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

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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.

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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*-methylenedioxymethamphetamine (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 5-HTR$_{1B}$ KO (KO$_{1B}$) mice, (ii) the ability of MDMA to mimic

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both anorexia and hyperactivity and (iii) siRNA- and viral-mediated knockdown and surplus strategies combined to molecular and behavioral techniques.

Methods

Animals. Male KO_{1B}, KO_4 and control mice (WT_{1B}, WT_4) from heterozygous breeding (129/SvTer)[19,20] were housed with food and water available *ad libitum*.[14] 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,[14] 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,[11,14,21] MDMA (10 mg kg^{-1}, Sigma, L'Isle d'Abeau Chesnes, Saint-Quentin-Fallavier, France) and selective dose of 5-HTR_4 antagonist, RS39604 (0.5 mg kg^{-1}, Tocris, Ellisville, USA) were dissolved in NaCl (9%) before acute intraperitoneal (i.p.) administration. The 5-HTR_4 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-HTR_4 (si5-HTR_4), siCART provoked 5-HTR_4 and CART downregulation compared with siRNA controls (siCt: 0.05 μg μl^{-1}), respectively; and of (ii) viral vector of *mHtr*4 gene (HSV-5-HTR_4; 10^7 infectious units per ml, 1 μl min^{-1}), an overexpression of 5-HTR_4 compared with HSV-LacZ construct (see Supplementary Information).

Biochemical analyses. As described,[22] 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)[23] of WT_4 and KO_4 mice sacrificed 5 min after the end of the open-field session. As reported in detail,[14,19] receptor autoradiography was performed using (^{125}I)SB207710 and (^{3}H)GR113808, two specific 5-HTR_4 antagonists (see Supplementary Information).

Quantitative Real-Time PCR. Mice were sacrificed 3-h after the different treatments and NAc (2×1.2 mm^3) and hypothalamus (3.9 mm^3) were micro-dissected from 1 mm-thick sections to treat total mRNA and treat complementary DNA in reactions containing CART or 5-HTR_4 primers, as described in detail.[14,24]

Activity. Naive or feeding-tested mice were tested in the open-field[19] after i.p. administration of NaCl or MDMA combined with (i) i.p. administration of RS39604 in KO_{1B},

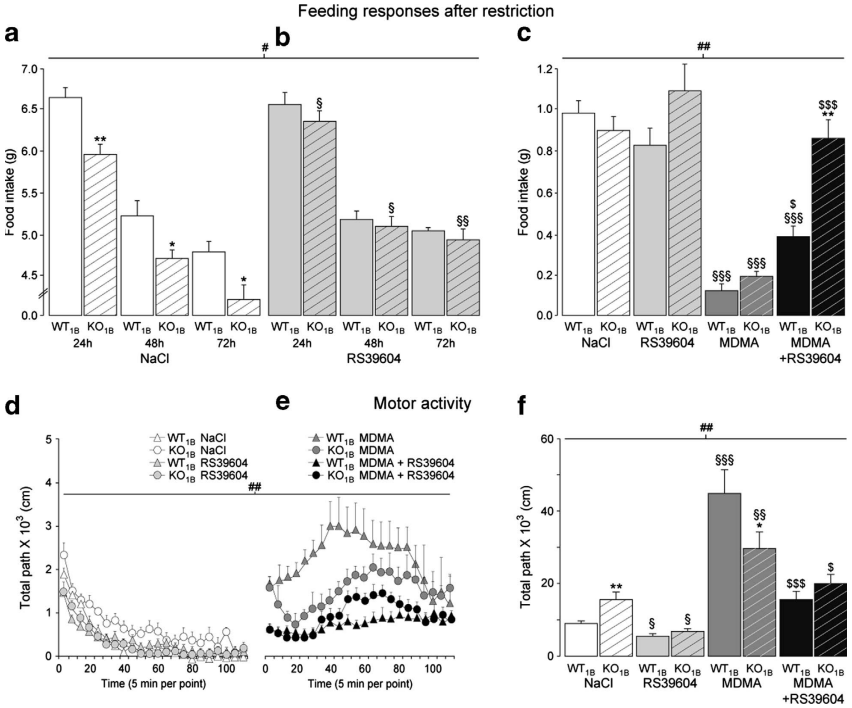

Figure 1 Anorexia-like symptoms in KO_{1B} mice are treated with RS39604, a 5-HTR_4 antagonist. (**a**–**c**) Total food intake of WT_{1B} and KO_{1B} mice following (**a**, **b**) 3 days of diet (−20%), over 24-h after NaCl or RS39604 (0.5 mg/kg) and (**c**) 24 h of 100% food deprivation, over 1 h after 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. *$P<0.05$, **$P<0.01$; $^{\S}P<0.05$, $^{\S\S}P<0.01$, $^{\S\S\S}P<0.001$; $^{\$}P<0.05$, $^{\$\$\$}P<0.001$ compared with WT_{1B}, NaCl and MDMA, respectively; $^{\#}P<0.05$, $^{\#\#}P<0.01$ genotype and treatment interaction.

KO_4, WT_{1B} and WT_4 mice and intra-accumbal infusion of (ii) si5-HTR_4, RS39604, siCt (or NaCl) as controls in WT 129 Sv/Pas mice and (iii) HSV-5-HTR_4, 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 paradigms[11,19] 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 WT_{1B} and KO_{1B} 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-HTR_4 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 RS39604, or injection in the NAc of NaCl or RS39604, 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-, RS39604-, or MDMA plus RS39604-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

KO_{1B} 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 KO_{1B} and WT_{1B} mice (2 months) were then selected[26,27] 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 WT_{1B}: 4.80 ± 0.09 vs KO_{1B}: 4.82 ± 0.16). When food was reintroduced and available *ad libitum* after the diet period, WT_{1B} mice were eating more than their normal meal size (Figure 1a). This rebound in food intake was reduced in KO_{1B} mice that even ate less than their predeprivation food ration after 3 days *ad libitum* (Figure 1a). Moreover, KO_{1B} mice did display increased locomotion compared with saline-injected WT_{1B} mice (Figures 1d and f), as reported.[26,28]

Following MDMA in KO_{1B} mice, anorexia (Figure 1c), and hyperactivity although reduced (Figures 1e and f), are still observed, consistently with a previous study using a 5-HTR_{1B} antagonist (GR127935).[11]

The absence of 5-HTR_{1B} then predisposes to anorexia-like symptoms in challenge situations. We next tested whether this predisposition requires 5-HTR_4.

Inactivating 5-HTR_4 in KO_{1B} mice suppressed their anorexia and hyperactivity. Selective inactivation of 5-HTR_4[14] in food-restricted KO_{1B} 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-HTR_4 suppressed anorexia (Figure 1c) and hyperactivity (Figures 1e and f) induced by MDMA in KO_{1B} compared with NaCl-treated KO_{1B} mice. Identical dose of antagonist only reduced both effects in WT_{1B} mice (Figures 1c–f), suggesting a gain-of-function of 5-HTR_4 owing the absence of 5-HTR_{1B}. To ensure this issue, we first assessed whether the gene defective-mutation of 5-HTR_4 reduce hyperactivity induced by novelty and MDMA. This is the observed effect (Supplementary Figure S1). We then evaluated the density of 5-HTR_4 sites and mRNA in the brain of KO_{1B} mice.

Only the NAc of KO_{1B} mice over-expressed both 5-HTR_4 and CART whereas its hypothalamus over-expressed 5-HTR_4 but down-expressed CART. Among brain areas examined (Supplementary Table S1), 5-HTR_4 density (Figure 2a) and mRNA content (Figure 2b) were higher in the NAc and hypothalamus of KO_{1B} compared with WT_{1B} mice. The levels of CART mRNA were higher in the NAc and weaker in the hypothalamus of KO_{1B} compared with WT_{1B} 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 KO_{1B} mice in baseline conditions.[11,30] Accordingly, the ability of KO_{1B} mice to self-restrict of food might depend on excessive NAc-5-HTR_4. We next focused on the NAc because additionally, marked increases in 5-HT metabolism were not detected in the NAc of KO_4 mice following the open-field session (Supplementary Table S2). To avoid bias of adaptive changes in KO mice and determine whether a 5-HTR_4 surplus within the NAc triggers both anorexia and hyperactivity, *mHtr*4 gene (HSV-5-HTR_4) was transferred in the NAc of WT mice.

Overexpression of 5-HTR_4 in the NAc ties anorexia to hyperactivity. Injecting HSV-5-HTR_4 in the NAc of WT mice increased the density of NAc-5-HTR_4 at 54-h postinjection (Figure 3a). The NAc-5-HTR_4 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-HTR_4

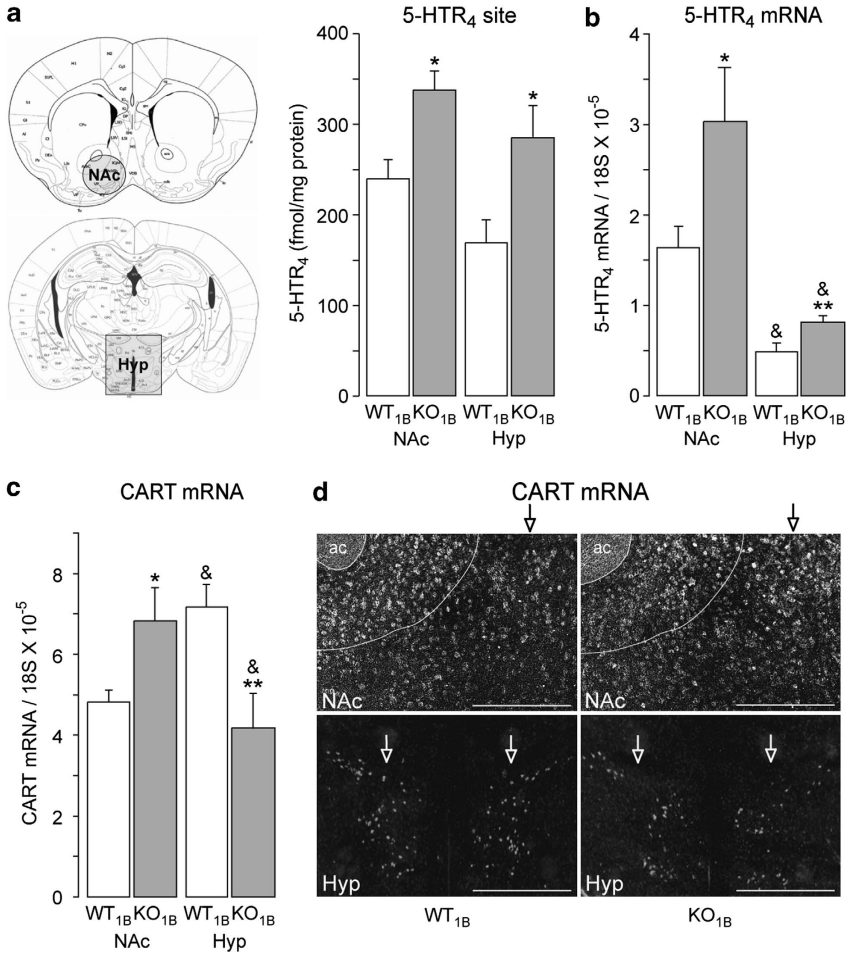

Figure 2 KO_{1B} mice over-expressed 5-HTR_4 and CART in the NAc. (**a**) The density of 5-HTR_4 binding site (^{3}H)GR113808) of KO_{1B} compared with WT_{1B} mice following analyses of 3–6 brain frontal sections per structure level and per mouse ($n=5$). (**b**) 5-HTR_4 and (**c**) CART mRNA content in the NAc and hypothalamus (Hyp) of KO_{1B} ($n=6$) and WT_{1B} 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 $P<0.05$ difference between the NAc and Hyp in either WT_{1B} or KO_{1B} mice; $^{*}P<0.05$, $^{**}P<0.01$ compared with WT_{1B}.

in the NAc, compared with controls. Stimulating NAc-5-HTR_4 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-HTR_4 decreased feeding (35%, Figure 3d) and enhanced motor activity (148%, Figure 3e). HSV-5-HTR_4 mice did further self-restrict after restriction compared with controls (Figure 3f), mimicking feeding responses of KO_{1B} mice, following 20% of their normal food rations for 3 days.

Subsequently, NAc-5-HTR_4 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-HTR_4 increases motor activity, and their blockade reduces hyperactivity. The distance covered in the open-field is enhanced following stimulation of NAc-5-HTR_4 with a specific dose of BIMU8, an agonist (198%), and unchanged following their specific blockade with antagonist or RNA interference (si5-HTR_4) infused in the NAc (Figures 4a and b). In contrast, antagonism or knockdown of NAc-5-HTR_4 reduced hyperactivity induced by i.p. administration of MDMA (Figure 4a).

CART knockdown in the NAc inhibits stimulating NAc-5-HTR_4-induced motor hyperactivity. We next examined whether CART in the NAc mediates the motor effects of BIMU8, a 5-HTR_4 agonist. Blocking CART with RNA interference (siCART) in the NAc suppressed the motor hyperactivity induced by stimulation of 5-HTR_4 (Figure 4b).

We finally tested whether MDMA's preference requires 5-HTR_4 because a rewarding effect could prevail over self-preservation.

Inactivating 5-HTR_4 suppressed MDMA's preference in WT and reduced it in KO_{1B} mice. Using the conditioned place preference test, we found that The KO_{1B} mice displayed a higher preference for MDMA than WT_{1B} mice (Figure 5a), which is reduced after i.p. administration of a 5-HTR_4 antagonist (Figure 5a). An absence of preference for MDMA is further shown when 5-HTR_4 is locally inactivated in the NAc of adult WT_4 mice (Figure 5b).

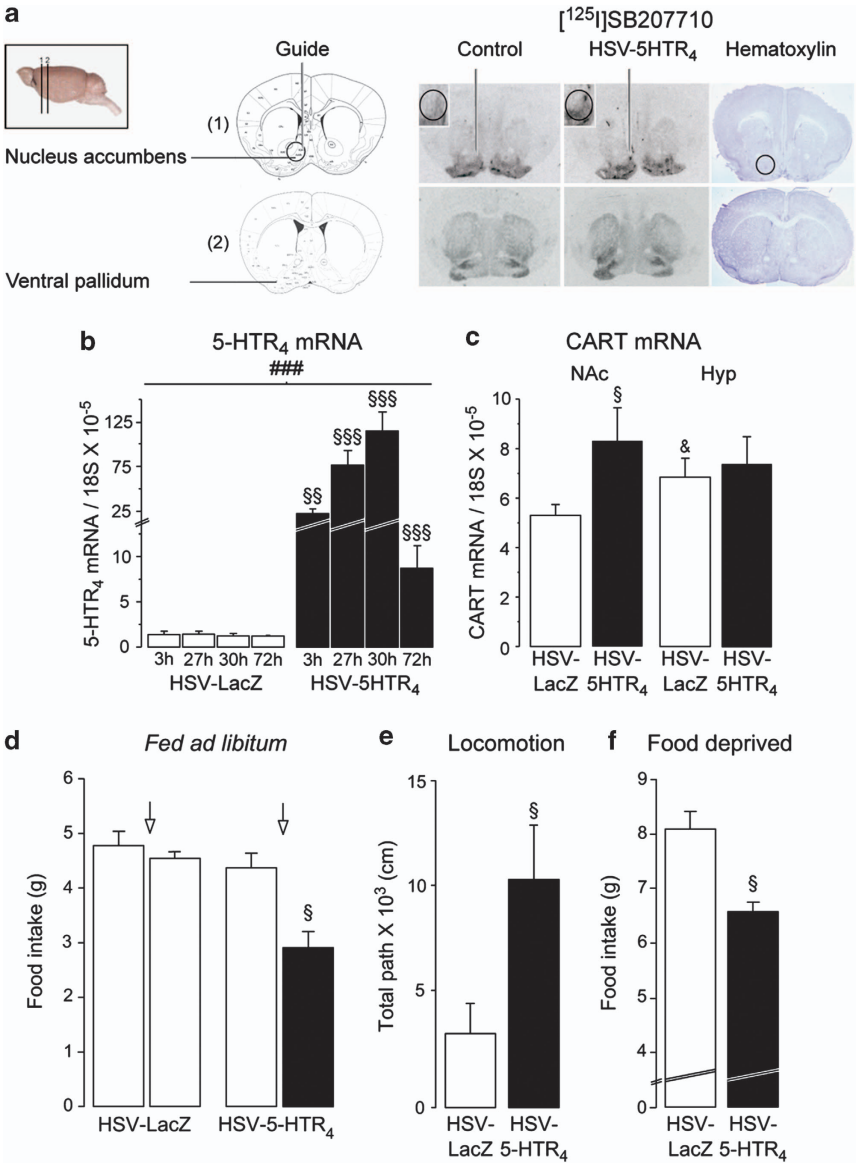

Figure 3 Overexpression of 5-HTR$_4$ in the NAc ties anorexia to hyperactivity in WT mice. (**a**) Increased density of NAc-5-HTR$_4$ binding site ([^{125}I]SB207710) in the NAc (1) but not in nearness structure (2: ventral pallidum) observed on transverse brain sections from mice infused in the NAc with HSV-5HTR$_4$ 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-HTR$_4$ mRNA content increased after infusion, in the NAc, of HSV-5-HTR$_4$ ($n=8$ mice for each time point for both conditions). (**c**) NAc- and Hyp-CART mRNA content 72 h after injection of HSV-5-HTR$_4$ 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-5-HTR$_4$ ($n=6$) or HSV-LacZ ($n=5$). (**e**) Total distance traveled. Data are means ± s.e.m.; $^{\S}P<0.05$, $^{\S\S}P<0.01$, $^{\S\S\S}P<0.001$ compared to HSV-LacZ; $^{\&}P<0.05$ differences between the NAc and hypothalamus; $^{\#\#\#}P<0.001$ interaction between time and treatment.

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[3,31–33] 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-HTR$_{1B}$ and/or endowed of a NAc-5-HTR$_4$ surplus because they self-restrict despite an upstream 'starter' period of restrictive diet, believed to trigger 'spiral' restrictions in humans.[34]

Animal models of anorexia-like symptoms predisposition, identified herein, mimic the activity-based anorexia rat model,[35] and are to the best of our knowledge, unique. It is noteworthy to observe that KO$_{1B}$ mice persist to self-restrict their intake of food. Excluding adaptive mechanisms, KO$_{1B}$ mice would be expected to consume a higher amount of food because stimulating 5-HTR$_{1B}$ decreases feeding.[11,36] This phenotype is apparently not related to the reduced activity of 5-HTR$_{2C}$ in KO$_{1B}$ mice[37] because stimulating 5-HTR$_{2C}$ decreases feeding.[38] In contrast, present results showed a

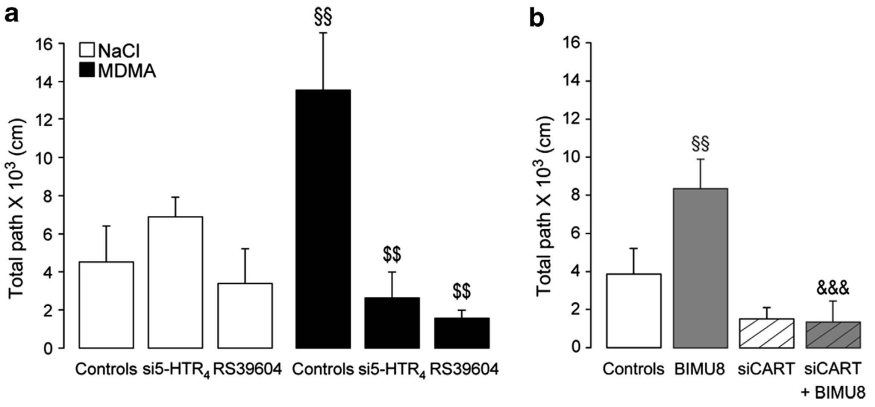

Figure 4 Motor hyperactivity induced by stimulation of NAc-5-HTR$_4$ requires CART. (**a**) Total distance traveled over 110 min after i.p. administration of NaCl or MDMA plus an intra-accumbal infusion of control solution (NaCl or si5-HTR$_4$ control: siCt), si5-HTR$_4$, 5-HTR$_4$ antagonist (RS39604) and (**b**) of WT mice after an intra-accumbal infusion of controls, 5-HTR$_4$ agonist (BIMU8), siCART or BIMU8 plus siCART. Data are means ± s.e.m.; $n = 5$–10 mice per each group, treated with MDMA (10 mg Kg^{-1}), siRNA (0.05 μg μl^{-1}), viral vector (10^7 infectious units per ml), RS39064 or BIMU8 (4×10^{-4} μg μl^{-1}). $^{§§}P<0.01$, $^{$$}P<0.01$, $^{&&&}P<0.001$ compared to controls, MDMA and BIMU8, respectively.

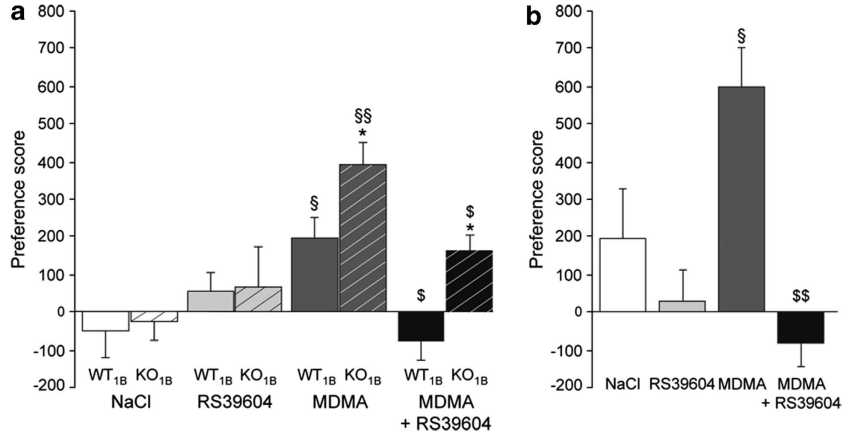

Figure 5 MDMA's preference involves 5-HTR$_4$ in an unbiased conditioned place preference test. (**a**) Score preference in WT$_{1B}$ and KO$_{1B}$ mice treated with i.p. administration of NaCl, MDMA (10 mg kg^{-1}), combined or not with RS39604 (0.5 mg kg^{-1}) and (**b**) in WT mice treated with i.p. administration of NaCl or MDMA (10 mg kg^{-1}) plus intra-accumbal infusion of NaCl or RS39604 (4×10^{-4} μg μl^{-1}). Data are means ± s.e.m.; $n = 7$–9 mice per group. $^{*}P<0.05$ compared to WT$_{1B}$; $^{§}P<0.05$, $^{§§}P<0.01$ compared with NaCl; $^{$}P<0.05$, $^{$$}P<0.01$ compared with MDMA.

gain-of-function of 5-HTR$_4$, consistent with the inhibitory influence of 5-HTR$_4$ on feeding.[14,19] Also, inactivating 5-HTR$_4$ suppressed motor hyperactivity in KO$_{1B}$ mice, consistently with the weaker efficacy of MDMA to enhance locomotion in KO$_4$ and 5-HTR$_4$ antagonist-treated WT mice.

The surplus of 5-HTR$_4$ in KO$_{1B}$ mice further suggests a negative 5-HTR$_{1B}$ control of 5-HTR$_4$ accordant with series of results; (i) The decreased levels of NAc-5-HT in KO$_{1B}$ mice[39] because lesion of 5-HT neurons, though in rats, upregulates 5-HTR$_4$ in brain areas including the NAc;[40] (ii) The 5-HTR$_{1B}$ and 5-HTR$_4$ location does not overlap (for example. in the striatum,[40,41] on 5-HT neurons[24,42,43]) likely related to their common binding to p11;[44,45] (iii) KO$_{1B}$ mice are hyperactive and less 'anxious'[46] while KO$_4$ mice are hypoactive and more 'anxious' under stress.[19,47]

Molecular events for driving self-restriction and motor hyperactivity are detected in the NAc. The NAc-5-HTR$_4$ surplus induced sustained anorexia and motor hyperactivity, mimicking the molecular and behavioral phenotypes of KO$_{1B}$ mice (NAc-5-HTR$_4$/CART surplus, anorexia, hyperactivity). Similarly, stimulation of NAc-5-HTR$_4$ decreases feeding[14] and increases locomotion.

As difference in feeding responses to activation of 5-HTR subtypes, stimulation of 5-HTR$_{1B,}$ 5-HTR$_{2C}$, 5-HTR$_{1\text{-}7}$ and 5-HTR$_6$ in the NAc did not change locomotion in basal conditions, however, in rats (Supplementary Figure S2).[48–50] Likewise, blocking or silencing NAc-5-HTR$_4$ did not change locomotion but suppressed hyperactivity induced by MDMA, in tune with the effect of the whole blockade of 5-HTR$_{1B}$, 5-HTR$_{2B}$ and 5-HTR$_{2C}$.[28,51–54] In rats, inactivating NAc-5-HTR$_4$ did not however, alter hyperactivity after MDMA,[50] suggesting differences between doses and species.[55,11]

To the end, stimulating NAc-5-HTR$_4$ in mice not only triggers anorexia but also hyperactivity, consistent with opposite changes in feeding and locomotion detected only in KO$_4$ 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-HTR$_4$ in KO$_{1B}$ mice upregulates NAc-CART,

as observed following stimulation of NAc-5-HTR_4.[14] A final experiment in our series bore out our hypothesis because NAc-CART knockdown suppressed not only anorexia[14] but also motor hyperactivity induced by NAc-5-HTR_4 stimulation. In addition, locomotion is unchanged following CART peptide[56] 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 CART[14,57–59] and might also express 5-HTR_4 (Supplementary Figure S2).[24,40,43,58] Injecting si5-HTR_4 in the NAc decreased the density of 5-HTR_4 not only in the NAc but also in the lateral hypothalamus (− 14%, not illustrated). The 5-HTR_4 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*.[15,60–63] Colocalization of 5-HTR_4/CART is more conceivable than in two different neuronal populations, considering the 5-HTR_4 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-HTR_{1B}, 5-HTR_{2C}) may provoke an anorexia associated or not with different changes in locomotion, as induced by fenfluramine[64,65] that increase,[51] decrease[66,67] or does not modify locomotion[68] while, 5-HTR_4 likely located on the afferent neurons of the NAc to the lateral hypothalamus may provoke an anorexia associated with motor hyperactivity.

Finally, the present study suggests that activation of the NAc-5-HTR_4 promotes a rewarding effect because (i) mice with NAc-5-HTR_4 surplus limit their food intake despite energy requirements; (ii) inactivating NAc-5-HTR_4 can reduce and even suppress the preference for MDMA, as also observed in 5-HTR_{2B} KO mice.[69] Chronic stimulation may desensitize 5-HTR_4[70] and has been excluded from our subtasks. Nonetheless, increased cAMP production in the NAc[71] upon stimulation of the 5-HTR_4 in freely moving mice[14] could trigger addiction.

In conclusion, motor hyperactivity is anorexia-dependent upon activation of the NAc-5-HTR_4/CART pathway. Probably, a rewarding effect associated with energy expenditure (anorexia/hyperactivity) may facilitate to limit excessive intakes (overeating/resting). Present and previous findings[6,14,64,72] 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-HTR_{2C}/CART pathway but, when motivation comes into play, the NAc-5-HTR_4/5-HTR_{1B}/CART pathway might prevail over the autonomic nervous control of feeding because NAc-5-HTR_4/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.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)