Cholinergic dysfunction in the dorsal striatum promotes habit formation and maladaptive eating

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Introduction

Eating disorders such as anorexia nervosa, bulimia nervosa, and binge eating disorder are severe psychiatric illnesses that are difficult to treat (1). Anorexia nervosa, in particular, has the highest mortality rate of all mental disorders (2). Anorexia nervosa includes persistent restriction of food intake (restrictive-only) and binge eating followed by purging. In contrast, bulimia nervosa and binge eating disorder involve the consumption of a large amount of food in a short period of time, accompanied by a loss of control over eating (3). Currently, there is an unmet need to develop specific treatments for eating disorders.

Eating disorders are attributed to a combination of genetic, neurobiological, psychological, and socio-educational factors (1, 4). Eating disorders are frequently associated with a lack of cognitive flexibility, aberrant reward processing, or obsessive-compulsive disorder (5, 6). The inability to interrupt a behavior despite negative consequences suggests a common basis with compulsive disorders (7). An imbalance between goal-directed behaviors and habitual behaviors has been suspected to be a central cause of eating disorders (8, 9).

Goal-directed behaviors are observed when individuals are engaged in reaching a specific objective. Hence, action selection is governed by its outcome. Habits emerge from goal-directed learning after behaviors become automated as a result of repetition. Habits are driven by environmental stimuli and are independent from outcomes (10). The dorsal striatum is a key hub for the regulation of goal-directed behaviors and habits (10). While the dorsomedial striatum (caudate in humans) is crucial for the acquisition of flexible goal-directed behaviors, the dorsolateral striatum (putamen in humans) modulates rigid habitual behaviors. Dopaminergic (DA) transmission is a pivotal regulator of the transition from goal-directed behaviors to habits (11, 12).

Dysregulation of habit formation has been recently proposed as pivotal to eating disorders. Here, we report that a subset of patients suffering from restrictive anorexia nervosa have enhanced habit formation compared with healthy controls. Habit formation is modulated by striatal cholinergic interneurons. These interneurons express vesicular transporters for acetylcholine (VACHT) and glutamate (VGLUT3) and use acetylcholine/glutamate cotransmission to regulate striatal functions. Using mice with genetically silenced VACHT (VACHT conditional KO, VACHTcKO) or VGLUT3 (VGLUT3cKO), we investigated the roles that acetylcholine and glutamate released by cholinergic interneurons play in habit formation and maladaptive eating. Silencing glutamate favored goal-directed behaviors and had no impact on eating behavior. In contrast, VACHTcKO mice were more prone to habits and maladaptive eating. Specific deletion of VACHT in the dorsomedial striatum of adult mice was sufficient to phenocopy maladaptive eating behaviors of VACHTcKO mice. Interestingly, VACHTcKO mice had reduced dopamine release in the dorsomedial striatum but not in the dorsolateral striatum. The dysfunctional eating behavior of VACHTcKO mice was alleviated by donepezil and by γ-DOPA, confirming an acetylcholine/dopamine deficit. Our study reveals that loss of acetylcholine leads to a dopamine imbalance in striatal compartments, thereby promoting habits and vulnerability to maladaptive eating in mice.
Cholinergic interneurons (ChIs) from the striatum are also major regulators of striatal functions and exert complex effects over DA efflux (13, 14). ChIs express both the vesicular acetylcholine transporter (VACHT; Slc18a3) and the atypical vesicular glutamate transporter type 3 (VGLUT3; Slc17a8) (15). Thus, ChIs release both acetylcholine (ACh) and glutamate (Glu) to regulate striatal functions (16, 17). ACh and Glu released from ChIs differentially affect DA transmission in the ventral striatum and reward-guided behaviors (13, 18). Interestingly, rodents with selective ablation of ChIs present deficits of behavioral flexibility and increased compulsive behavior (19, 20). However, how each individual neurotransmitter (ACh or Glu) released by ChIs affects the transition between goal-directed behaviors and habits, and therefore potentially influences maladaptive eating, is still obscure (21, 22).

Using a computer-based neurocognitive task to evaluate the balance between goal-directed behaviors and habits in humans (23, 24), we here confirm that a subgroup of patients with restrictive anorexia nervosa are more prone to habitual behaviors than healthy controls. Using mouse models, we show that disrupting ACh/Glu cotransmission, by genetically inactivating VAChT Cholinergic interneurons (ChIs) from the striatum are also major regulators of striatal functions and exert complex effects over DA efflux (13, 14). ChIs express both the vesicular acetylcholine transporter (VACHT; Slc18a3) and the atypical vesicular glutamate transporter type 3 (VGLUT3; Slc17a8) (15). Thus, ChIs release both acetylcholine (ACh) and glutamate (Glu) to regulate striatal functions (16, 17). ACh and Glu released from ChIs differentially affect DA transmission in the ventral striatum and reward-guided behaviors (13, 18). Interestingly, rodents with selective ablation of ChIs present deficits of behavioral flexibility and increased compulsive behavior (19, 20). However, how each individual neurotransmitter (ACh or Glu) released by ChIs affects the transition between goal-directed behaviors and habits, and therefore potentially influences maladaptive eating, is still obscure (21, 22).

Using a computer-based neurocognitive task to evaluate the balance between goal-directed behaviors and habits in humans (23, 24), we here confirm that a subgroup of patients with restrictive anorexia nervosa are more prone to habitual behaviors than healthy controls. Using mouse models, we show that disrupting ACh/Glu cotransmission, by genetically inactivating VAChT.
(VACHT−conditional KO mice, VACHTcKO mice), but not VGLUT3 (VGLUT3cKO mice), impairs behavioral flexibility, promotes habit formation, and consequently causes mice to be more susceptible to maladaptive eating. Specific deletion of VACHT from the dorsomedial striatum is sufficient to phenocopy the maladaptive eating of VACHTcKO mice. In parallel, we observed that DA efflux decreases in the dorsomedial striatum but remains unchanged in the dorsolateral striatum of VACHTcKO mice. This observation points to the possibility that uneven DA transmission in striatal compartments could underlie excessive habit formation and maladaptive eating. Indeed, increasing extracellular ACh with donepezil (an acetylcholine esterase inhibitor) or DA efflux with l-DO-PA abolishes the self-starvation phenotype of VACHTcKO mice in the activity-based anorexia model.

Our experiments reveal mechanisms underlying vulnerability to maladaptive eating and identify striatal ACh/Glu cotransmission as a potential target to correct excessive habitual behavior and pathological eating.

Results
Enhanced habit formation in restrictive-type anorexic patients. Whether patients with eating disorders have a dysregulated balance of goal-directed behaviors and habits is not clearly established (9, 25). Thus, we investigated habit formation in a group of patients with eating disorders using a computer-based neurocognitive task (24, 25). These experiments were conducted with a cohort of 25 healthy controls and 31 patients with eating disorders (Supplemental Table 1; supplemental material available online with this article; https://doi.org/10.1172/JCI138532DS1). Patients with eating disorders were divided in 2 categories: restrictive-type anorexia patients (AN-R, n = 21) and binge eating/purging eating disorder patients (ED-BP, n = 10). During the learning phase, accuracy increased over training sessions, and instrumental learning performances were similar across the 3 groups of subjects (Figure 1, A and B). During the initial phase, reaction time decreased across blocks of training and was not altered in patients with eating disorders (Supplemental Figure 1, A and B). In the second phase, participants were tested on their knowledge of response-outcome associations learned during the first stage. In phase 2, no differences were observed in response accuracy (Figure 1, C and D) or reaction time (Supplemental Figure 1, C and D) among groups. During the “slip-of-action” stage (phase 3), the percentage of responses was not significantly different between controls and patients for valued items or devalued items when eating disorder patients were considered altogether (Figure 1E). However, performance of the AN-R and ED-BP subgroups of patients was heterogeneous compared with that of healthy controls (Welch’s test, P = 0.01, and Brown-Forsythe test, P = 0.02; Supplemental Table 2). Some individuals suffering from AN-R persistently responded to stimuli associated with devalued outcomes (Figure 1E), while ED-BP patients responded similarly to controls (Figure 1E). This finding suggests that a subgroup of patients with restrictive-type anorexia was not able to withhold inappropriate responses for devalued outcomes, indicating a shift toward habitual behavior. Finally, in phase 4, inhibitory control was tested. During this phase, we found no significant differences between controls and patients with AN-R or ED-BP in responses to valued or devalued stimuli (Figure 1F). Hence, the habit bias observed during the slip-of-action stage in AN-R patients was not due to a deficit in response inhibition. Interestingly, the rate of responses for devalued outcome was positively correlated with the body mass index (BMI) in AN-R patients (Supplemental Figure 1, E–G). In contrast, neither Hospital Anxiety and Depression Scale score nor disease duration was correlated with the rate of devalued outcome responses (Supplemental Figure 1, H and I). Furthermore, in AN-R patients, but not ED-BP patients, the percentage of response to devalued outcomes was correlated with a lack of cognitive flexibility (measured with the Trail Making Test B minus A; Figure 1, G–I).

Women constituted the vast majority of healthy controls (19 of 25) and patients (29 of 31). This sex difference had only a small impact, as the global effect was close to the one observed when the cohort was restricted to women. In both cases, we found a significant interaction between condition (value) and group performance during phase 3 of the test.

Altogether, these results show that a subgroup of anorexic patients presents a disruption in the balance between goal-directed behaviors and habits, which is correlated with a lack of cognitive flexibility.

VACHT mutant mice have an impairment in flexibility and are more prone to habit formation. The dorsal striatum plays a pivotal role in the regulation of the balance between flexible goal-directed behaviors and inflexible habits (10, 26, 27). The striatal network, and its cognitive function, are powerfully regulated by ChIs (19, 28). However, how striatal functions are influenced by ACh or Glu cotransmission is not yet established. To specifically address the roles of ACh and Glu released by ChIs in behavioral flexibility, habit formation, and maladaptive eating, we used genetically modified mice in which VACHT or VGLUT3 was deleted selectively in ChIs using the Cre-Lox recombination approach. This method allowed the deletion of either VACHT (D2 driver) or VGLUT3 (ChAT or D2 driver) from ChIs (respectively, VACHTcKO mice, VGLUT3cKO mice, or VGLUT3cKO-D2Cre mice). Extensive anatomical characterization of mutant mice was performed (Supplemental Figures 2 and 3). Since the highest density of D2R expression in ChIs is found in the dorsal striatum of rodents (29, 30), the efficiency of D2-Cre-Lox deletion was maximal in the dorsal striatum of VACHTcKO mice (Supplemental Figure 2E). Extracellular ACh was dramatically reduced in the dorsal striatum of VACHTcKO mice (Supplemental Figure 2G). Silencing of VGLUT3 by D2-Cre or ChAT-Cre drivers was also maximal in the dorsal striatum (Supplemental Figures 2 and 3).

Behavioral flexibility allows for the adjustment of a behavioral response to unexpected conditions. Lack of behavioral flexibility is a well-established marker of eating disorders (6). We used a touchscreen pairwise visual discrimination and reversal learning task to explore behavioral flexibility in VGLUT3cKO mice and VACHTcKO mice. In the acquisition phase, mice were taught to discriminate and choose a rewarded over an unrewarded image (Figure 2A). After reaching the criterion (80% correct responses out of 30 trials in 2 consecutive sessions), the contingency was switched (Figure 2D), and mice had to extinguish the previous response and learn the new rewarded association. Both VGLUT3cKO mice and VACHTcKO mice did not differ from controls during the acquisition phase, indicating that learning was not significantly affected by...
made by VACHtKO mice was significantly higher in comparison with controls, indicating that VACHtKO mice have an impaired ability to maintain a new correct choice pattern. That is, after learning an action-outcome association, VACHtKO mice have difficulty in changing it (Figure 2H, Late). A similar deficit was observed in VACHtfl/fl mice that received bilateral AAV-Cre injection in the dorsal striatum resulting in a limited deletion of VACHt (Figure 2, I–T). Importantly, deletion of VACHt in the striatum did not affect attentional performance or inhibition control determined in the 5-choice serial reaction time task (5-CSRTT) (Supplemental Figure 5, A–C). Moreover, cohorts of VACHtKO mice and VGLUT3cKO mice that were previously tested in the reversal learning task were also evaluated in extinction tests. VGLUT3cKO mice showed no deficiency in the ability to stop responding (Figure 2, U and V; and Supplemental Figure 5, A–C). In contrast, the late phase of the task (sessions with >50% correct responses), the number of incorrect choices (regressive errors) made by VACHtKO mice was significantly higher in comparison with controls, indicating that VACHtKO mice have an impaired ability to maintain a new correct choice pattern. That is, after learning an action-outcome association, VACHtKO mice have difficulty in changing it (Figure 2H, Late). A similar deficit was observed in VACHtfl/fl mice that received bilateral AAV-Cre injection in the dorsal striatum resulting in a limited deletion of VACHt (Figure 2, I–T). Importantly, deletion of VACHt in the striatum did not affect attentional performance or inhibition control determined in the 5-choice serial reaction time task (5-CSRTT) (Supplemental Figure 5, A–C). Moreover, cohorts of VACHtKO mice and VGLUT3cKO mice that were previously tested in the reversal learning task were also evaluated in extinction tests. VGLUT3cKO mice showed no deficiency in the ability to stop responding (Figure 2, U and V; and
Supplemental Figure 6, A and B, for VGLUT3cKO-D2Cre), whereas VAChTcKO mice presented extinction deficits compared with controls (Figure 2, W and X). Taken together, these data suggest that silencing VGLUT3 and glutamate released by Chls had no effect on behavioral flexibility. In contrast, reducing VAChT and cholinergic signaling in the dorsal striatum significantly impaired behavioral flexibility and extinction.

VAChT-mutant mice are more prone to habit formation. We then tested whether the behavioral flexibility deficit observed in VAChTcKO mice was associated with an altered transition from goal-directed behaviors to habits. In order to bias mice toward goal-directed behaviors, they were trained for 12 days under continuous reinforcement using a fixed-ratio 1 paradigm (FR1; 1 nosepoke is associated with the delivery of 1 sucrose pellet) (Figure 3A, Training). Then, during the devaluation tests, the outcome was made less desirable by exposure of mice for 1 hour to sucrose pellets (devalued condition) or to regular chow (valued condition) (Figure 3A, Devaluation). A decrease in responding to the devalued condition compared with the valued condition indicates goal-directed behavior, whereas insensitivity to devaluation demonstrates habitual
behavior (31). Because of interindividual differences and variability during baseline training, devaluation results are shown as raw data (number of active nosepokes; Figure 3, C, E, G, and J) as well as normalized data (Supplemental Figure 7, A, D–G, I, and M).

Performance of VGLUT3cKO mice was similar to that of littermate controls during operant instrumental training (Figure 3B). During devaluation tests, both VGLUT3cKO mice and controls performed less in the devalued versus the valued condition (Figure 3C and Supplemental Figure 7B for VGLUT3-constitutive-KO mice), revealing goal-directed behaviors. Male VAChTcKO mice made significantly more nosepokes compared with controls during the last days of FR1 training (Figure 3F). Conversely, performance of female VAChTcKO mice was similar to that of controls throughout instrumental training (Figure 3I). In the devalued condition, control mice decreased their responding (goal-directed behavior). However, male and female VAChTcKO mice exhibited comparable performance in valued and devalued conditions (Figure 3, G and J; and Supplemental Figure 7, E–G, for normalized analysis). Since VAChTcKO male mice increased their performance during days 7–12 of FR1 training (as shown in Figure 3F), results of devaluation tests were also normalized to the performance of mice on the last day of FR1 training (Supplemental Figure 7F; normalized results for females are shown in Supplemental Figure 7G). Regardless of the type of analyses that were conducted, male and female VAChTcKO mice were significantly more affected than control mice. Two-way repeated-measures ANOVA (B–G) and post hoc comparison with the method of contrasts; Kaplan-Meier test (I and J) and post hoc comparison with log-rank Mantel-Cox and Gehan-Breslow-Wilcoxon tests. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 4. VAChTcKO mice develop binge- and anorexia-like phenotypes. (A) Diagram showing the binge-like sucrose overconsumption model. Mice alternated daily between 20-hour food restriction and 4-hour simultaneous access to food and to a highly concentrated sucrose solution. (B) Daily sucrose consumption by male VAGChTcKO mice (n = 12) and littermate controls (n = 12) during access to sucrose solution (H0–H4). (C) Daily sucrose consumption by male VAChTcKO mice and controls during the first hour of access to sucrose solution (H0–H1). (D) Daily sucrose consumption by male VAChTcKO mice and controls during the last 3 hours of access to sucrose solution (H1–H4). (E) Daily sucrose consumption by female VAChTcKO mice (n = 13) and littermate controls (n = 11) during access to sucrose solution (H0–H4). (F) Daily sucrose consumption by female VAChTcKO mice and littermate controls during the first hour of access to sucrose solution (H0–H1). (G) Daily sucrose consumption by female VAChTcKO mice and controls during the last 3 hours of access to sucrose solution (H1–H4). (H) Diagram of the activity-based anorexia (ABA) model. During baseline, mice were habituated to cages with running wheel for 7 days receiving ad libitum food. Then, mice underwent a progressive time-restricted access to food for 8 days. (I and J) Percentage of male (I, n = 12) and female (J, n = 12) VAChTcKO mice and controls (n = 12) that reached a critical level of less than 75% baseline BW compared with controls during ABA. Note that male and female VAChTcKO mice were significantly more affected than control mice. Two-way repeated-measures ANOVA (B–G) and post hoc comparison with the method of contrasts; Kaplan-Meier test (I and J) and post hoc comparison with log-rank Mantel-Cox and Gehan-Breslow-Wilcoxon tests. *P < 0.05, **P < 0.01, ***P < 0.001.
training, VGLUT3cKO mice showed decreased nosepoke activity relative to control mice (Figure 3D and Supplemental Figure 7H for VGLUT3-constitutive-KO mice). During devaluation tests after RI training, as expected, control mice favored habitual behavior, as they exhibited comparable performance in valued and devaluated conditions. In contrast, VGLUT3cKO mice and VGLUT3-constitutive-KO mice favored goal-directed behaviors, as they both performed less in the devaluated condition (Figure 3E and Supplemental Figure 7I and J). Male VAChTcKO mice nosepoke more than littermate control mice during RI training (Supplemental Figure 7K). During devaluation tests after RI training, both control mice and VAChTcKO mice (males) exhibited habitual behavior by performing similarly in valued and devaluated conditions (Supplemental Figure 7L and M). These results suggest that interfering with ChI cotransmission by silencing glutamate (VGLUT3cKO mice) causes mice to be less prone to develop habits, whereas shutting down ACh (VAChTcKO mice) increased the propensity of mice to habit formation. Thus, ACh and Glu released from ChIs have opposite roles in the modulation of goal-directed behaviors and habits.

Striatal cholinergic transmission regulates motivated behavior (32). To test whether increased habitual behavior demonstrated by VACHTcKO mice could be explained by changes in motivation, we compared male and female VAChTcKO mice...
controls during FR1 training. However, motivation was not a major determinant when VACHtcKO female mice performed in FR1. Furthermore, these behaviors were not due to increased locomotor activity, since VACHtCKO mice are not hyperactive in a novel or in a familiar environment (Supplemental Figure 7N). VGLUT3-deleted male mice showed no increased motivation in the PR test (Supplemental Figure 7O). Taken together, these findings in a progressive ratio (PR) test (Figure 3, H and K). Cohorts of mice used in PR tests were the same as those previously used for FR1 training and devaluation tests (Supplemental Figure 8). VACHtC KO males demonstrated enhanced motivation compared with controls (Figure 3H), whereas VACHtC KO females and controls showed a similar breaking point in the PR test (Figure 3K). Thus, motivation may explain why males nosepoked more than controls during FR1 training. However, motivation was not a major determinant when VACHtC KO female mice performed in FR1. Furthermore, these behaviors were not due to increased locomotor activity, since VACHtC KO mice are not hyperactive in a novel or in a familiar environment (Supplemental Figure 7N). VGLUT3-deleted male mice showed no increased motivation in the PR test (Supplemental Figure 7O). Taken together, these find-
Deficits of behavioral flexibility and an increased tendency to develop habits are associated with pathological eating (Figure 1, G–I; and refs. 7, 33, 34). Thus, we tested whether VACHTcKO mice (with a behavioral flexibility deficit and more prone to habits) or VGLUT3cKO mice (more inclined to maintain goal-directed behaviors) were susceptible to maladaptive eating.

We first assessed binge-like sucrose overconsumption (35). Mice were food-restricted for 20 hours and then exposed simultaneously to food and to a highly concentrated sucrose solution for 4 hours each day, for 16 days (Figure 4A). This protocol was

**Figure 4.** Percentage of mice that reached critical weight threshold for control (n = 10) or VACHTcKO mice (n = 10) treated with saline or -DOPA (15 mg/kg). A total of 9–10 were used in each group. *P < 0.05. **Figure 7.** Pharmacological modulation of ACh or DA neurotransmission reverses the self-starvation phenotype observed in VACHTcKO mice. (A and B) Percentage of mice that reached critical weight threshold for control (n = 10) or VACHTcKO mice (n = 10) treated with saline or -DOPA (15 mg/kg). A total of 9–10 were used in each group. (B and C) Chronic pharmacological treatment with donepezil (0.3 mg/kg). Percentage of mice that reached critical weight threshold for control (n = 10) or VACHTcKO mice (n = 10) treated with saline or donepezil. Note that donepezil was effective to reverse decreased food intake observed for VACHTcKO mice compared with saline-treated (NaCl-treated) VACHTcKO mice and had no effect on control mice. Kaplan-Meier test (A–D) and post hoc comparison with log-rank Mantel-Cox and Gehan-Breslow-Wilcoxon tests. **Figure 8.** Hypothetical model. Subcompartments of the striatum: (i) nucleus accumbens (NAc), reward-guided behaviors; (ii) dorsomedial striatum (DMS; or caudate in human), goal-directed behaviors; and (iii) dorsolateral striatum (DLS; or putamen in human), habits. Transitions from the NAc to the DMS and then to the DLS parallel the transition from voluntary reward-seeking behavior to compulsive behaviors. These transitions are finely tuned by dopamine (12). In VGLUT3cKO mice, DA effluxes are increased in the NAc/DMS but unchanged in the DLS. VGLUT3cKO mice are resistant to habit formation and remain in goal-directed behaviors. In contrast, in VACHTcKO mice, DA transmission is reduced by about 50%–70% in the NAc/DMS but remains unaltered in the DLS. Imbalanced DA levels in the NAc/DMS versus the DLS lead to rapid transitions from goal-directed behaviors to habits. In control conditions, VACHTcKO mice feed normally. Stressful conditions (e.g., restricted access to food) precipitate maladaptive eating behaviors in VACHTcKO mice.
designed to mimic some human features of bulimia-like binge eating (35). VGLUT3cKO mice consumed sucrose, water, and food to a similar extent compared with littermate controls (Supplementary Figure 9, A-D). In contrast, during the 16 days of the test, both male and female VACHTcKO mice progressively exhibited a significant increase in sucrose consumption during the 4-hour session when compared with respective controls (Figure 4, B and E). The difference in sucrose consumption between VACHTcKO mice and controls was due to increased sucrose consumption in the first hour of access to sucrose (H0–H1; Figure 4, C and F). Sucrose intake between the second and the fourth hour was only minimally altered in VACHTcKO mice (H1–H4; Figure 4, D and G). Water or food consumption during the first hour or during the total 4 hours of the test was similar between VACHTcKO mice and controls (Supplemental Figure 10, A–H). We also used saccharine, a non-caloric sweetener, in the binge-like model. VACHTcKO mice showed a small but significant tendency to binge more saccharine than controls (Supplemental Figure 10, I–L). These findings suggest that differences in homeostatic state do not contribute to sucrose overconsumption of VACHTcKO mice.

Eating high-caloric rewarding food without any physical hunger is an important feature of binge eating. Thus, we investigated whether palatability (hedonic effect) or homeostasis (hunger effect) contributed to the binge eating. The sucrose preference test was used to assess hedonia-driven motivation. Male VACHTcKO mice had a similar preference for the sucrose solution compared with control mice (Supplemental Figure 10N), indicating that the observed binge-like behavior was not caused by an increased sensitivity to the motivational properties of sucrose. We also examined the effect of reduced metabolic need (or hunger) on sucrose binge by measuring sucrose consumption after exposing animals to (a) an intraperitoneal injection of glucose solution 30 minutes before the session (Supplemental Figure 10, part O) or (b) ad libitum food for 1 hour (“chow preload”; Supplemental Figure 10P). In both conditions, VACHTcKO mice still binged significantly more sucrose than controls. Notably, VACHTcKO mice did not consume more food than controls during the chow preload period (Supplemental Figure 10Q). Furthermore, when appetitive food (high-fat, high-sugar pellets) was used instead of regular food, mutant mice continued to preferentially binge more sucrose solution than control mice (Supplemental Figure 10R), although they consumed the same amount of the alternative reward (Supplemental Figure 10S). These findings suggest that metabolic differences were not involved in the sucrose overconsumption exhibited by VACHTcKO mice during the first hour (H0–H1) of the test. Thus, VACHTcKO mice, which showed accelerated habit formation, exhibited a higher tendency to develop a binge-like sucrose overconsumption irrespectively of hunger or metabolic needs.

We next assessed VACHT- and VGLUT3-mutant mice in the activity-based anorexia (ABA) model, a well-validated rodent model of maladaptive eating endophenotype related to anorexia nervosa (Figure 4H) (36). Mice were habituated for 7 days to individual cages with free access to a running wheel. During this baseline period, mutant mouse lines and controls showed no differences of food intake or body weight (Supplemental Figure 11, A–F). A progressive time-restricted access to food was then introduced during the following 8 days, pushing mice to paradoxically combine overexercise and voluntary food restriction. During the ABA food restriction period, VGLUT3cKO mice and littermate controls showed similar food intake and reached 75% of their baseline body weight at comparable rates (Supplemental Figure 11, G and H). In contrast, both male and female VACHTcKO mice demonstrated significant alteration of food intake relative to control littermates (Supplemental Figure 11I). In addition, compared with controls, more male and female VACHTcKO mice reached a threshold of less than 75% of their baseline body weight (i.e., a critical weight loss as observed in patients with anorexia nervosa; ref. 1) (Figure 4, I and J). Together, these results suggest that, when VACHTcKO mice have limited access to food, they are more likely to develop maladaptive/compulsive eating behaviors.

Increased propensity to habit formation drives maladaptive eating in VACHTcKO mice. VACHTcKO mice demonstrated a propensity to develop habits as well as vulnerability to maladaptive eating. Since we used the same cohort of VACHTcKO mice and littermate controls to perform several behavioral tests (Supplemental Figure 8), we next assessed the link between habits, motivation, sex, and maladaptive eating behavior. We first performed Pearson correlations (Figure 5, A–D; and Supplemental Figure 12).

We then used correlational data to build a structural equation model (Figure 5, E and F) using latent variable analysis to test the effects of habits and motivation on anorexia- and bulimia-like behaviors, simultaneously. This analysis revealed that excessive habitual behavior predicts vulnerability of male and female VACHTcKO mice to maladaptive eating behavior (Figure 5, E and F).

Using logistic regression and receiver operating characteristic curves (ROCs), we then tested the diagnostic power of valuation/devaluation scores to identify mice’s vulnerability to binge-like behavior (Figure 5G) and anorexia-like behavior (Figure 5H). Valuation scores and sex were significant predictors of binge-like behaviors with a diagnostic power of 94.4% (area under the ROC curve), and devaluation scores were significant predictors of vulnerability to anorexia-like behavior with 92.1% diagnostic power (Figure 5H). Thus, our results suggest that cholinergic dysfunction due to VACHT deletion from ChIs drives habitual behaviors and vulnerability to maladaptive eating behaviors (binge- and anorexia-like) under specific environmental conditions. Our data also suggest that goal-directed and habitual behavior scores could be used to predict vulnerability to the development of binge-like and anorexia-like behaviors in mutant mice.

DA efflux is differentially affected across the dorsal striatum of VACHTcKO mice. Local DA neurotransmission plays a central role in the striatum and is thought to be pivotal in regulating reward, the balance between goal-directed behaviors and habits, and cognitive flexibility (12, 37). It is well established that cholinergic signaling regulates striatal DA neurotransmission (13, 14). In the nucleus accumbens, ACh and Glu released by ChIs differentially regulate DA efflux (13). To determine whether disruption of DA neurotransmission in VACHTcKO mice could be related to the maladaptive eating phenotypes, we measured DA efflux in the dorsal striatum. Microdialysis experiments showed that elimination of VACHT from ChIs decreased extracellular DA accumulation in the dorsal striatum by 50% (Supplemental Figure 13A). We then used in vivo voltammetry to measure DA efflux in the dorsomedial striatum and in the dorsolateral striatum of VACHTcKO mice, VGLUT3cKO.
mice, and littermate controls (Figure 6, A–H; and Supplemental Figure 13, B–Q). Following KC1-induced depolarization, DA release was decreased in the dorsomedial striatum of VACHtKO mice (~40%) (Figure 6, B and C; and Supplemental Figure 13P) compared with control mice. Conversely, it was increased in the dorsomedial striatum of VGLUT3cKO mice (~40%) (Supplemental Figure 13, B, C, and P, for VGLUT3cKO, and Supplemental Figure 13, F–H, for VGLUT3-constitutive-KO mice). In the dorsolateral striatum, DA efflux was not modified after deletion of either VACHt or VGLUT3 (Figure 6, F and G; and Supplemental Figure 13, D, E, and Q, for VACHtKO and VGLUT3cKO, and Supplemental Figure 13, I–K, for VGLUT3-KO mice). Therefore, disturbing ACh secretion from ChIs markedly affects DA release in the dorsomedial striatum without altering its levels in the dorsolateral striatum. Opposite changes are observed in the absence of glutamate secretion from ChIs. Overall, altering ACh/Glu cotransmission in ChIs creates an imbalance in DA release between subcompartments of the dorsal striatum.

**Deletion of VACHt in the dorsomedial striatum of male mice is sufficient to induce maladaptive eating behavior.** DA voltammetry experiments suggest that reduced levels of VACHt affect DA efflux in the dorsomedial striatum, but not in the dorsolateral striatum. Therefore, we tested whether altered VACHt levels in the dorsomedial striatum could be involved in maladaptive eating. To address this question, we specifically deleted VACHt from the dorsomedial striatum of adult male mice by infusing AAV-Cre virus in this striatal subcompartment (VACHtKO-DMS; Figure 6I) and tested them for maladaptive eating. Strikingly, in the binge-like sucrose overconsumption model, decreased expression of VACHt restricted to the dorsomedial striatum was sufficient to reproduce the increased sucrose intake (Figure 6, J–L) observed in VACHtKO mice (Figure 4, B–G). Likewise, throughout the 16 days of binge-like testing, neither food nor water intake was modified in VACHtKO-DMS mice relative to controls (Supplemental Figure 14, A and B). In addition, VACHtKO-DMS mice had a similar preference for the sucrose solution compared with controls (Supplemental Figure 14C). Furthermore, as reported above for VACHtKO mice, VACHtKO-DMS mice that were satiated by pre-exposure to chow for 1 hour still binged more sucrose than controls (Supplemental Figure 14D). Importantly, there was no difference in food intake during this chow preload period (Supplemental Figure 14E). Therefore, increased binge activity in VACHtKO-DMS mice was not related to hunger, but most likely was related to increased habitual behavior.

In the ABA model, more mice reached critical levels of weight loss following the deletion of VACHt in the dorsomedial striatum compared with controls (Figure 6M). Interestingly, we observed no correlation between the extent of VACHt deletion in the dorsomedial striatum and sucrose binge-like overconsumption or self-starvation phenotypes (Supplemental Figure 14, F and G). These correlative analyses suggest that even a partial deletion of VACHt in the dorsomedial striatum could be sufficient to increase vulnerability to maladaptive eating. Importantly, when we analyzed between-subject differences in sucrose binge-like overconsumption and ABA models, no correlation was found for control mice (Supplemental Figure 14H). Conversely, Pearson correlation analyses yielded a robust negative correlation between the quantity of sucrose consumed during the binge-like model and food intake in the ABA model for VACHtKO-DMS mice (Supplemental Figure 14I). Hence, VACHtKO-DMS mice that were more vulnerable to bulimia-like phenotype were also more susceptible to anorexia-like behavior 10 days later. These data show that a limited disruption of cholinergic activity in the dorsomedial striatum of adult mice is sufficient to increase vulnerability to maladaptive eating.

**Pharmacological modulation of ACh or DA neurotransmission reverses the ABA phenotype of VACHtKO mice.** To test whether the reduced DA release observed in VACHt mutants is related to maladaptive eating behaviors, we treated male VACHtKO mice and controls with l-DOPA (15 mg/kg) during the ABA test. We chose the ABA model because of the robust phenotype of VACHtKO mice in this test. During the baseline phase, the presence of the wheel did not alter food intake or body weight of VACHtKO mice and controls (Supplemental Figure 15, A–D). During the restriction phase, l-DOPA exacerbated the anorexia-like phenotype of control mice as shown by survival curves (Figure 7A and Supplemental Figure 15E) and as previously reported (38). In contrast, the percentage of VACHtKO mice reaching a critical weight loss was greatly reduced by l-DOPA pretreatment (Figure 7B and Supplemental Figure 15E). Therefore, increasing DA transmission with l-DOPA alleviated the self-starvation phenotype of VACHtKO mice, although an opposite effect was observed in littermate controls. These data indicate that increasing DA efflux can rescue maladaptive eating deficits in VACHt-deficient mice, suggesting that decreased DA is causally linked to the behavioral deficits in these mutants.

Donepezil is an acetylcholinesterase (AChE) inhibitor that reverses deficits of VACHt-mutant mice (39). To confirm that the phenotypes observed in VACHt-deficient mice could be due to reduced cholinergic signaling, we tested whether donepezil was able to reverse self-starvation phenotypes in the ABA model. Chronic treatment with donepezil (0.3 mg/kg) had no effect on the percentage of control mice reaching a critical threshold of less than 75% of their baseline body weight (Figure 7C and Supplemental Figure 15F). In contrast, donepezil significantly decreased the percentage of VACHtKO mice reaching the 75% threshold of baseline body weight (Figure 7D and Supplemental Figure 15F). Thereby, increasing cholinergic signaling by AChE inhibition was fully effective to treat the anorexia-like phenotype in VACHtKO mice.

Figure 7E summarizes our findings in animal models. In this hypothetical model, we suggest that an unbalanced DA transmission across striatal subcompartments alters the transition from goal-directed behaviors to habits and drives the vulnerability of VACHtKO mice to the development of environment-dependent maladaptive eating.

**Discussion**

ChIs have the remarkable ability to corelease ACh and glutamate (17, 40). In this study, we show a striking dissociation of neurochemical and behavioral actions modulated by these 2 neurotransmitters in the dorsal striatum. When glutamate transmission from ChIs is silenced by deletion of VGLUT3, DA efflux increases in the dorsomedial striatum but not in the dorsolateral striatum. In contrast, decreased release of ACh due to VACHt deletion results in decreased DA efflux in the dorsomedial striatum, but not in the dorsolateral striatum (as modeled in Figure 7E). Reducing gluta-
mature transmission from ChIs (VGLUT3cKO mice) favors goal-directed behaviors, and these mice are not more vulnerable to maladaptive eating than littermate controls. In contrast, VACHTcKO mice present a loss of behavioral flexibility and facilitated habit formation that predict their increased risk of developing maladaptive eating behaviors. Importantly, l-DOPA treatment reversed the self-starvation phenotype in VACHT-deficient mice, supporting the interpretation that reduced DA efflux may play a pivotal role in the maladaptive eating observed in these mice. We surmise that, in addition to signaling mechanisms elicited by ACh, favoring DA efflux in the dorsolateral striatum versus the dorsomedial striatum/nucleus accumbens could directly lead to excessive habits and bulimia-like or anorexia-like behaviors. Future experiments will help to establish whether specifically decreasing dopamine efflux in the dorsomedial striatum/nucleus accumbens or increasing it in the dorsolateral striatum (as described in Figure 7E) is sufficient to accelerate habit formation and maladaptive eating.

We previously found that silencing ACh or Glu released by ChIs differently affected DA release in the nucleus accumbens and reward-based behaviors (13, 18). Glutamate released from ChIs inhibits DA transmission in the nucleus accumbens by activation of a metabotropic glutamate receptor (mGluR) (13). Glutamate released by ChIs could operate on inhibitory group I mGluR receptors present on DA striatal fibers (41). In addition, it is well established that a combination of striatal nicotinic and muscarinic receptors accounts for the local regulation of DA signaling (13, 14, 41, 42). Our findings, combined with data from the literature, support a framework in which ACh/Glu cotransmission from ChIs exerts a dual effect on DA release in both the nucleus accumbens and the dorsomedial striatum, but not in the dorsolateral striatum (Figure 7E). This difference between the nucleus accumbens/dorsomedial striatum and the dorsolateral striatum is likely due to the presence of a distinct combination of mGluRs, nicotinic AChRs, and muscarinic AChRs in different striatal subcompartments (41). ACh and Glu from ChIs modulate the balance of DA transmission between striatal compartments and, by doing so, play a pivotal role in the regulation of the transition from goal-directed behaviors to habits. An imbalance of cotransmission favoring ACh release correlates with increased goal-directed behaviors, whereas an imbalance favoring glutamate release correlates with habitual behaviors.

Targeted deletion of VACHT in the dorsal striatum of adult mice using viral vectors recapitulates the loss of behavioral flexibility as well as sucrose overconsumption and self-starvation observed in VACHTcKO mice. These results rule out the involvement of developmental compensatory mechanisms in endophenotypes herein uncovered. In addition, specific deletion of VACHT in the dorsomedial striatum suggests that this striatal area is sufficient to drive maladaptive eating behaviors. However, these findings do not rule out the possibility that other brain areas could also fulfill similar functions. The striatum is part of the complex cortico-striato-thalamo-cortical loop, and the prefrontal cortex is a pivotal regulator of this neuronal network. Several reports from the literature suggest that the prefrontal cortex, and in particular the orbitofrontal cortex, are critical for the regulation of reward, compulsivity, and eating disorders (43, 44). In addition, several brain areas such as other structures from the cortico-basalo-cortical loop, the thalamus, the hippocampus, or the amygdala play an influential role in goal-directed or habitual actions (10, 22, 45). Our results combined with data from the literature suggest that the dorsal striatum could act as a final endpoint effector of a pathway driven by the prefrontal cortex.

In parallel, we found that the maladaptive eating of VACHTcKO mice cannot be attributed to locomotor hyperactivity, anhedonia, impulsivity, motivation, or metabolic need. We also observed that mice with VACHT deletion in the dorsomedial striatum that binged more sucrose also showed the lowest food intake in the ABA model a few days later. Altogether, these findings further suggest that maladaptive eating behaviors observed in VACHTcKO mice correlate with decreased ACh transmission in the dorsal striatum and increased habit formation. Interestingly, in a familiar environment (such as a regular home cage), feeding behavior of VACHTcKO mice and controls is similar (the present study and ref. 46). Abnormal eating behaviors of mice with dysfunctional striatal cholinergic transmission occur only in conditions such as restricted access to food. These findings illustrate how genetic and environmental factors may interact to drive abnormal eating behaviors.

There have been conflicting data over the involvement of excessive and maladaptive habits in eating disorders (25, 47). Using a self-reported habit index, Davis et al. recently found that the chronicity and severity of anorexia nervosa were related to the strength of habits (48). Using a neurocognitive task previously designed to assess habit formation in humans (24, 25), we showed that a subgroup of patients with restrictive anorexia nervosa present an increased tendency to habits. Our present findings are well in line with a recent report (44), which combined an instrumental motivation task and functional MRI in acutely underweight anorexic patients. This study unraveled the existence of a subgroup of anorexic patients favoring goal-directed actions (gAn) and a second subgroup that was more habit driven (hAn) (44).

In our study, we observed that enhanced habit formation was strongly correlated with decreased cognitive flexibility in restrictive anorexia nervosa patients but not binge-purge anorexia nervosa patients. The human test used herein is based on a go/no-go inhibitory task, whereas the mouse task relies on a pre-session outcome devaluation of the reinforcer. However, despite these differences, these tests support the idea that excessive habits can push both humans and mice to use automatic behaviors and to become less sensitive to the value of the outcome. In humans, these cognitive investigations suggest that the propensity to quickly develop habitual behavior combined with a lack of cognitive flexibility could serve as behavioral markers for a subgroup of restrictive anorexia patients.

Clinically it is well established that eating disorders, especially anorexia nervosa and bulimia nervosa, are more frequent among females than males (1). Unlike humans, VACHTcKO mice demonstrated only minor differences between males and females in maladaptive eating behavior. Nonetheless, our findings show that these mice recapitulate some of the major endophenotypes of eating disorders in humans. Another discrepancy between patients and our animal model is that habit formation in VACHTcKO mice predicted vulnerability to both anorexia-like and bulimia-like behaviors. Our data showed facilitation of habits in a subgroup of restrictive anorexia nervosa patients, whereas binge-purge anorexia nervosa patients demonstrated no change of habit for-
mation. This might reflect an intrinsic difference between human patients and the animal model. However, it could also be related to the fact that we assessed habits in a small cohort of patients with bulimia nervosa. The neurocognitive test was conducted with a large majority of anorexic patients (binge-purge anorexia or restrictive anorexia) and included only 7 patients with bulimia nervosa in the ED-BP group (Figure 1 and Supplemental Table 1).

To clarify whether excessive habits can also drive the symptoms of bulimia nervosa, further studies with a large cohort of subjects will be necessary.

Several reports previously proposed compulsivity as a transdiagnostic marker overlapping different eating disorders (5, 7, 25, 48). Our findings taken together with observations by Davis et al. (48, 49) suggest that increased habit formation could be a transdiagnostic marker for subgroups of anorexia nervosa as well as bulimia nervosa patients. Interestingly, excessive goal-directed behavior has also been proposed as a transdiagnostic marker for subgroups of eating disorders patients (44). On that note, it is tempting to hypothesize that in our study (Figure 1E), patients who do less than 10%-20% mistakes in devalued conditions could represent goal-driven patients. If this hypothesis is correct, evaluation of habits versus goal-directed behaviors could be an innovative way to more specifically stratify patients with eating disorders. Precisely defining these subgroups could be key to better understand and treat these pathologies.

The present study focuses on one endophenotype of anorexia, i.e., excessive habit formation, and on the network involving the caudate-putamen. However, it should be kept in mind that anorexia nervosa is a complex pathology and several important dimensions are not easily modeled in animal studies. This is the case, for example, with obsessional preoccupation with body image, severe body image distortions, or lack of insight about being ill. These dimensions have been proposed to involve other transmitters than ACh and DA and other brain areas than the cortico-basal-cortical loop (45). These additional dimensions and brain areas will have to be investigated with appropriate animal models in order to develop effective treatment of anorexia.

Deficits of DA or ACh transmission can be pharmacologically alleviated with L-DOPA or donepezil, respectively. Indeed, we found that L-DOPA was efficient in reducing anorexia-like symptoms in VACHTcKO mice. This observation supports the idea that a reduced DA efflux in the dorsomedial striatum/nucleus accumbens could play a causative role in establishing maladaptive eating behaviors in VACHTcKO mice (Figure 7E). However, L-DOPA markedly increased anorexia-like behaviors in control mice. In humans, L-DOPA induces a range of effects: it can lead to anorexic symptoms (50), cause antianorexic effects (51), or have no effect (52). An inverted U shape of dopamine effects has often been reported (53). Therefore, paradoxical effects of L-DOPA in controls versus VACHTcKO mice could be related to the notion that too much or too little dopamine are both detrimental. Therefore, DA-targeting drugs or L-DOPA should be used with care for the treatment of eating disorders.

In contrast, we observed that the AChE inhibitor donepezil prevented the self-starvation phenotype of VACHTcKO mice without altering eating behaviors of control mice. This finding supports the idea that abnormal striatal cholinergic signaling correlates with increased vulnerability to maladaptive eating in VACHTcKO mice. Donepezil was previously proposed to treat anorexia (54); however, the rationale underlying this suggestion emerged from cognitive parallels drawn between dementia and anorexia, which are quite different from the model herein proposed (Figure 7E). AChE inhibitors have also been proposed to rectify eating compulsions in Alzheimer’s disease (55, 56). However, the efficacy of anticholinergic drugs in the treatment of anorexia is still controversial (57, 58). Interestingly, donepezil has been demonstrated to be a well-tolerated and efficient add-on drug for treatment-resistant obsessive-compulsive disorder and pathological repetitive behaviors (tics) (59, 60). Our present findings suggest that it might be relevant to test AChE inhibitors in eating disorder–affected individuals who present increased habitual behaviors. However, despite a relatively favorable profile of side effects, AChE inhibitors can cause nausea and diarrhea and should therefore be used with caution to treat this population of patients. In particular, low doses should be favored in potential future clinical trials.

It could be worthwhile to assess whether subgroups of patients with eating disorders exhibit genetic alterations of VACHT or other cholinergic striatal markers before treating them with donepezil. Notably, VACHT probes used in positron emission tomography have been described (61). Subjects with altered expression of cholinergic markers and a higher propensity to habit formation would then be candidates to undergo cholinergic-enhancing therapies. It is important to note that altering the function of Chls is not the sole way to modify the balance between reward-guided goal-directed behavior and habit formation. This could also be achieved, for example, by modification of the activity of the orbitofrontal cortex (43, 62) (Figure 7) or by inhibition of cholinergic neurons from the midbrain projecting to the striatum (63).

In summary, our work indicates that enhanced habit formation can promote vulnerability to maladaptive eating in mice as well as in subgroups of human patients. Our data open avenues to the identification of a specific subgroup of patients suffering from restrictive anorexia nervosa or bulimia nervosa and suggest potential innovative targeted therapies for the treatment of these patients.

Methods

Human subjects. Description of patients and healthy controls is provided in Supplemental Methods.

The slip-of-action neurocognitive test. To evaluate the balance between goal-directed behaviors and habits in humans, we used a computer-based neurocognitive task as previously reported by (24, 25). The test is described in Supplemental Methods.

Animals. Housing and breeding of mouse colonies were performed as previously reported (13) and as described in Supplemental Methods.

Histology and cellular/molecular assays. Immunohistochemistry, Western blots, immunofluorescence, and quantitative PCR experiments were performed as previously reported (13, 64) and as described in Supplemental Methods. See complete unedited blots in the supplemental material.

Behavioral investigations. Touchscreen behavioral tasks, operant sucrose self-administration, sucrose binge-like overconsumption, sucrose preference, and ABA model were performed as previously reported (31, 35, 36, 65) and as described in Supplemental Methods.
Stereotaxic surgery and intracerebral virus infusions. Details regarding virus injections and histological evaluations of VAChT deletion for touchscreen experiments and for eating disorder models are provided in Supplemental Methods.

In vivo microdialysis. Details regarding in vivo microdialysis experiments are described in Supplemental Methods.

In vivo voltammetry. In vivo voltammetry was performed as previously reported (13) and as briefly described in Supplemental Methods.

Data and materials availability. All relevant data and analyses are within the paper and its supplemental material online. Raw data are available upon request. All unique biological materials used (knockout mice or antiserums) are readily available upon request.

Statistics. Statistical analyses are shown in Supplemental Tables 3–21. Details regarding statistical analysis and mathematical modeling are provided in Supplemental Methods.

Study approval. Clinical studies were approved by the local ethics committee (“Comité de Protection des Personnes,” CPP: AM7149-13321). A signed informed consent document was obtained from each participant before enrollment.

Animal care, animal handling, and all experiments were performed according to the guidelines of the Canadian Council on Animal Care (http://ccac.ca/en/standards/guidelines), approved by the Facility Animal Care Committee of the Douglas Research Center (protocols 2008-5643 and 2014-7479) or by the University of Western Ontario (protocols 2016-103 and 2016-104), and performed in accordance with the NIH guidelines for the care and use of animals and an approved animal protocol from the Duke University IACUC.

Author contributions
MF and HJ performed the behavioral experiments with the help of OK, XQM, CA, and SR. MF and HJ performed the anatomical experiments with the help of EV. MF performed in vivo DA voltammetry with the help of LM and AG. JIK programmed the computerized neuropsychological test. DJK performed the neuro-psychological test in humans with the help of PD and under the supervision of PG. JPRJ performed in vivo microdialysis under the supervision of MGC. PRN, LR, JYN, TAP, and MMH performed statistical analysis and mathematical models. MF, MAMP, VFP, and SEM designed the study and wrote the manuscript with the help of PRN, HJ, JG, DG, MGC, MMC, and MPB. All authors were involved in revising the manuscript for intellectual content. All authors read and approved the final manuscript. MF and HJ are co-first authors. MF is listed first because he contributed more to the conception of the project and the writing of the manuscript.

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