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Archival Report

Biological Psychiatry

Operant Training for Highly Palatable Food Alters Translating Messenger RNA in Nucleus Accumbens D_2 Neurons and Reveals a Modulatory Role of *Ncdn*

Enrica Montalban, Albert Giralt, Lieng Taing, Yuki Nakamura, Assunta Pelosi, Mallory Brown, Benoit de Pins, Emmanuel Valjent, Miquel Martin, Angus C. Nairn, Paul Greengard, Marc Flajolet, Denis Hervé, Nicolas Gambardella, Jean-Pierre Roussarie, and Jean-Antoine Girault

ABSTRACT

BACKGROUND: Highly palatable food triggers behavioral responses including strong motivation. These effects involve the reward system and dopamine neurons, which modulate neurons in the nucleus accumbens (NAc). The molecular mechanisms underlying the long-lasting effects of highly palatable food on feeding behavior are poorly understood.

METHODS: We studied the effects of 2-week operant conditioning of mice with standard or isocaloric highly palatable food. We investigated the behavioral responses and dendritic spine modifications in the NAc. We compared the translating messenger RNA in NAc neurons identified by the type of dopamine receptors they express, depending on the kind of food and training. We tested the consequences of invalidation of an abundant downregulated gene, *Ncdn*.

RESULTS: Operant conditioning for highly palatable food increased motivation for food even in well-fed mice. In wild-type mice, free choice between regular and highly palatable food increased weight compared with access to regular food only. Highly palatable food increased spine density in the NAc. In animals trained for highly palatable food, translating messenger RNAs were modified in NAc neurons expressing dopamine D_2 receptors, mostly corresponding to striatal projection neurons, but not in neurons expressing D_1 receptors. Knockout of *Ncdn*, an abundant downregulated gene, opposed the conditioning-induced changes in satiety-sensitive feeding behavior and apparent motivation for highly palatable food, suggesting that downregulation may be a compensatory mechanism.

CONCLUSIONS: Our results emphasize the importance of messenger RNA alterations in D_2 striatal projection neurons in the NAc in the behavioral consequences of highly palatable food conditioning and suggest a modulatory contribution of *Ncdn* downregulation.

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Food palatability is a potent driver for food intake even without actual caloric need. Excessive consumption of palatable food can disrupt the normal regulation of appetite (1), inducing the development of a compulsive-like approach to food intake (2,3). However, the mechanisms by which exposure to palatable food induces persistent behavioral alterations responsible for maladaptive food consumption remain poorly understood. One hypothesis is that exposure to palatable food recruits the brain reward system, first inducing a strong motivation and later switching food-seeking behavior from flexible, goal-directed actions to inflexible, compulsive-like responses and weight gain (4–7). Obese participants display deficits in reversal learning (8), perseveration in set-shifting tasks indicating decreased cognitive flexibility (9,10), decreased sensitivity to satiety-mediated devaluation of food (11,12), and stronger devaluation of delayed rewards (13). In obese individuals, these behavioral modifications are accompanied by alterations within the brain reward system. Functional imaging of frontostriatal circuits, a main substrate for inhibitory behaviors and cognitive control, reveals blunted activation in response to food (14) and food-associated cues (15,16). The functionality of these circuits depends on dopamine (DA) transmission, and evidence supports a role of DA as a sensor of peripheral metabolic signals (17) and in mediating the value of food-associated cues (18). Downstream of DA, striatal D_2 receptors (D2Rs, usually not distinguished from D3Rs) have attracted much attention, as D2R antagonists induce weight gain (19–22). Variable alterations in D2R availability have been

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reported in the striatum of morbidly obese individuals (23–25). Moreover, polymorphisms in *ANKK1/DRD2* (*DRD2* being the D2R gene) were associated with blunted signals in frontostriatal circuits in response to food stimuli, linked to weight gain (26).

The nucleus accumbens (NAc) is key in responses to both natural (food) and non-natural (drugs of abuse) rewards (27,28). NAc alterations are suggested to modify food motivation and decision making in obesity (29). The NAc comprises two populations of projection neurons: D1R- or D2R-expressing striatal projection neurons (SPNs, D1SPNs and D2SPNs), also known as medium-size spiny neurons (30). Early optogenetic studies indicated that D1SPNs and D2SPNs have opposing effects on reinforcement (31,32), but further work showed that in the NAc both populations can encode reward and aversion depending on stimuli and activity patterns (33).

In animal models, palatable—usually highly caloric—food exposure alters reward-seeking behaviors (34–36) with increased impulsivity (37) and impaired cognitive functions (38), which are at least partially independent of overweight. These behavioral dysfunctions are accompanied by modifications of DA transmission markers, with a decrease in D2R expression in the striatum (39,40). However, the amplitude, direction, and nature of these alterations differ between studies, possibly due to differences in the composition of palatable food and duration and age of exposure (41). Yet, consistent findings point to alterations in excitatory transmission and structural plasticity of glutamatergic synapses in the striatum (42–44). However, the molecular bases of these regulations are poorly understood, and the impact of palatable food exposure on the transcriptional landscape of striatal neurons is not known. In many studies, the effects of palatability per se are not distinguished from the metabolic and neurobiological modifications related to food (over)consumption. Moreover, the passive—or forced—access to highly palatable food in rodent models does not allow isolating the specific impact of palatability on the goal-directed component of food-seeking behavior crucial to developing compulsive eating.

Here, we used a combination of behavioral and genome-wide approaches to characterize alterations in the translatome associated with food-seeking behavior for standard or isocaloric highly palatable food in the main dopaminoceptive neuronal populations of the NAc. We investigated changes in D1SPNs and D2SPNs using translating ribosome affinity purification (45,46) followed by RNA sequencing in mice (47). We compared mice that learned to nose poke to obtain either regular or highly palatable food with yoked control mice that received the same food noncontingently. After identifying changes in translating mRNA in D2SPNs, we tested the consequences of the genetic manipulation of one of the genes differentially regulated by conditioning for highly palatable food, *Ncdn* (coding for neurochondrin, also known as norbin).

METHODS AND MATERIALS

Animals

For translatome analyses, we used male and female (ages 10–12 weeks) *Drd1*-EGFP/Rpl10a or *Drd2*-EGFP/Rpl10a mice (46), maintained as heterozygotes on a C57BL/6J background (Table S1). To generate *Ncdn* conditional knockout (*Ncdn*-cKO) mice, we crossed $Ncdn^{Flox/Flox}$ mice with $Ncdn^{Flox/+}$/*Camk2a*-$Cre^{*/+}$ double mutant mice (48) and used male and female 3- to 4 month-old littermates. C57BL/6 (wild-type) male mice purchased from Janvier Labs were used at 3 to 4 months of age. We performed animal protocols following the National Institutes of Health Guide for the Care and Use of Laboratory Animals, approved by The Rockefeller University Institutional Animal Care and Use Committee.

Behavioral Experiments

Operant conditioning experiments were carried out in operant chambers (Med Associates) with 2 nose-poke holes. Mice were randomly assigned to one of 4 groups: master highly palatable (mHP), master standard (mST), yoked highly palatable (yHP), yoked standard (yST) (Figure 1A). Access to food was restricted for all mice 5 days before the start of conditioning until the ninth operant training session, and then all mice had ad libitum food access. During the operant conditioning sessions, mice were presented with 20-mg dustless precision ST pellets (5UTM #1811143; TestDiet) or HP isocaloric pellets with a higher level of sucrose among carbohydrates and a chocolate flavor (5UTL #1811223; TestDiet). The progressive ratio (PR) schedule lasted 90 minutes (Supplemental Methods). For the free choice paradigm, mice were separated into two groups. In their home cage, one group had access only to ST food, while the other group had free access to HP and ST food. Their weight was monitored.

Cell Population–Specific mRNA Immunoprecipitation and Sequencing

Cell population–specific purification of translating mRNA was performed as described elsewhere (45,46) with some modifications (47). The brain was rapidly dissected and sliced on ice. Bilateral pieces punched out from NAc of 3 mice (Table S1) were homogenized in ice-cold lysis buffer (47) (Supplemental Methods). RNA quality was checked using a Bioanalyzer. Five nanograms of RNA were used for reverse transcription, ultrasonication, and library construction with a TruSeq RNA Prep Kit (Illumina). The libraries were sequenced on an Illumina HiSeq 2500 instrument (details in Supplemental Methods).

Bioinformatic Analysis

We assessed the quality of the raw data using FastQC (49) and mapped the libraries (37–62 million 50-bp paired-end reads) to the *Mus musculus* genome GRCm38/mm10 (University of California Santa Cruz Genome Browser; https://genome.ucsc.edu/index.html) using HISAT2 (50). Reads were quantified using the RNA-Seq pipeline of SeqMonk (51). Sequencing data are deposited in the National Center for Biotechnology Information Gene Expression Omnibus (52) (#GSE137153; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137153) (Table S1). Differentially expressed genes were identified using the Bioconductor package DESeq2 v1.30.1 (53). Genes with adjusted p value $< .05$ (54) were declared differentially expressed. Gene Ontology overrepresentation analyses were performed with Gene Ontology Data Archive (55). Network inference was performed with combined results of context likelihood of relatedness (56) and GENIE3 (57) as described in

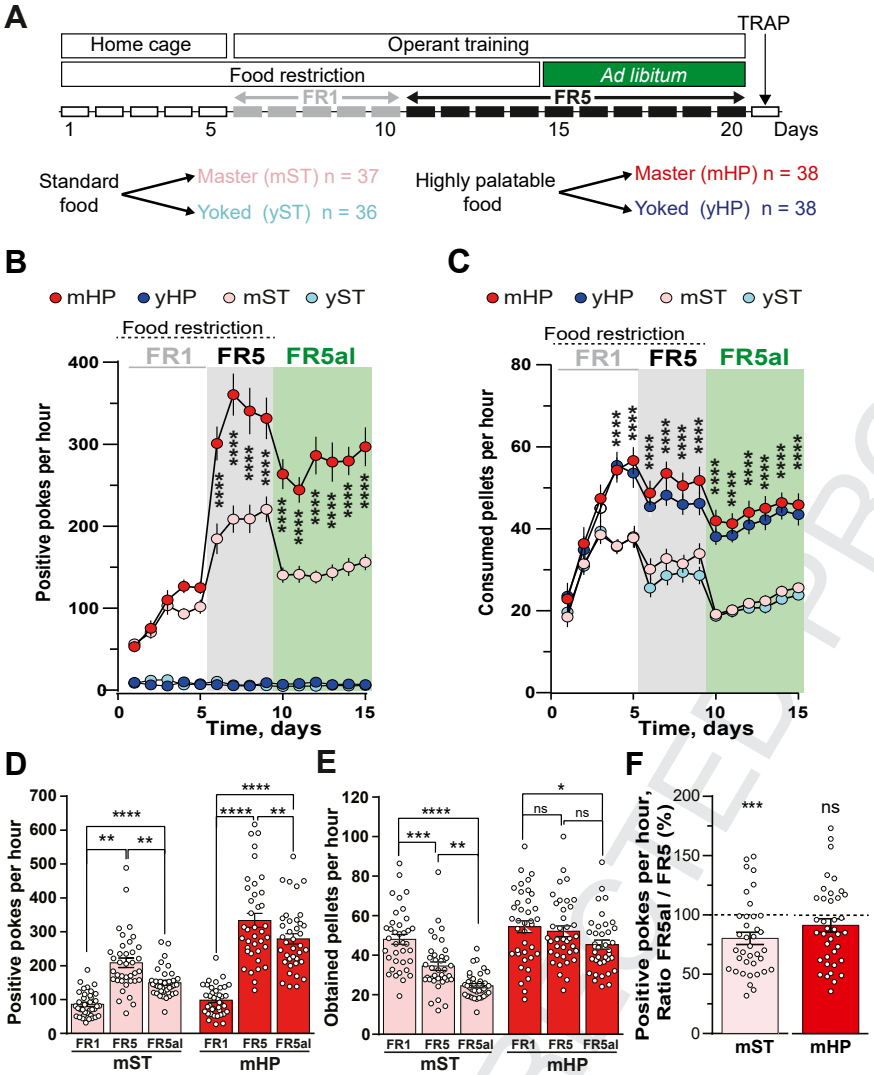

Figure 1. Operant training for standard and highly palatable food results in different behavioral changes. **(A)** Schematic description of the operant training protocol used in this study. **(B)** Time course of the number of positive pokes (i.e., in the active hole) during operant training as described in panel **(A)**. Two-way repeated-measures ANOVA for each phase (see Table S2 for all statistical results). Group effect, FR1: $F_{3,141} = 97.3$, $p < 10^{-4}$; FR5: $F_{3,144} = 161$, $p < 10^{-4}$; FR5al: $F_{3,144} = 234.5$, $p < 10^{-4}$. Holm-Sidak's multiple comparisons are indicated between mHP and mST. As expected, the yoked mice did not make positive pokes. **(C)** Time course of consumed pellets. Two-way repeated-measures ANOVA, group effect, FR1: $F_{3,142} = 8.25$, $p < 10^{-4}$; FR5: $F_{3,142} = 23.9$, $p < 10^{-4}$; FR5al: $F_{3,142} = 50.1$, $p < 10^{-4}$. Holm-Sidak multiple comparisons are indicated between mHP and mST. The yoked mice consumed virtually the same amount of food as their respective masters. **(D)** Summary of positive pokes per hour during the 3 phases for mHP and mST as in panel **(B)** (same data). Two-way ANOVA, food effect: $F_{1,219} = 70.9$, $p < 10^{-4}$; training effect: $F_{2,219} = 99.8$, $p < 10^{-4}$; interaction: $F_{2,219} = 13.2$, $p < 10^{-4}$. **(D, E)** Holm-Sidak multiple comparisons as indicated. **(E)** Summary of obtained pellets per hour during the 3 phases for mHP and mST. Two-way ANOVA, food type: $F_{1,219} = 59.4$, $p < 10^{-4}$; training phase: $F_{2,219} = 23.5$, $p < 10^{-4}$; interaction: $F_{2,219} = 5.0$, $p = .075$. **(F)** The satiety-induced decreased motivation for nose poking was evaluated by calculating for each mouse the ratio of the average number of positive pokes per day during the FR5al sessions divided by the average number of positive pokes per day during the FR5 sessions with food restriction. Data are plotted for mST (left, $n = 37$) and mHP (right, $n = 38$). One-sample t test, mST: $t_{36} = 3.8$, $p = .0005$; mHP: $t_{37} = 1.55$, $p = .13$, ns. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < 10^{-4}$. ANOVA, analysis of variance; FR, fixed ratio; FR5al, fixed ratio 5 with ad libitum food access in home cage; mHP, master highly palatable; mST, master standard; ns, not significant; TRAP, translating ribosome affinity-purification; yHP, yoked highly palatable; yST, yoked standard.

an earlier publication (47) and visualized and analyzed using Cytoscape (58). We retained only the 1% highest-ranking non-zero edges (313,944 edges). We filtered the list of edges to retain the linking genes differentially expressed between master and yoked animals fed with HP food (Supplemental Methods).

Spine Analysis

Spines were stained using the Golgi-Cox method (59) and counted as described in the Supplemental Methods by investigators blinded to the mouse group.

Statistical Analyses

We analyzed the data with GraphPad Prism 6 (GraphPad Software). Normality was checked with the D'Agostino and Pearson normality test. If n was <7 or the distribution significantly differed from normal, nonparametric tests were used. Complete statistical analyses results are presented in Table S2.

RESULTS

Food Palatability Induces Differential Behavioral Responses in an Operant Training Paradigm

The mice used for translatome profiling were trained in an operant paradigm for obtaining either ST (mST) or HP food (mHP) (Figure 1A). Yoked control mice (yST and yHP, respectively) were placed in the same conditions but received food pellets noncontingently when their paired master mouse obtained one. Under food restriction and low operant schedule (fixed ratio 1 [FR1], i.e., one pellet obtained for one poke), mHP mice displayed slightly more positive pokes than mST animals (Figure 1B) (for statistical analyses, see Table S2). As expected, yoked mice exerted no operant responses (Figure 1B),

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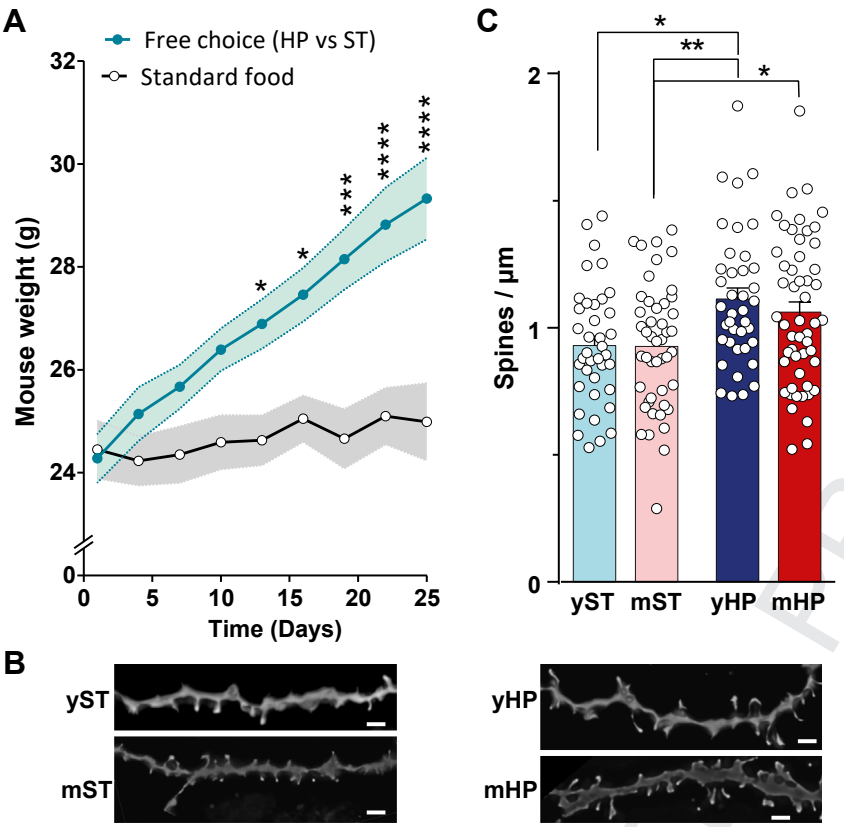

Figure 2. In wild-type mice, food free choice increases weight and HP food increases spine density in the nucleus accumbens. **(A)** Male 3- to 4-month-old wild-type C57BL/6 mice were kept in their home cage with free access to both HP and ST isocaloric food (free choice, n = 10). A different group had access only to ST food (n = 10). Weight of mice was monitored every third day for 24 days. Mice in the free choice conditions gained more weight than mice with access to ST food only. For each time point, within each group the data distribution was not different from normal. Two-way repeated measure analysis of variance, interaction: $F_{8,144} = 14.18$, $p < 10^{-4}$; time effect: $F_{8,144} = 26.30$, $p < 10^{-4}$; food type effect: $F_{1,18} = 10.09$, $p = .0052$. **(B)** Wild-type male mice were subjected to operant training as described in Figure 1A and killed 24 hours after the last session. Sections of the nucleus accumbens were stained using the Golgi-Cox method. Examples of dendrites from mice in each group are shown. Scale bar = 4 µm. **(C)** Sections through the nucleus accumbens stained with the Golgi method as in panel **(B)**, and the number of dendritic spines per micrometer were counted (38–52 dendrites counted per group, 8 mice per group). Two-way analysis of variance, interaction: $F_{1,171} = 0.40$, $p = .53$; food type: $F_{1,71} = 15.90$, $p < 10^{-4}$; role (yoked vs. master): $F_{1,171} = 0.47$, $p = .49$. Imaging **(B)** and spine analysis **(C)** were done by investigators blinded to the group. **(A, C)** Multiple post hoc comparisons Holm-Sidak test. *$p < .05$; **$p < .01$; ***$p < .001$; ****$p < 10^{-4}$. HP, highly palatable; mHP, master highly palatable; mST, master standard; ST, standard; yHP, yoked highly palatable; yST, yoked standard.

although they consumed as many pellets as their respective masters (Figure 1C). The ratio requirement increase (FR5) led to a higher augmentation in operant behavior in the mHP group than in the mST group (Figure 1B, D). The number of obtained rewards did not decrease in mHP mice compared with FR1 (Figure 1E). These data suggest that the motivational drive was enhanced in the mHP group, consistent with the incentive effect of palatability on operant responding (60). When mice no longer had food restricted in their home cage (ad libitum condition), both master groups tended to poke less (Figure 1B, D). However, this decrease was more pronounced in the mST than in the mHP group (Figure 1D, F), suggesting that mHP animals are less sensitive to satiety-induced devaluation of the food. To rule out differences due to weight changes, we monitored weight in an independent experiment and found that all mice recovered their initial weight within a day at the end of the restriction phase, with no difference between groups (Figure S1). These results demonstrate that, independently of the caloric intake, HP food enhances the motivational drive to seek food and blunts the effect of satiety on food-seeking behavior, two characteristics that are believed to be major culprits for the development of compulsive eating and obesity (1).

To determine whether HP food used for operant training induced a loss of control over food intake and weight gain, we evaluated wild-type mice, which had either access only to ST food or free choice between the ST and HP isocaloric foods for 30 days (Figure 2A). Exposure to ST/HP free choice led to a more pronounced weight gain than free access to ST food only. Together, these results indicate that mice, as expected, had a higher incentive drive for HP food.

The establishment and maintenance of goal-directed behavior have been related to morphological alterations in the corticostriatal pathway with increased spine density following operant training for drugs (61) or HP food (62). We examined whether the exposure to HP food or the operant training had measurable effects on NAc neuron spine density in our experimental conditions, using Golgi staining in wild-type C57BL/6 male mice (Figure 2B). We found a food type effect (two-way analysis of variance: $F_{1,171} = 15.90$, $p < 10^{-4}$) (Figure 2C; Table S2), indicating a predominant effect of food palatability on NAc neuron spines with an increased density after 2 weeks.

Translating Ribosome Affinity Purification Followed by RNA Sequencing Reveals Major Effects of Palatable Food Conditioning on Gene Expression in D2SPNs

As long-lasting behavioral adaptations depend on changes in gene expression (63), we investigated the translating mRNA in NAc D1SPNs and D2SPNs. We focused on relatively stable alterations by isolating cell population–specific translating mRNA 1 day after the last training session. We used 2 to 4

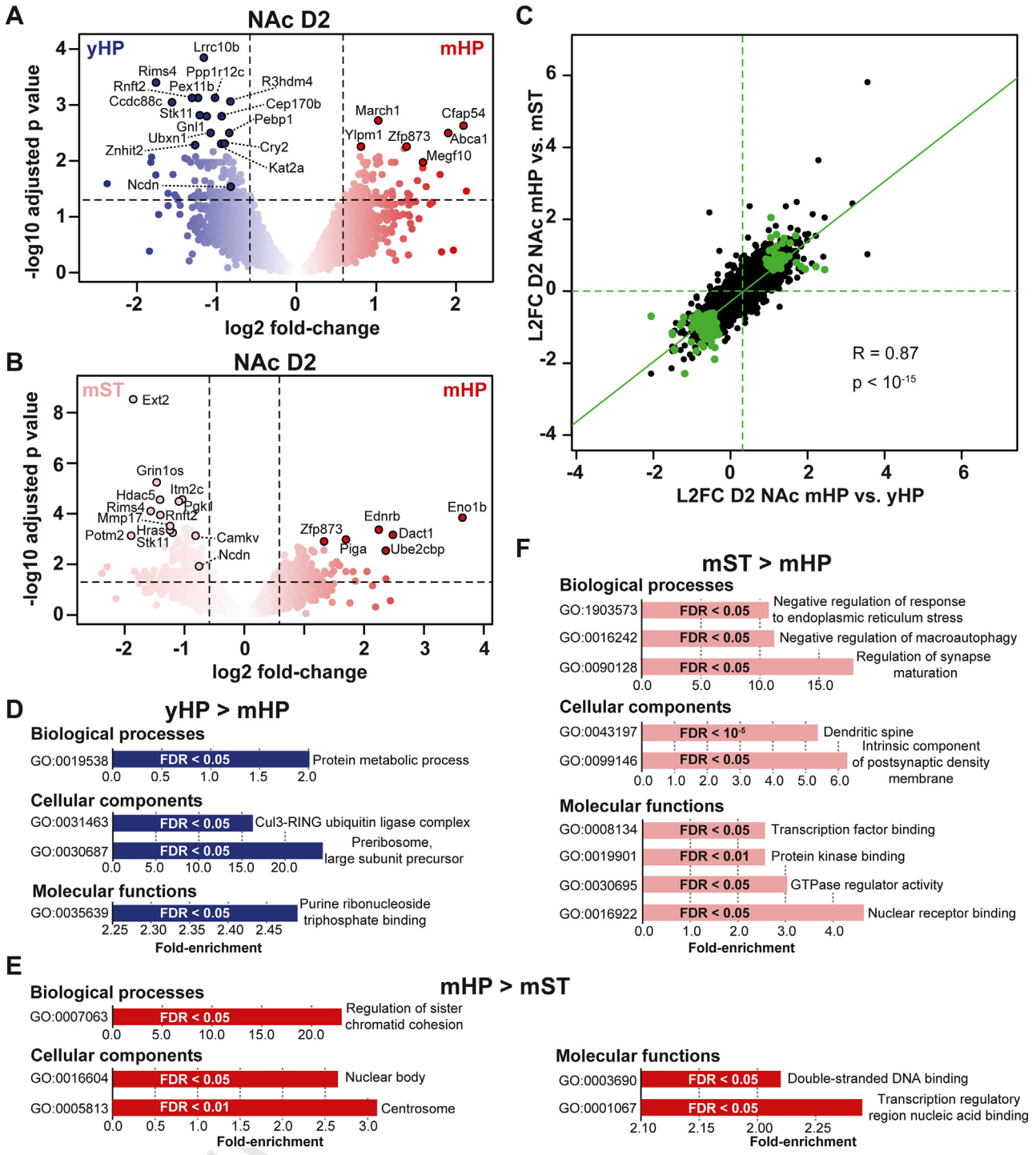

Figure 3. Effects of operant training for standard and highly palatable food on translating mRNA in D2R-expressing neurons in the NAc. *Drd2*-EGFP/Rpl10a transgenic mice trained as described in Figure 1A were killed 24 hours after the last training session, and the NAc was rapidly dissected. mRNA was immunopurified (3 mice pooled per sample) (Table S2) and quantified by the translating ribosome affinity purification followed by RNA sequencing method (Table 1; Tables S7 and S10). **(A)** Volcano plot of gene comparison between yHP and mHP groups in NAc D2R-expressing neurons. Names of the main differentially expressed genes are indicated. **(B)** Volcano plot of gene comparison between mST and mHP groups in NAc D2R-expressing neurons, with names of main differentially expressed genes indicated. **(C)** Scatter plot of the translating mRNA differences in the NAc D2R-expressing striatal projection neurons between mHP and yHP groups (x-axis) [see panel **(A)**] and between mHP and mST (y-axis). The correlation coefficient was calculated for all genes. mRNAs significantly different in both comparisons are indicated in green. **(D)** GO analysis of genes more expressed in yHP than in mHP in D2R-expressing neurons (Table S12). Only major nonredundant GO pathways are indicated. **(E, F)** GO analysis of genes more expressed in mHP than in mST **(E)** or more expressed in

samples per condition, each containing the left and right NAc from 3 mice (1–2 females per sample) (Table S1). Our experiment (Figure 1A) was designed to detect the mRNA footprints of 1) the goal-directed component of food-seeking behavior (master vs. yoked), 2) food palatability (yST vs. yHP), and 3) the interaction between food palatability and goal-directed behavior (mST vs. mHP). We retained the genes that exhibited an absolute change greater than 1.5-fold in either direction (absolute log2 fold change over 0.585), with a multiple comparisons–adjusted p value $< .05$ (summary in Table 1). We previously showed that the genes detected with this approach predominantly originate from SPNs (47).

We detected almost no differences in D1R-expressing neurons (Table 1; Tables S3–S6). In contrast, operant conditioning for HP food had striking effects in D2R-expressing neurons compared with the other groups (Table 1; Table S7). Although cholinergic interneurons also express D2Rs, the translating mRNA was mostly originating from D2SPNs as indicated by the low levels of markers of cholinergic interneurons, including Chat, Slc10a4, Slc18a3, Slc5a7, and Slc17a8 (Table S7), as previously reported (47). Changes in translating mRNA between mHP and yHP were detected in 213 genes (Figure 3A; Table S7). Operant conditioning for ST food had few effects on translating mRNA (4 genes) (Table 1; Table S8). The type of food caused almost no differences between yHP and yST mice (1 gene) (Table 1; Table S9). In contrast, modifications between mHP and mST mice were detected in 513 genes (Table 1; Figure 3B; Table S10). Thus, most changes in mRNA were observed in D2SPNs and in response to operant training for HP food. The complete results of the comparison between operant conditioning for HP food (mHP vs. yHP) and the comparison between conditioning with HP or ST food (mHP vs. mST) are highly correlated ($R = 0.87$, $p < 10^{-15}$) (Figure 3C). This reflects the predominant effects of conditioning with HP food on gene expression in NAc D_2 neurons (Table 1). Accordingly, among the 213 mRNAs differentially translated between mHP and yHP mice, 140 (66%) were also significantly changed between mHP and mST mice, all changing in the same direction (Table S11). Thus, most changes in translating mRNA induced by operant training were selectively taking place in D2SPNs of the mHP group.

Pathways and Gene Interaction Clusters Affected in NAc D2SPNs by Operant Conditioning

We used several approaches to evaluate the functional implications of the changes observed in translating mRNAs induced by operant conditioning for HP food in D2SPNs. First, Gene Ontology analysis showed that the pathways decreased in D2SPNs of mHP mice compared with the yHP group were related to ribosomes and ubiquitin ligase (Figure 3D; Table S12). In the comparison between mHP and mST groups, Gene Ontology terms increased in mHP mice were related to chromatin and centrosomes, whereas those that diminished included synaptic and signaling pathways (Figure 3E, F; Table S13). We then used the network inference performed as previously described (47) to identify gene clusters affected by operant conditioning with HP food. The output was a large connected network with only a few isolated edges (Figure 4). The main network presented two super-connected clusters of nodes with degrees over 10 (Figure 4). Both clusters comprise mostly genes whose expression was decreased by conditioning. One is enriched in genes coding proteins involved in calcium and cAMP (cyclic adenosine monophosphate) signaling (*Camkv, Mast3, Pde1b, Ppp1r12c, Tesc*), possibly indicating a modulation of signaling, including by DA. This cluster also contains *Gabrd* and *Gabra3*, higher in yHP and mHP, respectively. *Ncdn* is decreased in mHP and is also part of this cluster. The second cluster is enriched in proteins involved in protein production, from transcription (*Gtf2f1, Zpf622*) and splicing (*Puf60, Rrp1, Znhit2*) to translation (*Eif2b5, Rplp0, Trmt61a*), folding (*Cct7, Vbp1*), and protein modifications (*Gm16286, Ube3a*). This cluster analysis indicates that beyond synaptic function, conditioning also regulates basic cellular properties and suggests overall adaptation of cellular properties following operant conditioning for HP food.

Table 1. Number of Differentially Expressed Genes

Differences	D1SPNs	D2SPNs
mHP > yHP	2	84
mHP < yHP	3	129
mST > yST	0	1
mST < yST	0	3
yHP > yST	4	1
yHP < yST	2	0
mHP > mST	0	203
mHP < mST	0	310

D1SPNs, D_1 receptor–expressing striatal projection neurons; D2SPNs, D_2 receptor–expressing striatal projection neurons; mHP, master highly palatable; mST, master standard; yHP, yoked highly palatable; yST, yoked standard.

Role of *Ncdn* in Motivation for HP Food

We next sought to validate the relevance of our analysis by genetically manipulating one of the genes differentially regulated by conditioning for HP food. We focused on *Ncdn*, which was highly expressed and downregulated in NAc D2SPNs in the mHP group compared with yHP and mST groups (Table S1). Neurochondrin is an important modulator of morphological and synaptic plasticity through regulation of metabotropic glutamate receptor 5 membrane trafficking (48) and calcium/calmodulin-dependent protein kinase II activity (64). Moreover, forebrain-specific *Ncdn* KO generates a depressive-like phenotype (48). We hypothesized that deletion of *Ncdn* in neurons could mimic or alter some behavioral features of operant responding for HP food. We used *Ncdn*-cKO mice generated with *Camk2a-Cre* (48,65) and compared them with their $Ncdn^{Flox/Flox}$ littermates. Both *Ncdn*-cKO and

mST than in mHP **(F)** (Table S13), with only major nonredundant GO pathways indicated. **(D–F)** FDR values are indicated. D2R, D_2 receptor; FDR, false discovery; GO, Gene Ontology; GTPase, guanosine triphosphatase; L2FC, log2 fold change; mHP, master highly palatable; mRNA, messenger RNA; mST, master standard; NAc, nucleus accumbens; yHP, yoked highly palatable

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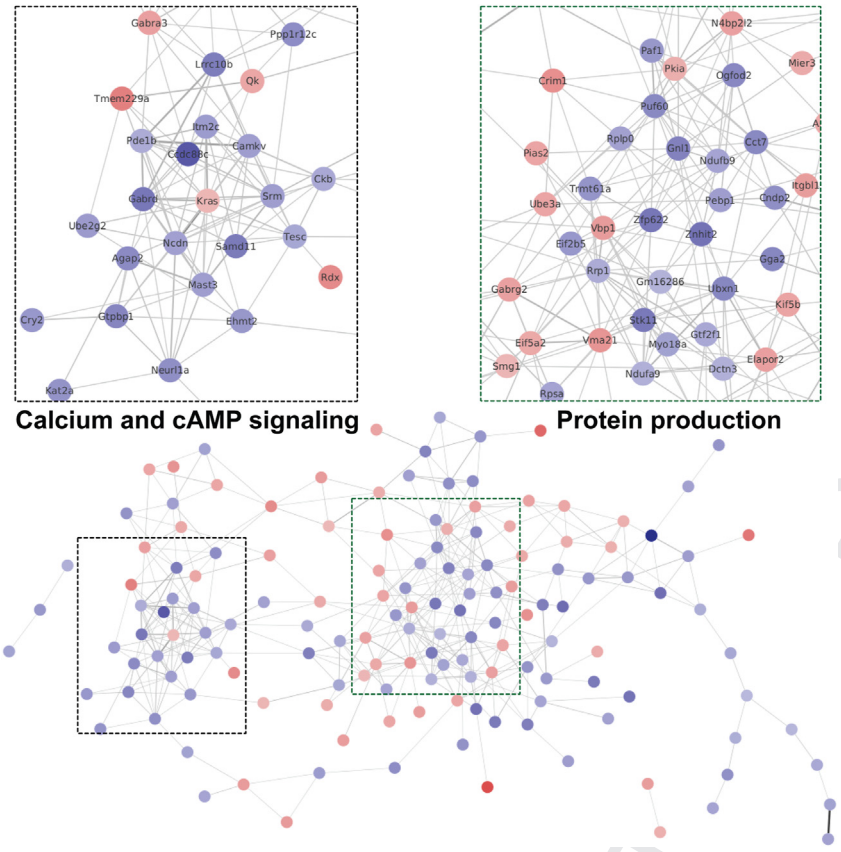

Figure 4. Main clusters of genes with translating messenger RNA regulated by conditioning for highly palatable food in the D_2 receptor–expressing neurons of the nucleus accumbens. Only the top 1% predicted interactions were retained and filtered using the genes with a mean expression greater than 30 counts per million, differentially expressed by at least 50% (log2 fold change greater than 0.585) at a false discovery rate less than 0.05. Almost all the remaining genes formed a supercluster. Two overconnected subclusters emerged, with degrees over 10. They comprised mostly genes suppressed by highly palatable food, involved either in calcium and cAMP signaling (left inset) or protein production (right inset). Nodes are colored with log2 fold changes of expression, blue genes being more expressed in yoked animals and red genes more expressed in master animals. Edge thickness and darkness are proportional to the interaction score. cAMP, cyclic adenosine monophosphate.

Ncdn$^{Flox/Flox}$ mice displayed higher operant responding when working for HP food than for ST food (Figure 5A, B; Table S2). The response of *Ncdn-cKO* mice for HP food was at some time points higher than that of *Ncdn*$^{Flox/Flox}$ mice (Figure 5A, B), showing that the mutation did not impair operant learning, but appeared to increase it during food-deprived FR1 and FR5 reinforcement schedules. During ad libitum access to food, we observed a decrease in positive pokes for ST food (Figure 5C), but not HP food (Figure 5D), in *Ncdn*$^{Flox/Flox}$ mice as above in wild-type mice (Figure 1F). In contrast, a pronounced decrease in pokes was observed in *Ncdn*-cKO mice working for HP food (Figure 5D). This result suggested that despite their stronger learning phase than *Ncdn*$^{Flox/Flox}$ mice (Figure 5A, B), the *Ncdn*-cKO mice were less motivated for HP food when they had ad libitum access in their home cage. We tested this hypothesis using a progressive ratio protocol 1 day after the conditioning paradigm (Figure 5E). The *Ncdn*-cKO mice displayed a lower breakpoint (i.e., the number of operant responses resulting in the last obtained reward) than the *Ncdn*$^{Flox/Flox}$ mice, confirming a decreased motivation (Figure 5E).

To determine whether these operant results depended on a change in reward sensitivity, we tested *Ncdn*$^{Flox/Flox}$ and *Ncdn*-cKO mice in a free choice paradigm over 43 days, during which mice of either genotype had free access to both ST and HP food. The analysis of cumulative caloric intake showed that in this free choice condition, both *Ncdn*$^{Flox/Flox}$ and *Ncdn*-cKO mice had an approximately 20-fold preference for HP over ST food (Figure 6A). However, HP food intake was lower in *Ncndn*-cKO mice than in *Ncdn*$^{Flox/Flox}$ control mice (Figure 6A). *Ncdn*$^{Flox/Flox}$ mice in the free choice condition displayed a significant weight gain compared with a matched group with access to ST food only (Figure 6B). In contrast, in *Ncdn*-cKO mice we did not observe any weight gain in the free choice condition compared with ST only (Figure 6B). Thus, deletion of *Ncdn*, a gene whose mRNA is less translated in NAc D2SPNs following operant conditioning for HP food, tends to increase operant behavior during food restriction but restores some satiety-induced devaluation and decreases motivation for HP food and weight gain in a free choice paradigm. These latter results suggest that decreased expression of *Ncdn* in the NAc could be a compensatory mechanism, counterbalancing the persistent effects of HP food on motivation.

DISCUSSION

HP foods recruit and have the potential to hijack the brain reward system, similar to drugs of abuse, leading to overeating, satiety-induced desensitization, and craving, thus resulting in weight increase and contributing to increased prevalence of obesity [see (3,6,23,66,67) for reviews]. The mechanisms of these effects of food are typically investigated in rodent models, using free access to fat- and/or sugar-rich food. Even though these paradigms mimic in part the

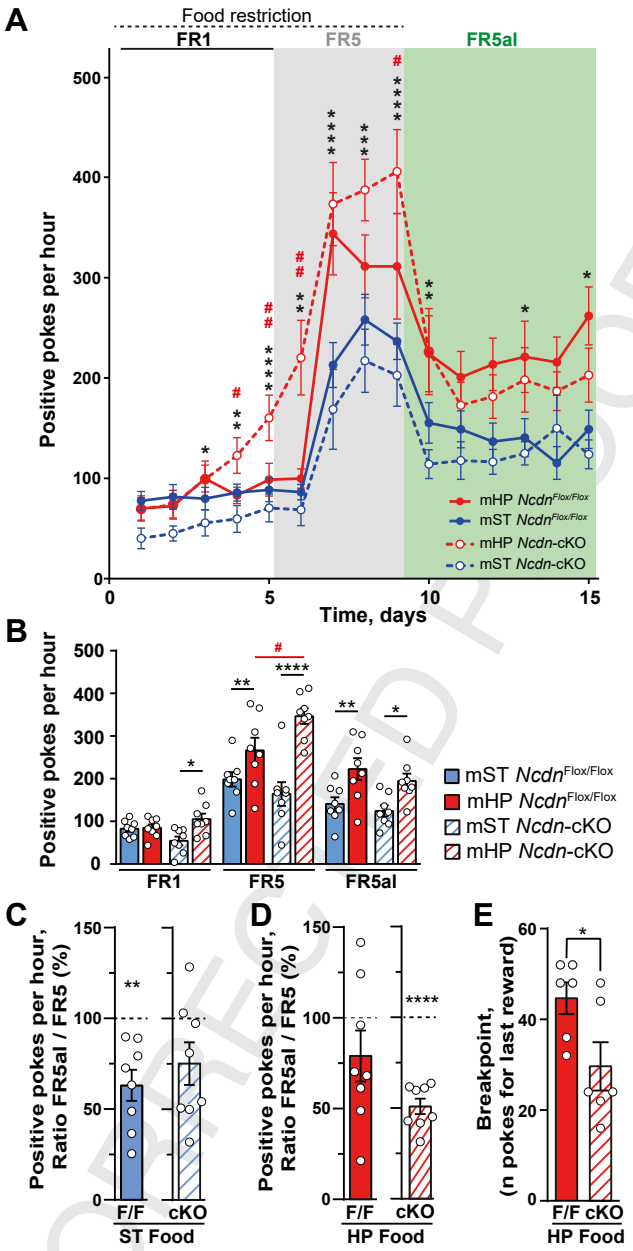

Figure 5. Role of *Ncdn* in satiety-induced devaluation. **(A)** Time course of the number of positive pokes (i.e., in the active hole) during operant training of *Ncdn*-cKO and control littermates (*Ncdn*$^{Flox/Flox}$) for ST or HP food (8 mice per group), using the schedule described in Figure 1A. Two-way repeated-measures analysis of variance for each phase (see Table S2 for complete statistical results). Group effect, FR1: $F_{3,28} = 4.6$, $p < .01$; FR5: $F_{3,28} = 11.42$, $p < 10^{-4}$; FR5al: $F_{3,28} = 6.17$, $p = .002$. Fisher's least significant difference between mST and mHP *Ncdn*-cKO (*) and mHP in *Ncdn*$^{Flox/Flox}$ vs *Ncdn*-KO (#). **(B)** Average positive pokes per 1-hour session during each period of conditioning for ST (mST) or HP (mHP) food in *Ncdn*-cKO and *Ncdn*$^{Flox/Flox}$ mice (8 per group; data from Figure 4A). Two-way analysis of variance, group (genotype/food) effect: $F_{3,63} = 17.5$, $p < 10^{-4}$; conditioning phase effect: $F_{2,21} = 86.9$, $p < 10^{-4}$; interaction: $F_{6,63} = 3.00$, $p = .012$. Fisher's least significant difference between mST and mHP *Ncdn*-cKO (*) and mHP in *Ncdn*$^{Flox/Flox}$ vs. *Ncdn*-KO (#). **(C, D)** In these mice (8 per group), satiety-induced decreased motivation for nose poking was evaluated as in Figure 1D by making the positive pokes ratio FR5al/FR5. **(C)** For ST food, one sample *t* test, *Ncdn*$^{Flox/Flox}$: $t_7 = 4.33$, $p = .0034$; Ncdn-cKO: $t_7 = 2.17$, $p = .069$. **(D)** For HP food, one sample *t* test, Ncdn$^{Flox/Flox}$: $t_7 = 1.50$, $p = .18$; Ncdn-cKO: $t_7 = 11.5$, $p < 10^{-4}$. **(E)** The motivation of Ncdn$^{Flox/Flox}$ and Ncdn-cKO mice (6 per group) trained for HP food as in panel **(A)** was evaluated in a progressive ratio protocol, in which the number of pokes necessary to obtain the reward is increased after each reward. The breaking point was the number of pokes achieved by each mouse to obtain the last pellet within the 90-minute session. Mann-Whitney test, $U = 5$, $p = .032$. #$p < .05$, *$p < .05$; ##$p < .01$, **$p < .01$; ***$p < .001$; ****$p < 10^{-4}$. cKO, conditional knockout; F/F, Flox/Flox; FR, fixed ratio; FR5al, fixed ratio 5 with ad libitum food access in home cage; HP, highly palatable; mHP, master highly palatable; mST, master standard; ST, standard.

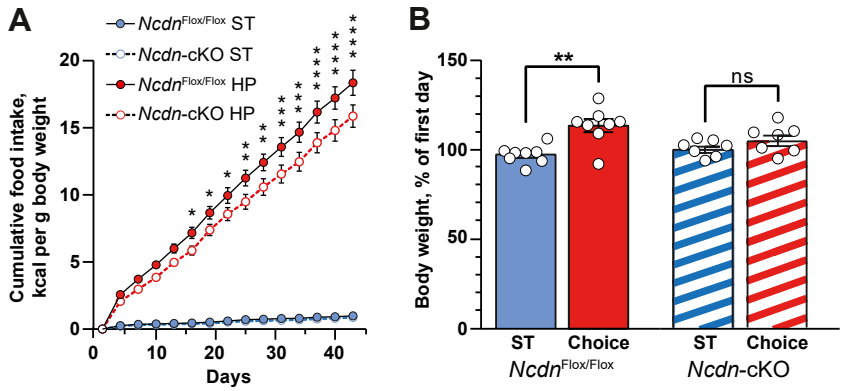

Figure 6. *Ncdn* cKO decreases HP food consumption and weight gain in food free choice conditions. **(A)** *Ncdn*-cKO mice consume less HP food than $Ncdn^{Flox/Flox}$ littermates in a free choice paradigm. For 6 weeks, mice in their home cage had free access to ST and HP food, and the consumption of each type of food was monitored ($Ncdn^{Flox/Flox}$, $n = 8$ mice; *Ncdn*-cKO, $n = 7$ mice). Although mice from both genotypes clearly preferred HP food, the *Ncdn*-cKO animals consumed less HP food than $Ncdn^{Flox/Flox}$ control mice. Two-way analysis of variance, interaction: $F_{42,364} = 207.3$, $p < 10^{-4}$; time effect: $F_{14,364} = 736.7$, $p < 10^{-4}$; group effect: $F_{3,26} = 201.3$, $p < 10^{-4}$. Post hoc multiple comparisons Holm-Sidak test results are shown between HP food consumption of *Ncdn*-cKO and $Ncdn^{Flox/Flox}$ mice. **(B)** Weight of mice in free choice conditions in panel **(A)** was compared to weight of mice of the same genotype with access only to ST food. Weight of $Ncdn^{Flox/Flox}$ mice was higher in free choice conditions than in ST food–only conditions, whereas this was not the case in *Ncdn*-cKO mice. Kruskal-Wallis test, $p = .0066$; Dunn's multiple comparisons test, $Ncdn^{Flox/Flox}$, $p = .0018$, $n = 8$ mice per group, and *Ncdn*-cKO, $p = .55$, $n = 7$ mice per group. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < 10^{-4}$. cKO, conditional knockout; HP, highly palatable; ns, not significant, ST, standard.

Western diet, they do not probe the effects of palatability on goal-directed actions. Here, using operant conditioning with food rewards that differ only by their palatability, we specifically monitored the effects of palatability on two major components of food-seeking behavior, which are believed to be altered in pathological feeding behavior, i.e., the incentive component and sensitivity to satiety. As expected, HP food enhanced motivation to exert effort in food-deprived and ad libitum conditions, as described for highly caloric/palatable foods (62). Although both the ST and the HP groups significantly decreased responding when switched from restricted to ad libitum access to food, the HP group was less sensitive to this satiety-induced devaluation, resembling the accelerated development of habit behavior in animals chronically exposed to a high-fat diet (68). This suggests that palatability, independently of caloric intake, could be sufficient to lead to compulsive-like behavior toward food. The reinforcing effects of rewards involve the meso-accumbens DA pathway (27,69). The involvement of the NAc in our experimental conditions is supported by the increase of dendritic spine density in this region after 2 weeks of exposure to highly palatable food, in line with previous reports of longer experiments (61,70).

Our study identifies alterations in the translatome of NAc D2SPNs 24 hours after the last operant conditioning session. Although FR5 operant training and high palatability reward could each be considered as relatively mild stimuli, with no effect on translatome in our conditions, their combination changed translating mRNA at this measurement time. This result indicates that a physiologically relevant combination of stimuli is able to alter gene expression in the NAc, presumably contributing to plasticity underlying learning. Translating mRNA alterations in response to operant conditioning for HP food were virtually restricted to D2SPNs. Despite the important early role of D1SPNs in food-induced long-term behavioral adaptations (71,72), we found virtually no significant changes in their translatome at the end of the operant protocol. Although the lack of change in D1SPNs could result from insufficient experimental power of the experiment, the fact that raising the significance threshold does not yield many more positive findings (Tables S3–S6) argues against this possibility. Rather, it is possible that translatome alterations in D1SPNs occur at an earlier time in conditioning, not explored in our study. The persistent changes in D2SPNs align with indirect observations that point to the role of these neurons in feeding disorders and obesity (14,73,74). Striatal hypofunction and increased body mass index are more frequent in individuals harboring the *ANKK1* TaqIA A1 allele, which is associated with decreased striatal availability of D2Rs (75–77). Data in rodents also support a role of D2Rs in the development of some features of obesity (39,40). Recent findings highlight a particular sensitivity of D2SPNs to circulating lipids (78,79) and implicate D2Rs and D2SPNs in the regulation of energy output (80,81), peripheral glucose levels, glucose-dependent reinforcement learning (82), and diet-induced obesity (83). Our findings also support the role of D2SPNs and identifies pathways and gene networks possibly underlying long-term adaptive modifications in these neurons, including downregulation of genes related to signaling and synaptic functions and upregulation of genes related to DNA and transcription.

To test the functional importance of the genes disclosed by our analysis, we focused on *Ncdn*, a highly expressed gene involved in synaptic function and downregulated in NAc D2SPNs. *Ncdn* neuronal deletion had restricted consequences on HP food–related behavior. *Ncdn*-cKO mice showed an increased initial operant response for HP food, suggesting that the basic mechanisms of reward-induced procedural learning were not impaired. However, the mutant mice displayed satiety-induced devaluation for HP food and decreased motivation for HP food in a progressive ratio task performed when mice were fed ad libitum. The same *Ncdn*-cKO mutation generates a depressive-like phenotype (48) and a decreased preference for sucrose (65). In our experiments, *Ncdn*-cKO mice preferred HP food to ST food and readily learned to work for it. Yet this preference was slightly blunted and did not result in the weight gain observed in control mice. These results suggest a partial alteration of motivation in *Ncdn*-mutant mice.

The molecular actions of neurochondrin in feeding behavior are not known and may combine several components. Neurochondrin inhibits melanin-concentrating hormone receptor 1 (84), which, in the NAc, stimulates feeding behavior (85),

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possibly contributing to the increased operant response in food-restricted cKO mice. In hippocampus, neurochondrin is important for metabotropic glutamate receptor 5 endocytosis and metabotropic glutamate receptor–mediated AMPA receptor endocytosis (48,86), and *Ncdn* cKO alters long-term depression and long-term potentiation of synaptic transmission (48). A similar plasticity defect in *Ncdn* cKO mice may interfere with the establishment of persistent motivation for HP food. In wild-type mice, *Ncdn* downregulation after HP food operant training might contribute to persistent increased feeding behavior and provide a stabilizing mechanism after initial plasticity, leading to persistent behavioral alterations.

Our study has methodological limitations that include using *Camk2a* promoter–dependent *Ncdn* cKO mice in which the lack of neurochondrin in diverse neuronal populations or brain regions other than the NAc may contribute to the behavioral phenotype. Further studies are also needed to identify the SPN populations in which spines are increased and their relation to mRNA alterations. Yet, our study provides the first description of the footprint of HP food–operant conditioning on the translating mRNA landscape in dopaminoceptive neurons of the NAc. It underlines the involvement of D2SPNs and identifies networks of implicated genes. Downregulation of *Ncdn* in response to HP food may play a dual role by increasing feeding behavior and modulate the conditioning-induced persistent motivational drive to eat HP food. Further exploration of *Ncdn* actions is warranted to explore novel approaches to counteract overeating and its contribution to obesity.

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J-AG, EM, and J-PR conceived and J-AG and J-PR supervised the project. EM, J-PR, AG, DH, EV, MM, NG, and J-AG designed the experiments. EM, AG, YN, BdP, AP, MB, and J-PR performed experiments. EM, J-PR, AG, ACN, EV, DH, and J-AG analyzed data; LT and NG performed, analyzed, and interpreted bioinformatics analyses; EM, AG, LT, YN, LG, Q20 ACN, EV, DH, NG, PG, MF, J-PR, and J-AG discussed the data and provided input and corrections to the manuscript. EM, NG, J-PR, and J-AG wrote the manuscript. All the authors except PG approved the final version of the manuscript.

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ARTICLE INFORMATION

Q21 From the Institut National de la Santé et de la Recherche Médicale Unite Mixte de Recherche-S 1270, Paris, France (EM, AG, LT, YN, AP, BdP, DH, J-AG); Faculty of Sciences and Engineering, Sorbonne Université, Paris, France (EM, AG, LT, YN, AP, BdP, DH, J-AG); Institut du Fer à Moulin, Paris, France (EM, AG, LT, YN, AP, BdP, DH, J-AG); Laboratory of Molecular and Cellular Neuroscience, Rockefeller University, New York, New York (MB, PG, MF, J-PR); Institut de Génomique Fonctionnelle, University of Montpellier, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Montpellier, France (EV); Eurecat, Centre Tecnològic de Catalunya, Unitat de Nutrició i Salut, Reus, Spain (MM); Instituto de investigaciones médicas Hospital del Mar, Barcelona, Spain (MM); Department of Psychiatry, Yale School of Medicine, Connecticut Mental Health Center, New Haven, Connecticut (ACN); and aSciStance Ltd, Great Chesterford, United Kingdom (NG).

EM is currently affiliated with the Unite Mixte de Recherche 1286 Q22 NutriNeuro-INRAE, Université de Bordeaux, Bordeaux, France. AG is currently affiliated with the Departament de Biomedicina, Facultat de Medicina i Ciències de la Salut, Institut de Neurociències, Universitat de Barcelona, Barcelona, Spain; Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, Madrid, Spain; Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain; and Production and Validation Center of Advanced Therapies (Creatio), University of Barcelona, Barcelona, Spain. LT is currently affiliated with the Unite Mixte de Recherche 1166, Faculté de Médecine, Sorbonne University, Paris, France. YN is currently affiliated with the Department of Pharmacology, Kurume University School of Medicine, Kurume, Fukuoka, Japan. AP is currently affiliated with the Department of Neuroscience, Pasteur Institute, Paris, France. MB is currently affiliated with the Department of Molecular and Comparative Pathobiology, Research Animal Resources, Johns Hopkins University School of Medicine, Baltimore, Maryland. BdP is currently affiliated with the Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel. J-PR is currently affiliated with the Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts.

AG and LT contributed equally to this work.

PG and J-AG contributed equally to this work as joint senior authors.

Address correspondence to Enrica Montalban, Ph.D., at enrica.montalban@inrae.fr, or Jean-Antoine Girault, M.D., Ph.D., at jean-antoine.girault@inserm.fr.

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