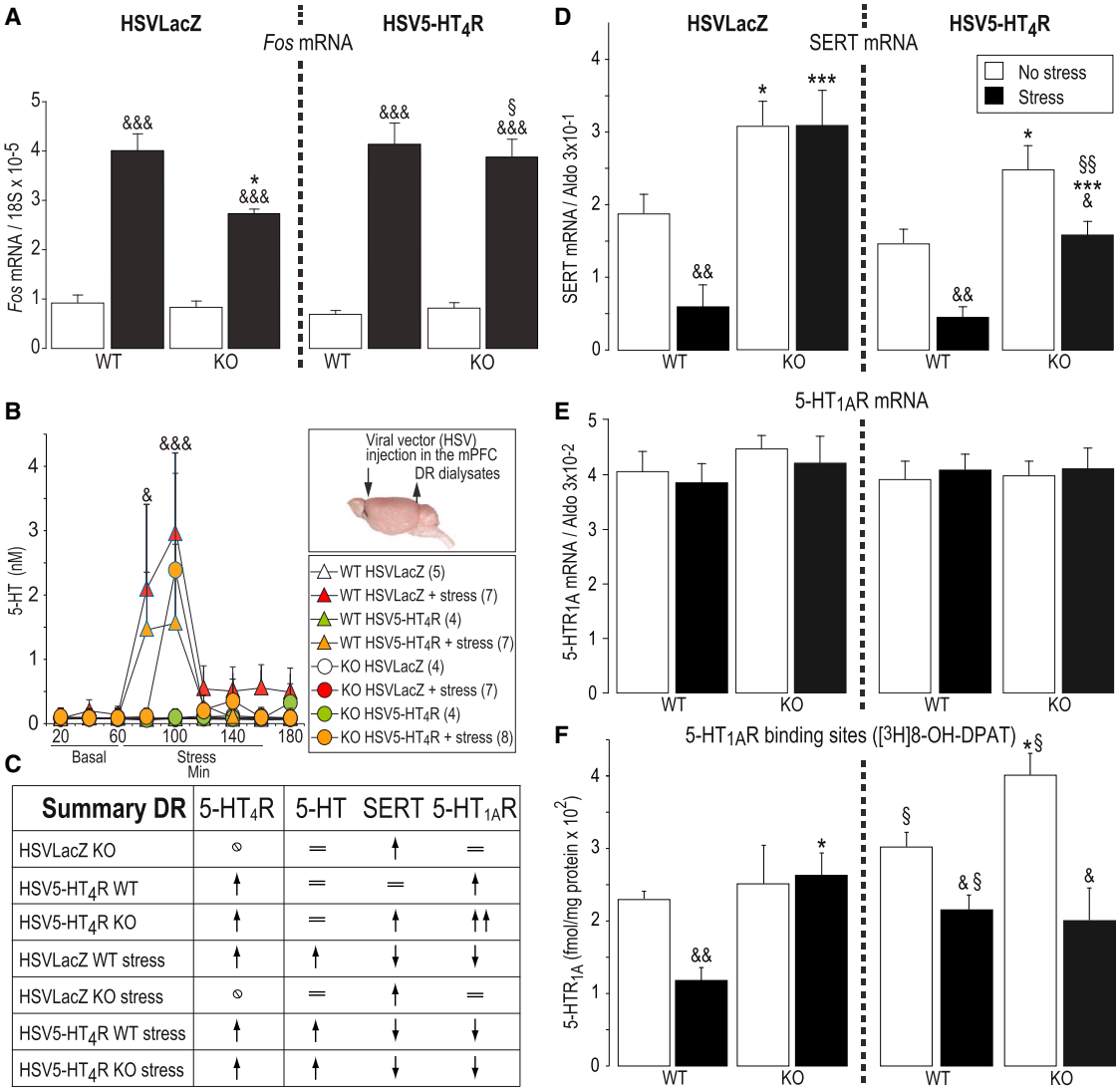

Figure 3. Stress-Induced Changes in the 5-HT Parameters in the DR Depends on mPFC-5-HT$_4$Rs

(A) *Fos* mRNA in the DR/MR immediately after stress.

(B) Extracellular 5-HT in the DR. Data corresponding to "HSVLacZ- and HSV5-HT$_4$R-transduced WT and, HSVLacZ- and HSVLacZ-transduced KO 5-HT$_4$R + stress" are graphically grouped behind the green circles.

(C) Summary: ⦸ absent, ↑ increases, ↑↑, high increases, ↓ decreases, = unchanged.

(D and E) Respective levels of SERT (D) and 5-HT$_{1A}$R (E) mRNA in the DR/MR immediately after stress.

(F) 5-HT$_{1A}$R binding sites at the DR/MR level.

$^{\&}p < 0.05$, $^{\&\&}p < 0.01$, and $^{\&\&\&}p < 0.001$ stress effect; $^{*}p < 0.05$, $^{***}p < 0.001$ differences between genotypes; and $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ treatments following two-way ANOVA: (A) genotype × stress $F(1,35) = 4.3$, $p < 0.05$; treatment × stress $F(1,35) = 5.4$, $p < 0.05$; genotype $F(1,35) = 5.1$, $p < 0.05$; stress $F(1,35) = 27.0$, $p < 0.0001$; (B) genotype × stress × treatment × time $F(1,29) = 3.2$, $p < 0.01$; genotype × stress × treatment $F(1,29) = 6.8$, $p < 0.05$; stress $F(1,29) = 16.2$, $p < 0.0004$; (D) genotype × stress × treatment $F(1,31) = 4.2$, $p < 0.05$; genotype × stress $F(1,31) = 3.9$, $p < 0.05$; genotype × treatment $F(1,31) = 7.3$, $p < 0.01$; genotype $F(1,31) = 64.2$, $p = 0.0001$; stress $F(1,31) = 21.9$, $p = 0.0001$; treatment $F(1,31) = 14.6$, $p = 0.0006$; (F) genotype × stress × treatment $F(1,13) = 9.9$, $p < 0.01$; stress × treatment $F(1,13) = 5.9$, $p < 0.05$; genotype $F(1,13) = 11.1$, $p < 0.01$; stress $F(1,13) = 32.6$, $p < 0.0001$; treatment $F(1,13) = 14.9$, $p < 0.01$. n = 5–7 mice/group. See also Figure S3 and Table S1.

The concomitant changes in DR-5-HT release and SERT/5-HT$_{1A}$R (summary: Figure 3C) fit with those seen in mice lacking either SERT (DR-5-HT$_{1A}$R reduction) (Fabre et al., 2000), monoamine oxidase-A (main catabolism enzyme of 5-HT) (5-HT elevation, DR-SERT/5-HT$_{1A}$R desensitization) (Evrard et al., 2002), or central 5-HT (undetectable 5-HT, 5-HT$_{1A}$R elevation) (Araragi and Lesch, 2013), as this is also seen in rats (Compan et al., 1998). We therefore suggest the existence of 5-HT release-dependent mPFC-5-HT$_4$R negative and positive control of DR-SERT and DR-5-HT$_{1A}$R, respectively. Notably, in stressed 5-HT$_4$R KO mice, the levels of DR-5-HT$_{1A}$R remained at control values, whereas they decreased in stressed WT

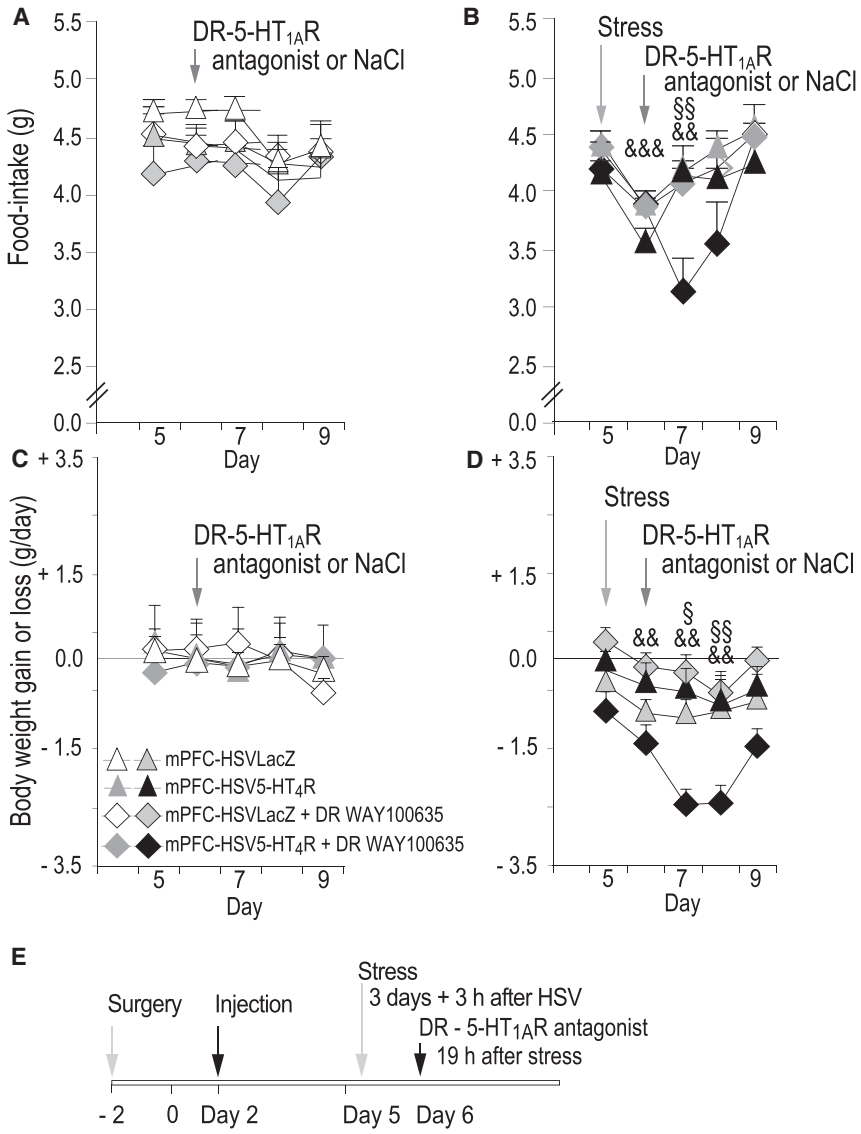

Figure 4. Early Anorexia in Stressed Mice with mPFC-5-HT$_4$R Overexpression and DR-5-HT$_{1A}$R Blockade

(A and B) Daily food intake for 21 hr.

(C and D) In identical mice, body weight gain or loss (body weight evaluated each day minus the first day of the test).

(E) Experimental design.

$^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ stress effect; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ differences between DR treatments in stressed mice with mPFC-HSV5-HT$_4$Rs following two-way repeated-measures ANOVA: (A and B) stress × food intake $F(1,51) = 7.0$, $p < 0.01$; time $F(4,204) = 8.3$, $p < 0.0001$; effect of DR treatment over time $F(4,204) = 2.6$, $p < 0.05$. ANOVA analyses for each day: stress, day 2 $F(1,51) = 40.7$, $p < 0.0001$; day 3 $F(1,51) = 14.0$, $p < 0.001$; and day 7, mPFC $F(1,51) = 4.8$, $p < 0.05$, and DR $F(1,51) = 7.2$, $p < 0.01$ treatment effect on food intake; (C and D) stress $F(1,51) = 7.0$, $p < 0.01$; time $F(4,204) = 4.7$, $p < 0.01$; and mPFC × DR treatment $F(1,51) = 3.8$, $p < 0.05$. ANOVA analyses for each day: stress, day 2 $F(1,51) = 8.5$, $p < 0.01$; day 3 $F(1,51) = 8.1$, $p < 0.001$; day 4 $F(1,51) = 5.5$, $p < 0.05$; and day 7 mPFC × DR treatment $F(1,51) = 5.1$, $p < 0.05$. n = 6–10 mice/group.

See also Figure S4.

mice. A suggested mPFC-5-HT$_4$Rs positive tonic control of DR-5-HT$_{1A}$Rs (limit a reduction) agrees with earlier observations (Amigó et al., 2016; Conductier et al., 2006).

In addition, mPFC-5-HT$_4$Rs may relay an earlier increase in DR-5-HT release, as the increase was restored in stressed mPFC-HSV5-HT$_4$R-transduced 5-HT$_4$R KO mice at 100 and not at 80 min during stress. As we discussed elsewhere (Compan et al., 2004), the hyperactivity of the hypothalamo-pituitary adrenal axis could first intervene (Kirby et al., 2000), considering the unchanged levels of corticosterone in stressed mutant mice.

An interesting point is that reduced levels of DR-5-HT$_{1A}$R increased 5-HT release, and stimulation of the mPFC-5-HT$_4$Rs can limit depressive-like states (Lucas et al., 2007; Richardson-Jones et al., 2010). The neural substrates of a transient hypophagia following stress are then included in a neural network of self-preservation (e.g., antidepressant-like behavior), consistently with the abilities of 5-HT$_4$Rs to limit anhedonia (Amigó et al., 2016) and to favor a rewarding anorexia (Jean et al., 2007, 2012). Accordingly, the reciprocal 5-HT/GLU neural pathways between the mPFC and the DR (Figure S1A) prevent the implementation of neural basis of depressive-like behavior induced by adverse stressors (Amat et al., 2005; Duman et al., 2016). Here, stress failed to provoke hypophagia when the DR-*N*-methyl-*D*-aspartate (NMDA) receptor was blocked (Figure S4A); this was consistent with the ability of the mPFC-GLU pathway to increase DR-5-HT release through NMDA receptors (Celada et al., 2001; de Kock et al., 2006). Thus, there are commonalities between adaptive neural responses to stress and the effects of particular antidepressants (SERT blockade, 5-HT accumulation, 5-HT$_{1A}$R-desensitization). In humans, chronic antidepressant treatment induces 5-HT$_{1A}$R-desensitization, reduces food intake in rats, and attenuates bulimia in humans (Jackson et al., 2010; McGuirk et al., 1992).

How the brain supports adapted behavior for escaping adverse effects of stressors (controllable stress) is worth mentioning here, because these behavioral strategies (i.e., learning responses indicating the end or a means to escape from a stressor) are associated with attenuated DR 5-HT cell activity and subsequent depression-like behavior (i.e., learned helplessness) provoked by uncontrollable stress (Amat et al., 2005; Grahn et al., 1999; Maswood et al., 1998). Learned

helplessness following uncontrollable (but not following controllable) stress is absent in animals when the activity of DR 5-HT cells has been reduced (Maier et al., 1995). When mice escape from stress, the mPFC inhibits stress-induced activation of DR 5-HT cells (Amat et al., 2005). The present conclusion integrates well with this scientific context; in this study, limitation of DR 5-HT cell hyperactivity by DR-5-HT_{1A}R prevented subsequent behavioral pathology, i.e., the transition from transient to persistent hypophagia owing to an uncontrollable stressor. In line with these results, enhancing 5-HT_{1A}R negative feedback prevents learned helplessness when animals are physically more active (Greenwood et al., 2003), highlighting potentiation of physical activity on stress resistance and antidepressant efficiency (Babyak et al., 2000; Salmon, 2001). Although the current study focuses on food intake in an uncontrollable condition, analyzing the physical activity of stressed mice treated with 5-HT_4R pharmaceuticals was tempting. Mice lacking 5-HT_4Rs in the whole brain, or only in the NAc, are less physically active in the open field (Compan et al., 2004; Jean et al., 2012). Accordingly, mice show less motor activity when the mPFC-5-HT_4Rs are blocked under basal conditions (Figure S2F). Mice are more active in the open field when NAc-5-HT_4Rs are stimulated (or overexpressed) (Jean et al., 2012), but not when mPFC-5-HT_4Rs are stimulated in unstressed mice. Thus, mPFC-5-HT_4Rs may serve to positively maintain motor reactivity to novelty without enhancing it. Accordingly, reduced motor activity in stressed mice is related to combined (and physiological) over- and down-expression of mPFC- and NAc-5-HT_4Rs, respectively (Figure S2C). Stimulating the mPFC-5-HT_4Rs in stressed mice only attenuated the weakness of motor reactivity in stressed mice compared with that in controls (Figure S2F). In summary, when mPFC-5-HT_4Rs are blocked, stressed mice eat more and still move less, showing all the behavioral ingredients to install depressive-like behavior. In contrast, activating the mPFC-5-HT_4Rs in stressed mice reduces food intake, favors physical activity following the increase in activity of DR 5-HT neurons accompanied by low levels of DR-SERT/5-HT_{1A}R, thereby showing better resistance to stress. Accordingly, SERT and 5-HT_{1A} KO mice are less active in home cages and open fields, respectively (Holmes et al., 2002; Ramboz et al., 1998).

Finally, even though this study demonstrates a causal relationship, the mechanisms by which mPFC-5-HT_4Rs interact with GLU and GABA transmission in the mPFC and DR to adapt feeding and energy balance following controllable and/or chronic uncontrollable stress remain to be investigated.

In this study, we found that neural adaptive responses to stress, known to reduce impaired behavior of self-preservation (depression), initiates persistent hypophagia following stress. An "early anorexia" could then favor self-preservation via neural pathways concerning stress, whereas obesity often exists in conjunction with depression (reviewed in Duman et al., 2016). Considering the relevance in certain circumstances of modeling behavioral traits of mental disease (reviewed in Donaldson and Hen, 2015), even though our models were rather simplistic by necessity, this study introduces a primary mechanism whereby individuals could shift from transient to persistent food restriction as seen in anorexia nervosa (Walsh, 2013) and suggests 5-HT_4Rs as possible targets for treating this currently incurable disease.

EXPERIMENTAL PROCEDURES

Animals

Maintenance and experiments were conducted with male 129SvPas WT, 129SvTer 5-HT_4R KO, and WT mice (4–6 months old) (Compan et al., 2004) under standard conditions consistent with those in the Guide for Care and Use of Laboratory Animals (MENESR agreements D3417213 and 34-408, authorization 00905.01) described in the Supplemental Experimental Procedures.

Microsurgery

A stainless steel guide cannula was implanted and fixed in a specific brain area (mPFC, NAc, DR) described in the Supplemental Experimental Procedures and elsewhere (Jean et al., 2007) 48 hr before the onset of the habituation period of any experiment. A stainless steel cannula connected to a microsyringe nanopump was inserted into the guide and, each compound was infused through a microcatheter for 1 min at a rate of 1 μL/min in freely moving animals.

Nucleic Acid Treatments

Methods for 5-HT_4R overexpression and knockdown strategies were previously established (Jean et al., 2012) and are described in the Supplemental Experimental Procedures.

Real-Time qPCR

Brains from stressed and unstressed mice of both genotypes were removed 5 and 77 hr after respective injection of siRNA and HSV, frozen, and dissected at −20°C of (mPFC, 1.2 mm^3) to isolate total mRNA and cDNA in reactions containing 5-HT_4R primers (Jean et al., 2007) and described in the Supplemental Experimental Procedures.

Biochemical Analyses

The Supplemental Experimental Procedures include methods reported in Compan et al. (1998, 2004), Doly et al. (2008), and Dusticier and Nieoullon (1987) utilized to, respectively, (1) label 5-HT_{1A}R and 5-HT_4R in frontal brain sections, and (2) evaluate the levels of tissue 5-HT and a metabolite thereof in the DR/MR or extracellular 5-HT in the DR of unstressed and stressed mice of both genotypes.

Feeding Test

As detailed in the Supplemental Information, experiments include three periods: the baseline, the day of treatments and restraint stress, and the recovery. An initial handling for weighing at t = 0 min preceded the injection of si5-HT_4R or siCt (50 ng/μL), BIMU8 or RS39604 (40 ng/μL), and NaCl (0.9%) into the mPFC at t = 10 min and the onset of stress at t = 3 hr for 110 min (total duration: 5 hr). The injection of HSV5-HT_4R or HSVLacZ (10^7 infectious units/mL) into the mPFC was performed 3 days and 3 hr before stress alone or combined with infusion in the DR of either WAY100635 (45 ng/μL, day 6: 19 hr after the end of the stress period) or MK801 (0.5 ng/μL) immediately after stress, and NaCl (0.9%).

Statistical Analysis

Data, presented as mean ± SEM, were obtained in multiple sessions over time (food intake) and were analyzed using two-way repeated-measures ANOVA (STATVIEW 5, SAS Institute, San Francisco, CA). When effects of independent variables (treatment, genotype, time, stress), or interactions were significant, one-way ANOVAs (treatment, genotype, time, or stress) were performed. For multiple comparisons, the Scheffé F-test was used. Differences with $p < 0.05$ were considered significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, one table, and one movie and can be found with this article online at https://doi.org/10.1016/j.celrep.2017.10.003.

AUTHOR CONTRIBUTIONS

A.J., L.L., S. Delaunay, S. Doly, N.D., and R.N. performed experiments. A.J. produced initial version of figures. S. Doly, D.L., L.M., R.N. and A.N. assisted with interpretation. R.N. and D.L. assisted with writing. V.C. conceived and designed the overall work, interpreted data, wrote the manuscript, produced figures, and the movie with the assistance of N. Scarpa and L. Janondy (BINOME, France).

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REFERENCES

Amat, J., Baratta, M.V., Paul, E., Bland, S.T., Watkins, L.R., and Maier, S.F. (2005). Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. Nat. Neurosci. *8*, 365–371.

Amigó, J., Díaz, A., Pilar-Cuéllar, F., Vidal, R., Martín, A., Compan, V., Pazos, A., and Castro, E. (2016). The absence of 5-HT4 receptors modulates depression- and anxiety-like responses and influences the response of fluoxetine in olfactory bulbectomised mice: adaptive changes in hippocampal neuroplasticity markers and 5-HT1A autoreceptor. Neuropharmacology *111*, 47–58.

Araragi, N., and Lesch, K.P. (2013). Serotonin (5-HT) in the regulation of depression-related emotionality: insight from 5-HT transporter and tryptophan hydroxylase-2 knockout mouse models. Curr. Drug Targets *14*, 549–570.

Azmitia, E.C. (1999). Serotonin neurons, neuroplasticity, and homeostasis of neural tissue. Neuropsychopharmacology *21* (*2, Suppl*), 33S–45S.

Babyak, M., Blumenthal, J.A., Herman, S., Khatri, P., Doraiswamy, M., Moore, K., Craighead, W.E., Baldewicz, T.T., and Krishnan, K.R. (2000). Exercise treatment for major depression: maintenance of therapeutic benefit at 10 months. Psychosom. Med. *62*, 633–638.

Bailer, U.F., Frank, G.K., Henry, S.E., Price, J.C., Meltzer, C.C., Mathis, C.A., Wagner, A., Thornton, L., Hoge, J., Ziolko, S.K., et al. (2007). Exaggerated 5-HT1A but normal 5-HT2A receptor activity in individuals ill with anorexia nervosa. Biol. Psychiatry *61*, 1090–1099.

Bevilacqua, L., Doly, S., Kaprio, J., Yuan, Q., Tikkanen, R., Paunio, T., Zhou, Z., Wedenoja, J., Maroteaux, L., Diaz, S., et al. (2010). A population-specific HTR2B stop codon predisposes to severe impulsivity. Nature *468*, 1061–1066.

Bonaventure, P., Hall, H., Gommeren, W., Cras, P., Langlois, X., Jurzak, M., and Leysen, J.E. (2000). Mapping of serotonin 5-HT(4) receptor mRNA and ligand binding sites in the post-mortem human brain. Synapse *36*, 35–46.

Celada, P., Puig, M.V., Casanovas, J.M., Guillazo, G., and Artigas, F. (2001). Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-1A, GABA(A), and glutamate receptors. J. Neurosci. *21*, 9917–9929.

Compan, V. (2013). Under- to overeating: what role do serotonin receptors play? Future Neurol. *8*, 701–714.

Compan, V., Daszuta, A., Salin, P., Sebben, M., Bockaert, J., and Dumuis, A. (1996). Lesion study of the distribution of serotonin 5-HT4 receptors in rat basal ganglia and hippocampus. Eur. J. Neurosci. *8*, 2591–2598.

Compan, V., Segu, L., Buhot, M.C., and Daszuta, A. (1998). Differential effects of serotonin (5-HT) lesions and synthesis blockade on neuropeptide-Y immunoreactivity and 5-HT1A, 5-HT1B/1D and 5-HT2A/2C receptor binding sites in the rat cerebral cortex. Brain Res. *795*, 264–276.

Compan, V., Zhou, M., Grailhe, R., Gazzara, R.A., Martin, R., Gingrich, J., Dumuis, A., Brunner, D., Bockaert, J., and Hen, R. (2004). Attenuated response to stress and novelty and hypersensitivity to seizures in 5-HT4 receptor knockout mice. J. Neurosci. *24*, 412–419.

Compan, V., Walsh, B.T., Kaye, W., and Geliebter, A. (2015). How does the brain implement adaptive decision making to eat? J. Neurosci. *35*, 13868–13878.

Conductier, G., Dusticier, N., Lucas, G., Côté, F., Debonnel, G., Daszuta, A., Dumuis, A., Nieoullon, A., Hen, R., Bockaert, J., and Compan, V. (2006). Adaptive changes in serotonin neurons of the raphe nuclei in 5-HT(4) receptor knock-out mouse. Eur. J. Neurosci. *24*, 1053–1062.

de Kock, C.P., Cornelisse, L.N., Burnashev, N., Lodder, J.C., Timmerman, A.J., Couey, J.J., Mansvelder, H.D., and Brussaard, A.B. (2006). NMDA receptors trigger neurosecretion of 5-HT within dorsal raphe nucleus of the rat in the absence of action potential firing. J. Physiol. *577*, 891–905.

Descarries, L., Beaudet, A., and Watkins, K.C. (1975). Serotonin nerve terminals in adult rat neocortex. Brain Res. *100*, 563–588.

Doly, S., Valjent, E., Setola, V., Callebert, J., Hervé, D., Launay, J.M., and Maroteaux, L. (2008). Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. J. Neurosci. *28*, 2933–2940.

Donaldson, Z.R., and Hen, R. (2015). From psychiatric disorders to animal models: a bidirectional and dimensional approach. Biol. Psychiatry *77*, 15–21.

Dourish, C.T., Kennett, G.A., and Curzon, G. (1987). The 5-HT1A agonists 8-OH-DPAT, buspirone and ipsapirone attenuate stress-induced anorexia in rats. J. Psychopharmacol. (Oxford) *1*, 23–30.

Duman, R.S., Aghajanian, G.K., Sanacora, G., and Krystal, J.H. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat. Med. *22*, 238–249.

Dusticier, N., and Nieoullon, A. (1987). Comparative analysis of the effects of in vivo electrical stimulation of the frontal cortex and gamma-butyrolactone administration on dopamine and dihydroxyphenyl acetic acid (DOPAC) striatal contents in the rat. Neurochem. Int. *10*, 275–280.

Evrard, A., Malagié, I., Laporte, A.M., Boni, C., Hanoun, N., Trillat, A.C., Seif, I., De Maeyer, E., Gardier, A., Hamon, M., and Adrien, J. (2002). Altered regulation of the 5-HT system in the brain of MAO-A knock-out mice. Eur. J. Neurosci. *15*, 841–851.

Fabre, V., Beaufour, C., Evrard, A., Rioux, A., Hanoun, N., Lesch, K.P., Murphy, D.L., Lanfumey, L., Hamon, M., and Martres, M.P. (2000). Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. Eur. J. Neurosci. *12*, 2299–2310.

Grahn, R.E., Will, M.J., Hammack, S.E., Maswood, S., McQueen, M.B., Watkins, L.R., and Maier, S.F. (1999). Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. Brain Res. *826*, 35–43.

Greenwood, B.N., Foley, T.E., Day, H.E., Campisi, J., Hammack, S.H., Campeau, S., Maier, S.F., and Fleshner, M. (2003). Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. J. Neurosci. *23*, 2889–2898.

Haahr, M., Rasmussen, P., Madsen, K., Marner, L., Ratner, C., Gillings, N., Baaré, W., and Knudsen, G. (2012). Obesity is associated with high serotonin 4 receptor availability in the brain reward circuitry. Neuroimage *61*, 884–888.

Hardaway, J.A., Crowley, N.A., Bulik, C.M., and Kash, T.L. (2015). Integrated circuits and molecular components for stress and feeding: implications for eating disorders. Genes Brain Behav. *14*, 85–97.

Holmes, A., Murphy, D.L., and Crawley, J.N. (2002). Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology (Berl.) *161*, 160–167.

Jackson, C.W., Cates, M., and Lorenz, R. (2010). Pharmacotherapy of eating disorders. Nutr. Clin. Pract. *25*, 143–159.

Jean, A., Conductier, G., Manrique, C., Bouras, C., Berta, P., Hen, R., Charnay, Y., Bockaert, J., and Compan, V. (2007). Anorexia induced by activation of

serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. Proc. Natl. Acad. Sci. USA *104*, 16335–16340.

Jean, A., Laurent, L., Bockaert, J., Charnay, Y., Dusticier, N., Nieoullon, A., Barrot, M., Neve, R., and Compan, V. (2012). The nucleus accumbens 5-HTR_4-CART pathway ties anorexia to hyperactivity. Transl. Psychiatry *2*, e203.

Kennett, G.A., Dourish, C.T., and Curzon, G. (1987). Antidepressant-like action of 5-HT1A agonists and conventional antidepressants in an animal model of depression. Eur. J. Pharmacol. *134*, 265–274.

Kirby, L.G., Rice, K.C., and Valentino, R.J. (2000). Effects of corticotropin-releasing factor on neuronal activity in the serotonergic dorsal raphe nucleus. Neuropsychopharmacology *22*, 148–162.

Laurent, L., Jean, A., Manrique, C., Najimi, M., Chigr, F., and Compan, V. (2012). Anorexia and drugs of abuse abnormally suppress appetite, the result of a shared molecular signal foul-up. In Animal Model of Eating Disorders, N. Avena, ed. (Springer Book, Humana Press).

Lucas, G., Rymar, V.V., Du, J., Mnie-Filali, O., Bisgaard, C., Manta, S., Lambas-Senas, L., Wiborg, O., Haddjeri, N., Piñeyro, G., et al. (2007). Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. Neuron *55*, 712–725.

Maier, S.F., Grahn, R.E., and Watkins, L.R. (1995). 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear conditioning and interference with escape produced by exposure to inescapable shock. Behav. Neurosci. *109*, 404–412.

Maswood, S., Barter, J.E., Watkins, L.R., and Maier, S.F. (1998). Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. Brain Res. *783*, 115–120.

McGuirk, J., Muscat, R., and Willner, P. (1992). Effects of chronically administered fluoxetine and fenfluramine on food intake, body weight and the behavioural satiety sequence. Psychopharmacology (Berl.) *106*, 401–407.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., and Hen, R. (1998). Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc. Natl. Acad. Sci. USA *95*, 14476–14481.

Ratner, C., Ettrup, A., Bueter, M., Haahr, M.E., Compan, V., le Roux, C.W., Levin, B., Hansen, H.H., and Knudsen, G.M. (2012). Cerebral markers of the serotonergic system in rat models of obesity and after Roux-en-Y gastric bypass. Obesity (Silver Spring) *20*, 2133–2141.

Richardson-Jones, J.W., Craige, C.P., Guiard, B.P., Stephen, A., Metzger, K.L., Kung, H.F., Gardier, A.M., Dranovsky, A., David, D.J., Beck, S.G., et al. (2010). 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. Neuron *65*, 40–52.

Salmon, P. (2001). Effects of physical exercise on anxiety, depression, and sensitivity to stress: a unifying theory. Clin. Psychol. Rev. *21*, 33–61.

Walsh, B.T. (2013). The enigmatic persistence of anorexia nervosa. Am. J. Psychiatry *170*, 477–484.

West, G.B., Brown, J.H., and Enquist, B.J. (1997). A general model for the origin of allometric scaling laws in biology. Science *276*, 122–126.

Yadav, V.K., Oury, F., Suda, N., Liu, Z.W., Gao, X.B., Confavreux, C., Klemenhagen, K.C., Tanaka, K.F., Gingrich, J.A., Guo, X.E., et al. (2009). A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. Cell *138*, 976–989.