The nucleus accumbens 5-HTR$_4$-CART pathway ties anorexia to hyperactivity

A Jean$^{1,2,3,9}$, L Laurent$^{1,2,3,9}$, J Bockaert$^{1,2,3}$, Y Charnay$^5$, N Dusticier$^8$, A Nieoullon$^6$, M Barrot$^7$, R Neve$^8$ and V Compan$^{1,2,3,4}$

In mental diseases, the brain does not systematically adjust motor activity to feeding. Probably, the most outlined example is the association between hyperactivity and anorexia in Anorexia nervosa. The neural underpinnings of this ‘paradox’, however, are poorly elucidated. Although anorexia and hyperactivity prevail over self-preservation, both symptoms rarely exist independently, suggesting commonalities in neural pathways, most likely in the reward system. We previously discovered an addictive molecular facet of anorexia, involving production, in the nucleus accumbens (NAc), of the same transcripts stimulated in response to cocaine and amphetamine (CART) upon stimulation of the 5-HT$_4$ receptors (5-HTR$_4$) or MDMA (ecstasy). Here, we tested whether this pathway predisposes not only to anorexia but also to hyperactivity. Following food restriction, mice are expected to overeat. However, selecting hyperactive and addiction-related animal models, we observed that mice lacking 5-HTR$_{1B}$ self-imposed food restriction after deprivation and still displayed anorexia and hyperactivity after ecstasy. Decryption of the mechanisms showed a gain-of-function of 5-HTR$_4$ in the absence of 5-HTR$_{1B}$, associated with CART surplus in the NAc and not in other brain areas. NAc-5-HTR$_4$ overexpression upregulated NAC-CART, provoked anorexia and hyperactivity. NAC-5-HTR$_4$ knockdown or blockade reduced ecstasy-induced hyperactivity. Finally, NAC-CART knockdown suppressed hyperactivity upon stimulation of the NAc-5-HTR$_4$. Additionally, inactivating NAc-5-HTR$_4$ suppressed ecstasy’s preference, strengthening the rewarding facet of anorexia. In conclusion, the NAC-5-HTR$_4$/CART pathway establishes a ‘tight-junction’ between anorexia and hyperactivity, suggesting the existence of a primary functional unit susceptible to limit overeating associated with resting following homeostasis rules.

Translational Psychiatry (2012) 2, e203; doi:10.1038/tp.2012.131; published online 11 December 2012

Introduction

In mental diseases (for example, depression, anxiety, eating disorders), the brain does not systematically adjust energy expenditures to intakes, as highlighted by the ‘paradoxical’ association between restrictive diet and motor hyperactivity in Anorexia nervosa.$^{1-3}$ Here, we set out to study potential neural underpinnings of this apparent homeostatic failure. We reasoned that if at least one single molecular pathway triggers both anorexia and motor hyperactivity, its abnormal activation could prevail over homeostasis rules. In this situation, interpreting motor hyperactivity as an ‘intention’ of patients with anorexia could be challenged because their motor hyperactivity would be anorexia-dependent. In contrast, if two parallel and different pathways trigger anorexia on one hand, and motor hyperactivity on the other hand, a complex coincidence of two parallel impairments in both the feeding and motor neural networks could be in cause.

Among the cumulative neural events related to anorexia, as in most eating disorders, altered 5-HT volume transmission$^4$ is at the forefront of investigations.$^5$ With exceptions, regardless stimulation of 5-HT$_{1A}$ and 5-HT$_{2B}$ receptors (5-HTR$_{1A}$, 5-HTR$_{2B}$) in the hypothalamus,$^6$ increased activity of 5-HT transmission in brain following treatments classically reduces feeding and body weight.$^7$ For instance, the 3,4-N-methylenedioxyxymethamphetamine (MDMA, ecstasy) diminishes feeding in rodents and humans, and enhances motor hyperactivity.$^8-11$

The hypothalamus appears central in regulating feeding behavior,$^{12}$ but motivation disorders related to self-imposed food restriction despite energy demand (anorexia) may involve disturbances in the nucleus accumbens (NAc),$^{7,13,14}$ a brain structure involved in reward and feeding.$^{15-18}$ Considering the ability of 5-HT$_4$ receptors (5-HTR$_4$) knockout (KO) mice to better resist stress-induced anorexia, we detected a first example of an addictive molecular facet of anorexia.$^{14,19}$ Indeed, stimulating NAC-5-HTR$_4$, as MDMA, provokes anorexia only if production of the same transcripts stimulated in response to cocaine and amphetamine (CART) is increased in the NAc.$^{14}$

We investigated, here, whether the NAC-5-HTR$_4$/CART molecular pathway triggers not only anorexia but also motor hyperactivity. To address this possibility, we used (i) an addiction- and hyperactive-related animal model: the NAC-5-HTR$_{1B}$ KO (KO$_{1B}$) mice, (ii) the ability of MDMA to mimic...
both anorexia and hyperactivity and (iii) siRNA- and viral-mediated knockdown and surplus strategies combined to molecular and behavioral techniques.

Methods

Animals. Male KO1B, KO4 and control mice (WT1B, WT4) from heterozygous breeding (129/SvTer) were housed with food and water available ad libitum. Male WT 129/SvPas mice were used when KO mice were not required. All experiments were performed on mice aged of 4–6 months, except a set, aged of 2 months (Figures 1a and b), following the Guide for Care and Use of Laboratory Animals (authorization n°21CAE011) (see Supplementary Information).

Surgery. As described in detail, a sterile 26-gauge stainless steel guide was unilaterally implanted in the left shell NAc for infusing 1 μl of each compound in freely moving mice (1 μl/min). The localization of the injection site was assessed in each mouse (see Supplementary Information).

Pharmacological and nucleic acid treatments in freely moving mice. As established, MDMA (10 mg kg⁻¹, Sigma, L’Isle d’Abeau Chesnes, Saint-Quentin-Fallavier, France) and selective dose of 5-HTR4 antagonist, RS39604 (0.5 mg kg⁻¹, Tocris, Ellisville, USA) were dissolved in NaCl (9%) before acute intraperitoneal (i.p.) administration. The 5-HTR4 agonist BIMU8 (Tocris, Ellisville, USA) and RS39604 was injected in the NAc at selective dose (4 × 10⁻⁴ μg μl⁻¹). Acute injection in the NAc of (i) double-stranded siRNA-5-HTR4 (si5-HTR4), siCART provoked 5-HTR4 and CART downregulation compared with siRNA controls (siCt: 0.05 μg μl⁻¹), respectively; and of (ii) viral vector of mHtr4 gene (HSV-5-HTR4: 1.07 infectious units per ml, 1 μl min⁻¹), an overexpression of 5-HTR4 compared with HSV-LacZ construct (see Supplementary Information).

Biochemical analyses. As described, the levels of 5-HT and 5-HIAA were evaluated in brain tissue samples containing the NAc (+1.6 mm), striatum (+1.0 mm), dorsal hippocampus (+2.2 mm) and amygdala (+3.2 mm from the bregma) of WT4 and KO4 mice sacrificed 5 min after the end of the open-field session. As reported in detail, receptor autoradiography was performed using (125I)SB207710 and (3H)GR113808, two specific 5-HTR4 antagonists (see Supplementary Information).

Quantitative Real-Time PCR. Mice were sacrificed 3-h after the different treatments and NAc (2 × 1.2 mm³) and hypothalamus (3.9 mm³) were micro-dissected from 1 mm-thick sections to treat total mRNA and treat complementary DNA in reactions containing CART or 5-HTR4 primers, as described in detail.

Activity. Naive or feeding-tested mice were tested in the open-field after i.p. administration of NaCl or MDMA combined with (i) i.p. administration of RS39604 in KO1B, KO4, WT1B, WT4, NaCl and MDMA, respectively; **P < 0.01, ***P < 0.001 genotype and treatment interaction.

Figure 1 Anorexia-like symptoms in KO1B mice are treated with RS39604, a 5-HTR4 antagonist. (a–c) Total food intake of WT1B and KO1B mice following (a, b) 3 days of diet (−20%), over 24-h after NaCl or RS39604 (0.5 mg/kg) and (c) 24 h of 100% food deprivation, over 1 h after NaCl, MDMA (10 mg kg⁻¹, RS39604 alone, or combined with MDMA. (d-f) Total distance traveled (d, e) every 5 min (f) over 110-min after MDMA combined with RS39604 or not compared with NaCl. Data are means ± s.e.m.; n = 7–11 per group of mice treated with i.p. administration of each compound. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; $P < 0.05, $$$P < 0.001 compared with WT1B, NaCl and MDMA, respectively; $P < 0.05, $$$P < 0.001 genotype and treatment interaction.
KO4, WT1B, and WT4 mice and intra-accumbal infusion of (ii) si5-HTR4, RS93604, siCt (or NaCl) as controls in WT 129 Sv/Pas mice and (iii) HSV-5-HTR4, BIMU8 combined or not with siCART, compared with controls (NaCl, HSV-LacZ, siCt) in WT 129 Sv/Pas mice. Ten min after RS39604 injection, 3 h after injection of the siRNAs or BIMU8, or 1 day after viral infection, the traveled path length was monitored.19

Feeding tests. Classic feeding paradigms11,19 were used in fed mice or, following (i) 100% food deprivation for 24 h or (ii) 20% food-restriction for 3 consecutive days. Four days before the experiments, mice were isolated in metabolic cages for baseline period with ad libitum access to food (pellet form, 16.5% crude proteins, 3.6% crude fat, 4.6% crude fibers, 5.2% ash). Food-deprived WT1B and KO1B mice were treated with i.p. administration of NaCl or RS39604 combined or not with MDMA. WT129Sv/Pas mice received acute infusion of HSV-5-HTR4 or HSV-LacZ in the NAc and were 20% food-deprived for 3 days. The amount of food consumed (not include the spillage) was measured with 1 mg precision.

Place conditioning paradigm. An unbiased place conditioning protocol was adapted.25 Mice received i.p. administration of NaCl, MDMA combined or not with RS93604, or injection in the NAc of NaCl or RS93604, 30 min before being confined to a single conditioning zone on alternate conditioning days. A preference score is the difference between times spent by each mouse in the MDMA-, NaCl-, RS93604-, or MDMA plus RS93604-paired zone during the preconditioning and testing phases (see Supplementary Information).

Statistical analysis. Data obtained in multiple sessions over time (food intake, locomotion) were analyzed using repeated measures analysis of variance (STATVIEW 5 software, SAS Institute Inc., San Francisco, CA, USA). When effects of independent variables (treatment, genotype, time), or interactions were significant, one-way analysis of variance (treatment, time or genotype) analyses were performed. For multiple comparisons, the Scheffé F-test was used. Differences with P<0.05 were considered significant.

Results

KO1B self-imposed food restriction following restriction and displayed hyperactivity: Anorexia-like symptoms still observed after MDMA. Considering the influence of 5-HT in the potential rewarding facet of anorexia,14 we tested whether an animal model predisposes to abuse of cocaine, and to be hyperactive persists to self-restrict following food restriction. Young KO1B and WT1B mice (2 months) were then selected26,27 and deprived of 20% of their normal food rations for 3 days in their home cages (means ± s.e.m. of normal food ration for 24 h expressed in g. in WT1B: 4.80 ± 0.09 vs KO1B: 4.82 ± 0.16). When food was reintroduced and available ad libitum after the diet period, WT1B mice were eating more than their normal meal size (Figure 1a). This rebound in food intake was reduced in KO1B mice that even ate less than their predeprivation food ration after 3 days ad libitum (Figure 1a). Moreover, KO1B mice did display increased locomotion compared with saline-injected WT1B mice (Figures 1d and f), as reported.26,28

Following MDMA in KO1B mice, anorexia (Figure 1c), and hyperactivity although reduced (Figures 1e and f), are still observed, consistently with a previous study using a 5-HTR1B antagonist (GR127935).11

The absence of 5-HTR1B then predisposes to anorexia-like symptoms in challenge situations. We next tested whether this predisposition requires 5-HTR4.

Inactivating 5-HTR4 in KO1B mice suppressed their anorexia and hyperactivity. Selective inactivation of 5-HTR414 in food-restricted KO1B mice restores adaptive feeding and motor responses because the mutant did not self-restrict (Figure 1b) and were not hyperactive anymore (Figures 1d and f). Inactivating 5-HTR4 suppressed anorexia (Figure 1c) and hyperactivity (Figures 1e and f) induced by MDMA in KO1B compared with NaCl-treated KO1B mice. Identical dose of antagonist only reduced both effects in WT1B mice (Figures 1c–f), suggesting a gain-of-function of 5-HTR4 owing the absence of 5-HTR1B. To ensure this issue, we first assessed whether the gene defective-mutation of 5-HTR4 reduce hyperactivity induced by novelty and MDMA. This is the observed effect (Supplementary Figure S1). We then evaluated the density of 5-HTR4 sites and mRNA in the brain of KO1B mice.

Only the NAc of KO1B mice over-expressed both 5-HTR4 and CART whereas its hypothalamus over-expressed 5-HTR4 but down-expressed CART. Among brain areas examined (Supplementary Table S1), 5-HTR4 density (Figure 2a) and mRNA content (Figure 2b) were higher in the NAc and hypothalamus of KO1B compared with WT1B mice. The levels of CART mRNA were higher in the NAc and weaker in the hypothalamus of KO1B compared with WT1B mice (Figures 2c and d). Because CART in both the NAc and hypothalamus decreases feeding,14,29 its opposite changes could underlie the adequate feeding behavior of KO1B mice in baseline conditions.11,30 Accordingly, the ability of KO1B mice to self-restrict of food might depend on excessive NAc-5-HTR4 activity, mHtr4 activity,5-HT in the potential rewarding facet of anorexia,14 we tested

Overexpression of 5-HTR4 in the NAc ties anorexia to hyperactivity. Injecting HSV-5-HTR4 in the NAc of WT mice increased the density of NAc-5-HTR4 at 54-h postinjection (Figure 3a). The NAc-5-HTR4 mRNA content was still higher at 72 h than in control mice (HSV-LacZ), with the highest level observed at 30-h postinjection (Figure 3b). Consistently, CART mRNA content at 72-h postinjection was increased in the NAc (Figure 3c) and unchanged in the hypothalamus (Figure 3c) following injection of HSV-5-HTR4.
in the NAc, compared with controls. Stimulating NAc-5-HTR4 also increases CART mRNA content in the NAc but not in the hypothalamus.14

The feeding and motor behaviors were then analyzed. At 24-h postinjection, overexpressing NAc-5-HTR4 decreased feeding (35%, Figure 3d) and enhanced motor activity (148%, Figure 3e). HSV-5-HTR4 mice did further self-restrict after restriction compared with controls (Figure 3f), mimicking feeding responses of KO1B mice, following 20% of their normal food rations for 3 days.

Subsequently, NAc-5-HTR4 surplus increased CART, decreased feeding and increased motor activity. To circumvent the ectopic expression after viral vector injection, potential conclusion was ensured using pharmacological and RNA interference approaches, as we established.14

**In the NAc, stimulation of 5-HTR4 increases motor activity, and their blockade reduces hyperactivity.** The distance covered in the open-field is enhanced following stimulation of NAc-5-HTR4 with a specific dose of BIMU8, an agonist (198%), and unchanged following their specific blockade with antagonist or RNA interference (si5-HTR4) infused in the NAc (Figures 4a and b). In contrast, antagonism or knockdown of NAc-5-HTR4 reduced hyperactivity induced by i.p. administration of MDMA (Figure 4a).

**CART knockdown in the NAc inhibits stimulating NAc-5-HTR4-induced motor hyperactivity.** We next examined whether CART in the NAc mediates the motor effects of BIMU8, a 5-HTR4 agonist. Blocking CART with RNA interference (siCART) in the NAc suppressed the motor hyperactivity induced by stimulation of 5-HTR4 (Figure 4b).

**Inactivating 5-HTR4 suppressed MDMA’s preference in WT and reduced it in KO1B mice.** Using the conditioned place preference test, we found that The KO1B mice displayed a higher preference for MDMA than WT1B mice (Figure 5a), which is reduced after i.p. administration of a 5-HTR4 antagonist (Figure 5a). An absence of preference for MDMA is further shown when 5-HTR4 is locally inactivated in the NAc of adult WT4 mice (Figure 5b).

---

**Figure 2** KO1B mice over-expressed 5-HTR4 and CART in the NAc. (a) The density of 5-HTR4 binding site ([^3H]GR113808) of KO1B compared with WT1B mice following analyses of 3–6 brain frontal sections per structure level and per mouse (n = 5). (b) 5-HTR4 and (c) CART mRNA content in the NAc and hypothalamus (Hyp) of KO1B (n = 6) and WT1B mice (n = 7). (d) In situ hybridization of CART mRNA (scale bar: NAc, 100 μm; Hyp, 1 mm; arrows point to changes). Data are means ± s.e.m.; and *P < 0.05, **P < 0.01 compared with WT1B.
Discussion

Over the last ten decades, parallel neural systems have been described to control feeding and motor behaviors. Here, we found a first example of a molecular signal foul-up between motor hyperactivity and anorexia, providing a common pathway of control. This would lead us to reconsider the belief that patients with anorexia nervosa intend to accelerate their weight loss with over-exercise because hyperactivity could be more inevitable than deliberate.

These findings strengthen the addictive facet of restrictive diet, now also observed in mice, dispossessed of 5-HTR1B and/or endowed of a NAc-5-HTR4 surplus because they self-restrict despite an upstream ‘starter’ period of restrictive diet, believed to trigger ‘spiral’ restrictions in humans.

Animal models of anorexia-like symptoms predisposition, identified herein, mimic the activity-based anorexia rat model, and are to the best of our knowledge, unique. It is noteworthy to observe that KO1B mice persist to self-restrict their intake of food. Excluding adaptive mechanisms, KO1B mice would be expected to consume a higher amount of food because stimulating 5-HTR1B decreases feeding. This phenotype is apparently not related to the reduced activity of 5-HTR2C in KO1B mice because stimulating 5-HTR2C decreases feeding. In contrast, present results showed a

Figure 3 Overexpression of 5-HTR4 in the NAc ties anorexia to hyperactivity in WT mice. (a) Increased density of NAc-5-HTR4 binding site ([125]SB207710) in the NAc (1) but not in nearness structure (2: ventral pallidum) observed on transverse brain sections from mice infused in the NAc with HSV-5HTR4 compared with control (HSV-LacZ) and sacrificed 54-h postinfusion. Circles highlight changes and delineate the injection site in a brain section stained with hematoxylin (right upper panel), indicating an absence of damage tissue. (b) NAc-5-HTR4 mRNA content increased after infusion, in the NAc, of HSV-5HTR4 (n = 8 mice for each time point for both conditions). (c) NAc- and Hyp-CART mRNA content 72 h after injection of HSV-5HTR4 or HSV-LacZ. (d, f) Total food intake in (d) fed and (f) food-deprived (3 days, 20%) mice (d) 24 h and (f) 3 days after infusion, in the NAc, of HSV-5HTR4 (n = 6) or HSV-LacZ (n = 5). (e) Total distance traveled. Data are means ± s.e.m.; ns, P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001 compared to HSV-LacZ; & P < 0.05 differences between the NAc and hypothalamus; ### P < 0.001 interaction between time and treatment.

Translational Psychiatry
gain-of-function of 5-HTR4, consistent with the inhibitory influence of 5-HTR4 on feeding. Also, inactivating 5-HTR4 suppressed motor hyperactivity in KO1B mice, consistent with the weaker efficacy of MDMA to enhance locomotion in KO4 and 5-HTR4 antagonist-treated WT mice. The surplus of 5-HTR4 in KO1B mice further suggests a negative 5-HTR1B control of 5-HTR4 accordant with series of results; (i) The decreased levels of NAc-5-HT in KO1B mice39 because lesion of 5-HT neurons, though in rats, upregulates 5-HTR4 in brain areas including the NAc; 40 (ii) The 5-HTR1B and 5-HTR4 location does not overlap (for example, in the striatum,40,41 on 5-HT neurons24,42,43) likely related to their common binding to p11,44,45 (iii) KO1B mice are hyperactive and more ‘anxious’ under stress.19,47

Molecular events for driving self-restriction and motor hyperactivity are detected in the NAc. The NAc-5-HTR4 surplus induced sustained anorexia and motor hyperactivity, mimicking the molecular and behavioral phenotypes of KO1B mice (NAc-5-HTR4/CART surplus, anorexia, hyperactivity). Similarly, stimulation of NAc-5-HTR4 decreases feeding14 and increases locomotion. As difference in feeding responses to activation of 5-HTR subtypes, stimulation of 5-HTR1B, 5-HTR2C, 5-HTR1-7 and 5-HTR6 in the NAc did not change locomotion in basal conditions, however, in rats (Supplementary Figure S2).48–50 Likewise, blocking or silencing NAc-5-HTR4 did not change locomotion but suppressed hyperactivity induced by MDMA, in tune with the effect of the whole blockade of 5-HTR1B, 5-HTR2B and 5-HTR2C.28,51–54 In rats, inactivating NAc-5-HTR4 did not however, alter hyperactivity after MDMA,50 suggesting differences between doses and species.55,11

To the end, stimulating NAc-5-HTR4 in mice not only triggers anorexia but also hyperactivity, consistent with opposite changes in feeding and locomotion detected only in KO4 mice, compared with other 5-HTR KO mice (Supplementary Figure S2).

The present study extends observations at a molecular level. Ectopic (viral mHtr4 gene) or ‘physiological’ surplus of NAc-5-HTR4 in KO1B mice upregulates NAc-CART,
as observed following stimulation of NAc-5-HTR₄.¹⁴ A final experiment in our series bore out our hypothesis because NAc-CART knockdown suppressed not only anorexia¹⁴ but also motor hyperactivity induced by NAc-5-HTR₄ stimulation. In addition, locomotion is unchanged following CART peptide⁵⁶ or siCART injection in the NAc. Identifying the cellular origin of this action would require long investigations. Nonetheless, NAc-neurons containing GABA projecting to the lateral hypothalamus express CART¹⁴,⁵⁷–⁵⁹ and might also express 5-HTR₄ (Supplementary Figure S2).²⁴,⁴⁰,⁴³,⁵⁸ Injecting si5-HTR₄ in the NAc decreased the density of 5-HTR₄ not only in the NAc but also in the lateral hypothalamus (– 14%, not illustrated). The 5-HTR₄ located on these neurons may influence feeding and locomotion (Supplementary Figure S2) because the lateral hypothalamus, in relation to the NAc, controls feeding and its stimulation enhances locomotion in the activity-based rat model for anorexia nervosa.¹³,⁶⁰–⁶³ Collo- localization of 5-HTR₄/CART is more conceivable than in two different neuronal populations, considering the 5-HTR₄ control of CART within the NAc via a cAMP/PKA signaling pathway.¹⁴ Interestingly, it appears that 5-HT receptors expressed in the different subnuclei of the hypothalamus (arcuate nucleus: 5-HT₁B, 5-HT₂C) may provoke an anorexia associated or not with different changes in locomotion, as induced by fenfluramine⁶⁴,⁶⁵ that increase, ¹⁹ serotonergic activity: viral transfections, increased cAMP production in the NAc⁷¹ upon activation of serotonin 5-HT₄ receptors is mediated by increases in CART in the nucleus accumbens. Proc Natl Acad Sci U S A 2007; 104: 16335–16340. 5-HT₄ receptors exert positive feedback on serotonergic activity: viral transfections, increased cAMP production in the NAc⁷¹ upon stimulation of the 5-HT₄ in freely moving mice could trigger addiction.

In conclusion, motor hyperactivity is anorexia-dependent upon activation of the NAc-5-HTR₄/CART pathway. Probably, a rewarding effect associated with energy expenditure (anorexia/hyperactivity) may facilitate to limit excessive intakes (overeating/resting). Present and previous findings bring out at least two modes of action of 5-HT to regulate feeding. In baseline conditions, feeding may be regulated via the hypothalamic 5-HT₄/CART pathway but, when motivation comes into play, the NAc-5-HT₄/5-HT₁B/CART pathway might prevail over the autonomic nervous control of feeding because NAc-5-HT₄/CART surplus makes the brain ‘silent’ to energy loss. Finally, it is conceivable that an anorectic-rewarding pathway of the NAc predisposes animals to a possible dependence on restrictive diet and hyperactivity, two hallmarks of anorexia nervosa.

Conflict of interest
Authors declare no conflict of interest.
31. Holtkamp K, Hebebrand J, Herpertz-Dahlmann B. The contribution of anxiety and food restriction on physical activity levels in acute anorexia nervosa. Int J Eat Disord 2004; 36: 163–171.

32. Konttinen H, Silvertonen K, Sarlio-Lahteenkorva S, Mannisto S, Haukkala A. Emotional eating and physical activity self-efficacy as pathways in the association between depressive symptoms and adiposity indicators. Am J Clin Nutr 2010; 92: 1031–1039.

33. Steanovv TS, Vekova AM, Kurktschiev DP, Temelkova-Kurktschiev TS. Relationship of 5-HT1B knockout mice lacking the 5-HT1B receptor. Psychopharmacology (Berl) 1999; 141: 154–161.

34. van Kuyck K, Casteels C, Vermaelen P, Bormans G, Nuttin B, Van Laere K. Motor- and food-related metabolic cerebral changes in the activity-based rat model for anorexia nervosa: a voxel-based mPET study. Neuroimage 2007; 35: 214–221.

35. van Kuyck K, Castels C, Vermaelen P, Bormans G, Nuttin B, Van Laere K. Motor- and food-related metabolic cerebral changes in the activity-based rat model for anorexia nervosa: a voxel-based mPET study. Neuroimage 2007; 35: 214–221.

36. Vickers DP, Dourish CT, Kennett GA. Evidence that hypophagia induced by d-fenfluramine and d-norfenfluramine in the rat is mediated by 5-HT2C receptors. Neuropeptides 2001; 41: 200–209.

37. Clifton PG, Lee MD, Somervuele EM, Kennett GA, Dourish CT. 5-HT1B receptor knockout mice show a compensatory reduction in 5-HT2C receptor function. Eur J Neurosci 2003; 17: 185–190.

38. Kennett GA, Curzon G. Evidence that hypophagia induced by mCPP and TFMP requires 5-HT1C and 5-HT1B receptors; hypophagia induced by RU 24969 only requires 5-HT1B receptors. Psychopharmacology (Berl) 1988; 96: 93–100.

39. Ase AR, Reader TA, Hen R, Radl M, Descamps L. Altered serotonin and dopamine metabolism in the CNS of 5-HT1A(−/−) or 5-HT1B(−/−) knockout mice. J Neurochem 2000; 75: 2415–2426.

40. Compan V, Daszuta A. Selective increases in serotonin 5-HT4 receptors in rat basal ganglia and hippocampus. Eur J Neurosci 1996; 8: 2593–2598.

41. Compan V, Segui L, Buhot MC, Daszuta A. Selective increases in serotonin 5-HT1B/1D and 5-HT2A/2C binding sites in adult rat basal ganglia following lesions of serotonergic neurons. Brain Res 1998; 793: 103–111.

42. Doucet E, Pohl M, Fattacchin CM, Adrien J, Mestekawy SE, Hamon M. In situ hybridization evidence for the synthesis of 5-HT1B receptor in serotonergic neurons of anterior cingulate cortex in the rat brain. Synapse 1995; 19: 16–28.

43. Victor MT, Cortes R, Mengo G. Serotonin 5-HT4 receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. J Neurosci 2005; 25: 418–439.

44. Vickers DP, Dourish CT, Kennett GA. Evidence that hypophagia induced by d-fenfluramine and d-norfenfluramine in the rat is mediated by 5-HT2C receptors. Neuropeptides 2001; 41: 200–209.

45. Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, Qi H, Svenningsson P. 5-Hydroxytryptamine 5-HT4 receptors in the nucleus accumbens are specifically involved in the appetite suppressant and not locomotor stimulant effects of MDMA (ecstasy). Psychopharmacology (Berl) 2010; 213: 355–363.

46. Bankson MG, Cunningham KA. 3,4-Methylenedioxymethamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. J Pharmacol Exp Ther 2001; 297: 846–852.

47. Geyer MA. Serotonergic functions in arousal and motor activity. Behav Brain Res 1996; 73: 31–38.

48. Baumann MH, Clark RD, Rothman RB. Locomotor stimulation produced by 3,4-methylenedioxymethamphetamine (MDMA) is correlated with dialysate levels of serotonin and dopamine in rat brain. Pharmacol Biochem Behav 2008; 90: 208–217.

49. Doly S, Valjent E, Setola V, Callebert J, Herve D, Launay JM et al. Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. J Neurosci 2008; 28: 2933–2940.

50. Colado ML, O’Shea E, Green AR. Acute and long-term effects of MDMA on cerebral dopamine biochemistry and function. Psychopharmacology (Berl) 2004; 173: 249–263.

51. Jaworski JN, Kozel MA, Philpot KB, Kuhr MJ. Intracerebral injection of CART (cocaine-amphetamine regulated transcript) peptide reduces cocaine-induced locomotor activity. J Pharmacol Exp Ther 2003; 307: 1038–1044.

52. Yang SC, Sheih KR, Lu HY. Cocaine- and amphetamine-regulated transcript in the nucleus accumbens participates in the regulation of feeding behavior in rats. Neuroscience 2005; 133: 841–851.

53. Hubert GW, Kuhr MJ. Co-localization of CART with substance P but not enkephalin in the rat nucleus accumbens. Brain Res 2005; 1050: 8–14.

54. Hubert GW, Manvich DP, Kuhr MJ. Cocaine and amphetamine-regulated transcript-containing neurons in the nucleus accumbens project to the ventral pallidum and in may inhibit cocaine-induced locomotion. Neuroscience 2009; 165: 179–187.

55. Maldonado-frizany CS, Swanson CJ, Kelley AE. Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. J Neurosci 1995; 15: 6779–6788.

56. Stratford TR, Kelley AE. Evidence of a functional relationship between the nucleus accumbens shell and lateral hypothalamus subserving the control of feeding behavior. J Neurosci 1999; 19: 11040–11048.

57. Stratford TR, Swanson CJ, Kelley A. Specifc changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. Behav Brain Res 1998; 93: 43–50.

58. Verfagen LA, Luijendijk MC, de Groot JW, van Dommelen LP, Klimstra AG, Adan RA et al. Anticipation of meals during restricted feeding increases activity in the hypothalamus in rats. Eur J Neurosci 2011; 34: 1485–1491.

59. Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL et al. Activation of central melanocortin pathways by fenfluramine. Science 2002; 297: 609–611.

60. Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-Jones E et al. Serotonin reciprocally regulates melanocortin neurons to modulate food intake. Neuro 2006; 51: 239–249.

61. Aulakhs CS, Hii JL, Wozniak NM, Murphy DL. Fenfluramine-suppressed food intake and locomotor activity is differentially altered by the selective type A monoamine oxidase inhibitor dihydroxy. Psychopharmacology (Berl) 1986; 95: 313–317.

62. Heffernan TG, Seiden LS. Possible involvement of serotonergic neurons in the reduction of locomotor hyperactivity caused by amphetamine in neonatal rats depleted of brain dopamine. Brain Res 1982; 234: 81–90.

63. Vickers SP, Benwell KR, Porter RH, Bickerdike MJ, Kennett GA. Dourish CT. Comparative effects of continuous infusion of mCPP, 50–5075 and d-fenfluramine on food intake, water intake, body weight and locomotor activity in rats. Br J Pharmacol 2000; 130: 1305–1314.

64. Doly S, Bertran-Gonzalez J, Callebert J, Bruneau A, Banas SM, Belmer A et al. Role of serotonin via 5-HT2B receptors in the reinforcing effects of MDMA in mice. PLoS ONE 2003; 4: e7952.

65. Dumais A, Bouhelal R, Sebbini M, Cory R, Bockaert J. A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. Mol Pharmacol 1988; 34: 880–887.

66. Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ et al. Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J Neurosci 1998; 18: 1848–1859.

67. Rogge G, Jones D, Hubert GW, Lin Y, Kuhr MJ. CART peptides: regulators of body weight, reward and other functions. Nat Rev Neurosci 2008; 9: 747–758.

Translational Psychiatry is an open-access journal published by Nature Publishing Group. This work is licensed under the Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 Unported License. To view a copy of this license, visit creativescommons.org/licenses/by-nc-nd/3.0/