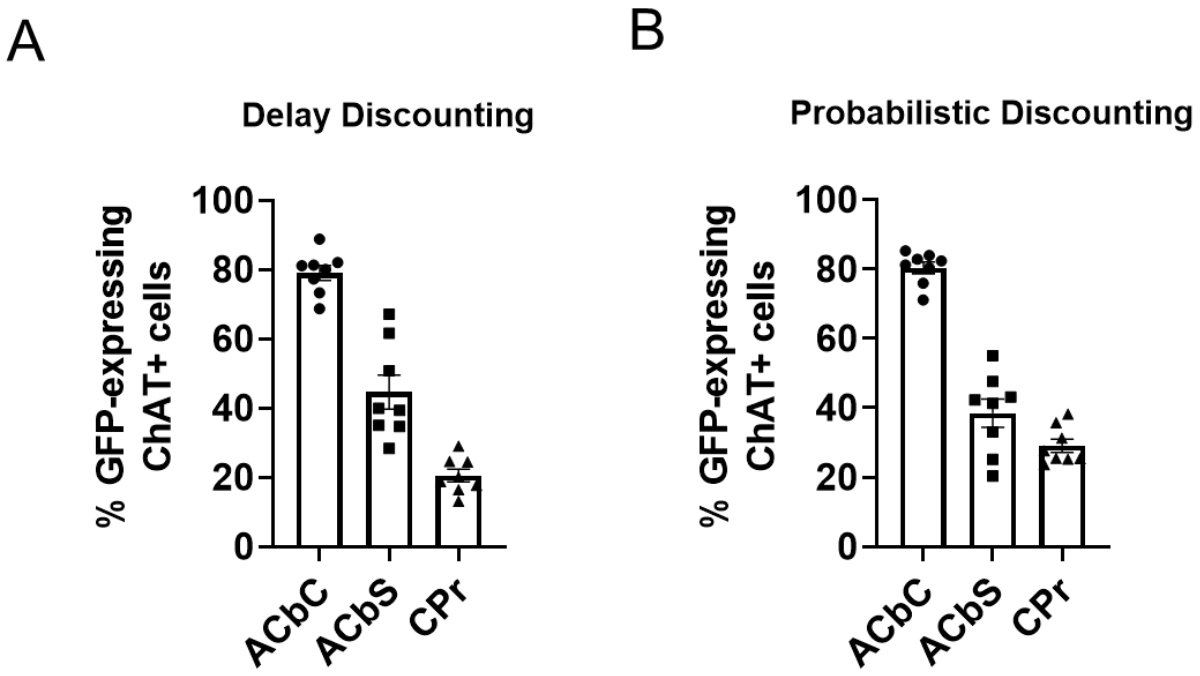

Figure S1. D2R virus expression in CINs in striatal sub-regions. Quantification of the percentage of ChAT+ neurons that co-expressed D2R-overexpressing AAV (DIO-D2-P2A-EGFP) in mice that performed delay discounting (**A**) and probabilistic discounting (**B**) tasks. ACbC, nucleus accumbens core; ACbS, nucleus accumbens shell; CPr, rostral caudoputamen.

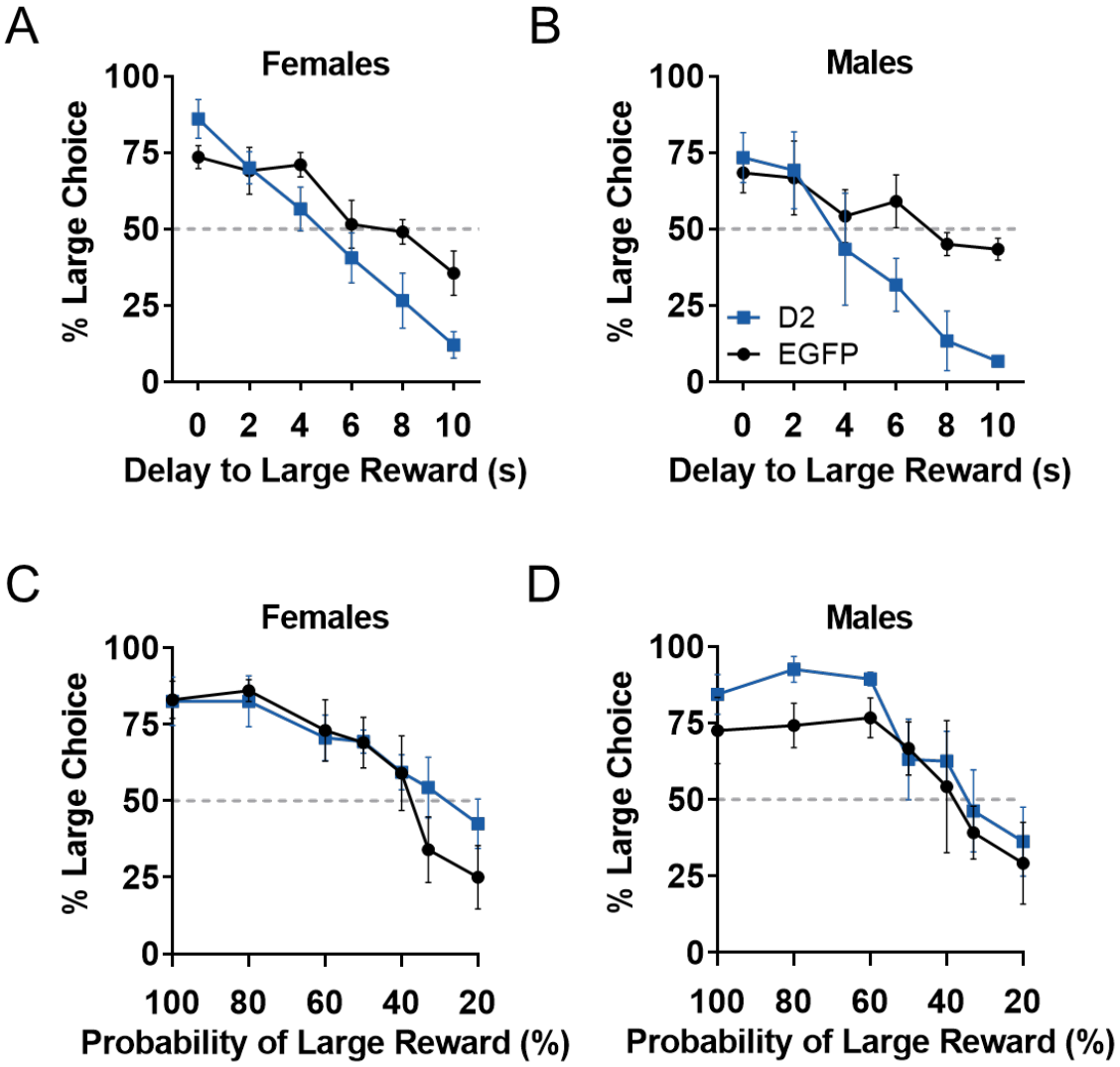

Figure S2. Analysis of delay and probabilistic discounting by sex following D2R upregulation. A. Percent preference for the large reward in delay discounting (related to Fig. 1D) by sex. In females (n = 5/group), two-way ANOVA showed significant main effects of virus ($F_{(1, 48)} = 6.620$, $p = 0.0132$) and delay ($F_{(5, 48)} = 21.16$, $p < 0.0001$). No significant virus x delay interaction was found ($F_{(5, 48)} = 2.329$, $p = 0.0567$). **B.** In males (n = 3/group), significant main effects of virus ($F_{(1, 24)} = 8.863$, $p = 0.0066$) and delay ($F_{(5, 24)} = 7.853$, $p = 0.0002$) were found, but there was not a significant virus x delay interaction effect ($F_{(5, 24)} = 1.746$, $p = 0.1624$). **C.** Percent preference for the large reward in probabilistic discounting (related to Fig. 2C) by sex. Females (EGFP, n = 5; D2, n = 4) showed a significant main effect of probability ($F_{(6, 42)} = 17.83$, $p < 0.0001$) but not a significant main effect of virus ($F_{(1, 7)} = 0.2542$, $p = 0.6296$) or probability x virus interaction effect ($F_{(6, 42)} = 1.210$, $p = 0.3204$). **D.** Males (EGFP, n = 3; D2, n = 4) showed a significant main effect of probability ($F_{(6, 30)} = 19.47$, $p < 0.0001$), but no significant main effects of virus ($F_{(1, 5)} = 0.4908$, $p = 0.5148$) or probability x virus interactions ($F_{(6, 30)} = 0.5544$, $p = 0.7627$) were observed.

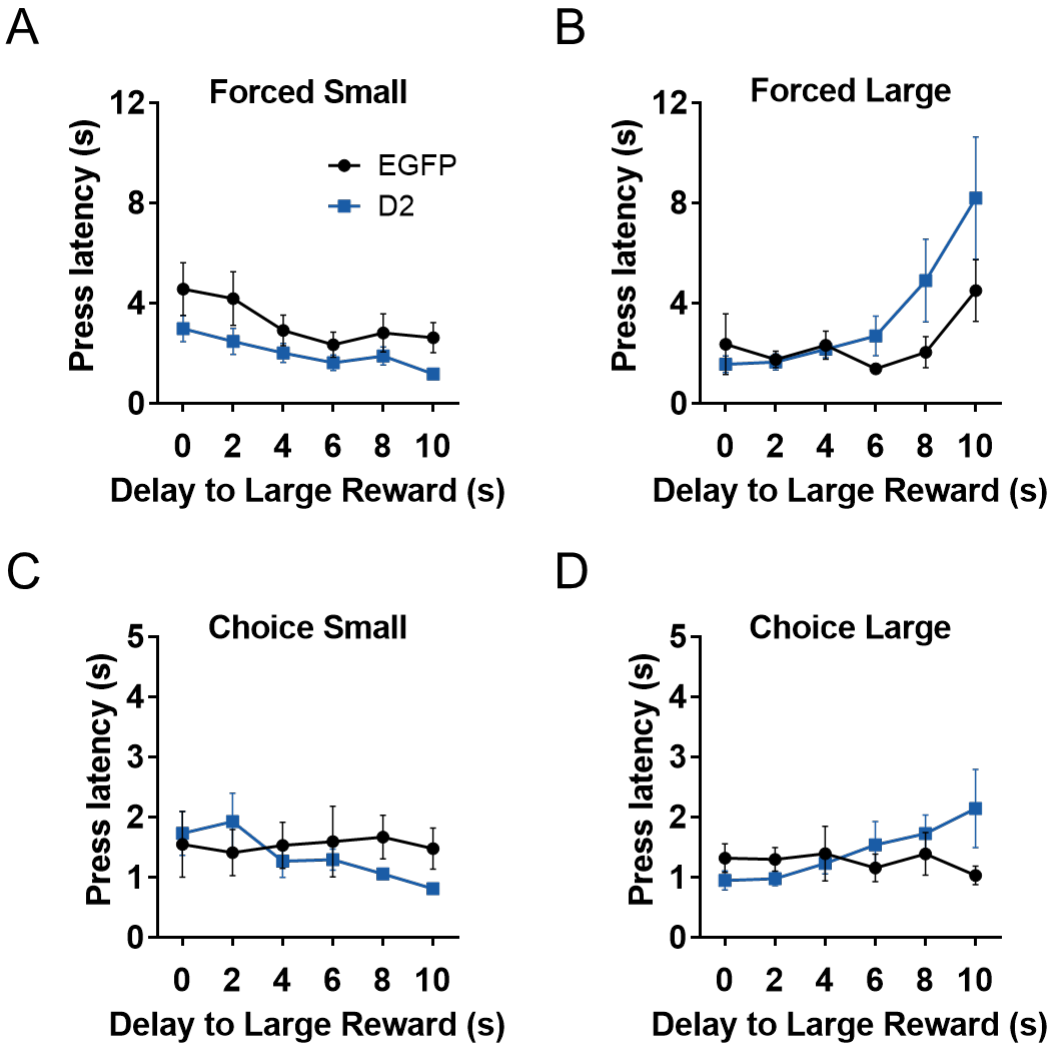

Figure S3. Analysis of median press latency for large and small rewards in delay discounting following D2R upregulation. A. Press latency in forced trials for EGFP- and D2R-expressing mice (related to Fig. 1E) broken down by large and small rewards. A significant main effect of delay was observed in forced small trials ($F_{(5, 70)} = 4.411$, $p = 0.0015$). No significant main effects of virus ($F_{(1, 14)} = 3.995$, $p = 0.0654$) or delay x virus interactions ($F_{(5, 70)} = 0.3318$, $p = 0.8921$) were found. **B.** A significant main effect of delay was observed in forced large trials ($F_{(5, 70)} = 7.277$, $p < 0.0001$). No significant main effects of virus ($F_{(1, 14)} = 1.594$, $p = 0.2273$) or delay x virus interactions ($F_{(5, 70)} = 1.910$, $p = 0.1036$) were found. **C.** Press latency in free choice trials for EGFP- and D2R-expressing mice (related to Fig. 1F) broken down by large and small rewards. No significant main effects of virus ($F_{(1, 14)} = 0.4012$, $p = 0.5367$), delay ($F_{(5, 70)} = 0.6677$, $p = 0.6492$), or delay x virus interactions ($F_{(5, 70)} = 0.9462$, $p = 0.4569$) were observed in the small choice. **D.** In large choice trials, there were no significant main effects of delay ($F_{(5, 69)} = 1.261$, $p = 0.2907$) or virus ($F_{(1, 14)} = 0.3170$, $p = 0.5823$), but a significant delay x virus interaction was found ($F_{(5, 69)} = 2.497$, $p = 0.0388$).

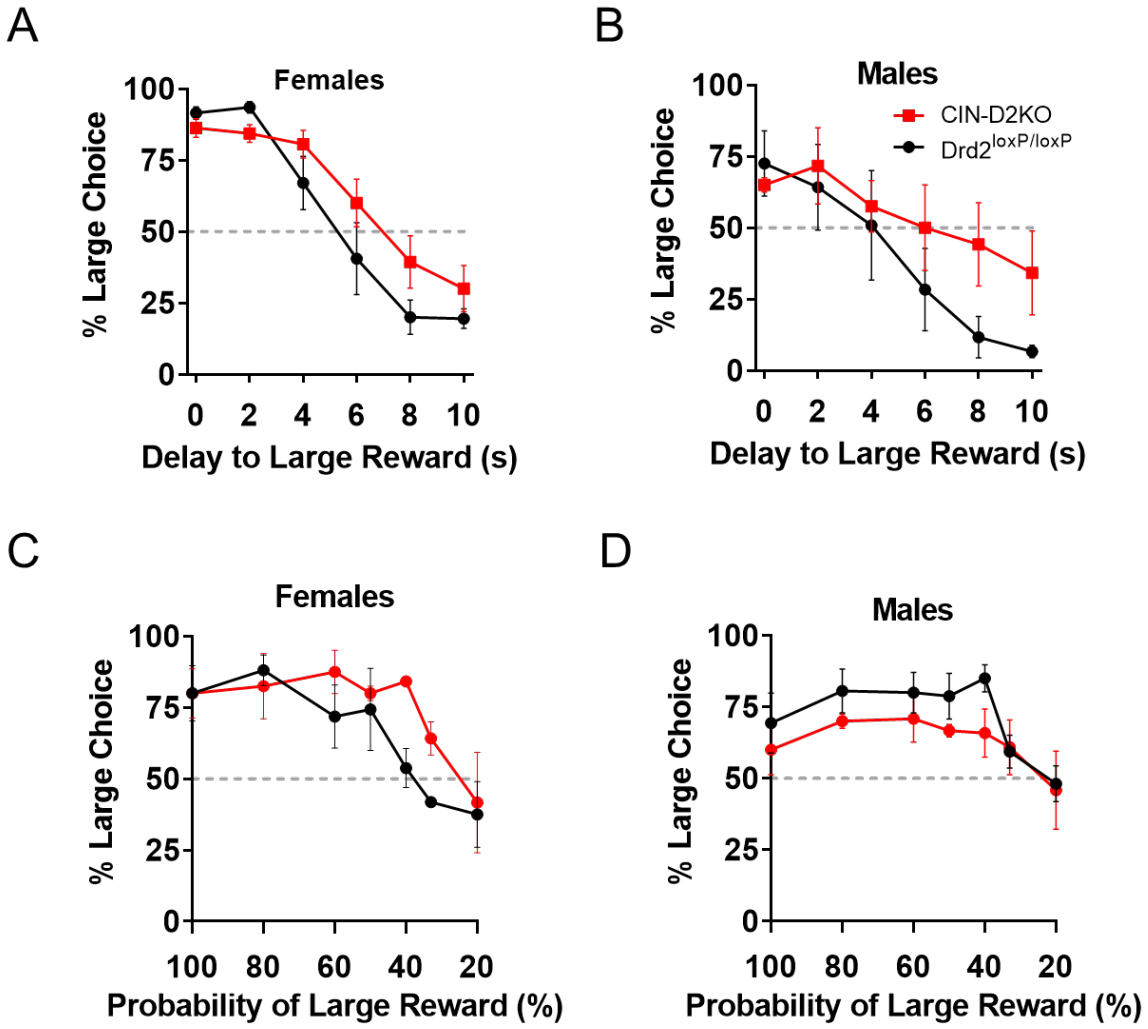

Figure S4. Analysis of delay and probabilistic discounting by sex following CIN D2R deletion. A. Percent preference for the large reward in delay discounting (related to Fig. 4B) by sex. In females (CIN-D2KO, n = 4; $Drd2^{loxP/loxP}$, n = 5), two-way ANOVA showed a significant main effect of delay ($F_{(5, 35)} = 55.59$, $p < 0.0001$) and a delay x genotype interaction effect ($F_{(5, 35)} = 2.594$, $p = 0.0425$), but no significant main effects of genotype ($F_{(1, 7)} = 1.317$, $p = 0.2889$) were observed. **B.** In males (CIN-D2KO, n = 3; $Drd2^{loxP/loxP}$, n = 3), there was a significant main effect of delay ($F_{(5, 20)} = 22.76$, $p < 0.0001$) and a delay x genotype interaction effect ($F_{(5, 20)} = 3.097$, $p = 0.0314$), but no significant main effects of genotype ($F_{(1, 4)} = 0.8422$, $p = 0.4107$) were found. **C.** Percent preference for the large reward in probabilistic discounting (Fig. 4H) broken down by sex. Females (CIN-D2KO, n = 3; $Drd2^{loxP/loxP}$, n = 4) showed a significant main effect of probability ($F_{(6, 30)} = 11.80$, $p < 0.0001$) but no significant main effects of genotype ($F_{(1, 5)} = 1.003$, $p = 0.3625$) or probability x genotype interactions ($F_{(6, 30)} = 1.739$, $p = 0.1462$) were observed. **D.** Males (CIN-D2KO, n = 3; $Drd2^{loxP/loxP}$, n = 4) showed a significant main effect of probability ($F_{(6, 30)} = 5.601$, $p = 0.0005$) but no significant main effects of genotype ($F_{(1, 5)} = 1.379$, $p = 0.2932$) or probability x genotype interactions ($F_{(6, 30)} = 0.5419$, $p = 0.7721$) were found.

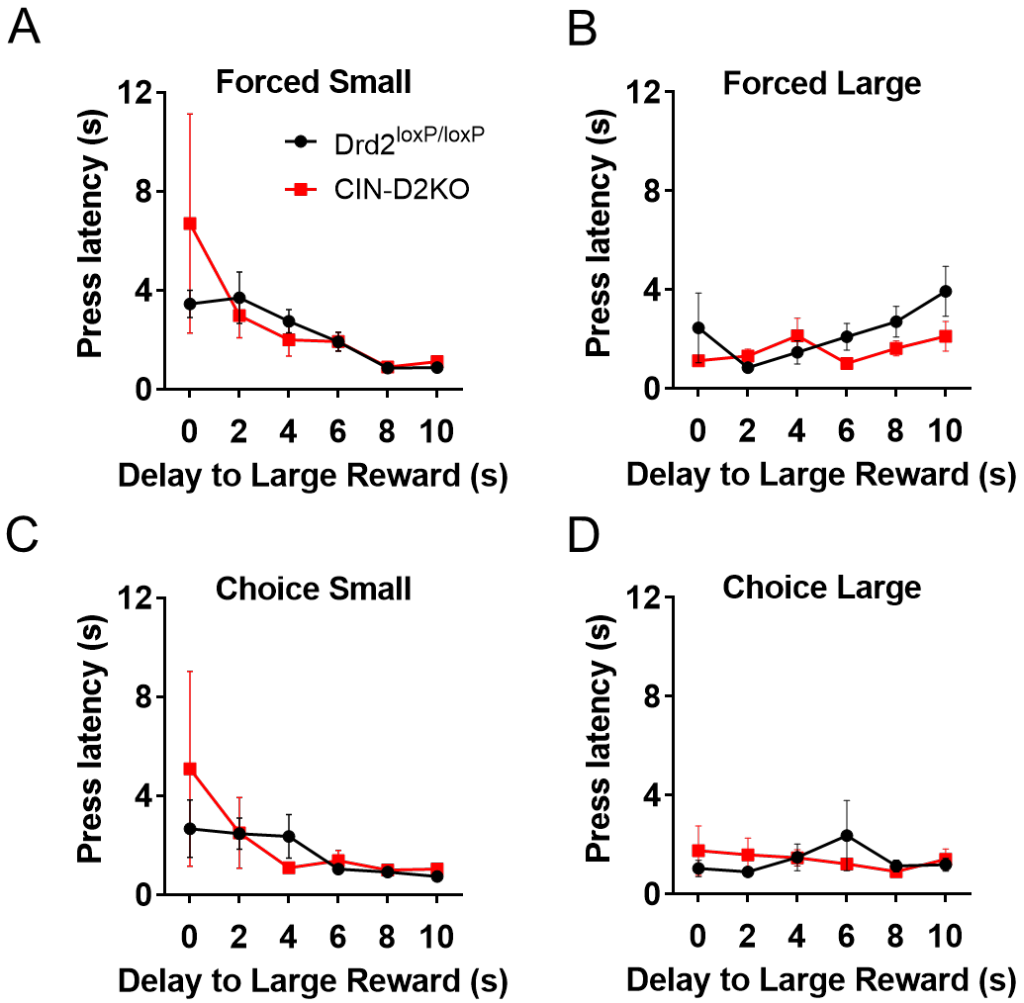

Figure S5. Analysis of median press latency for large and small rewards in delay discounting following CIN D2R deletion. **A.** Press latency in forced trials for control and D2R-knockout mice (related to Fig. 4C) broken down by large and small rewards. A significant main effect of delay was observed in forced small trials ($F_{(5, 65)} = 3.571$, $p = 0.0065$). No significant main effects of genotype ($F_{(1, 13)} = 0.1156$, $p = 0.7392$) or delay x genotype interactions ($F_{(5, 65)} = 0.7795$, $p = 0.5681$) were observed. **B.** In forced large trials, no significant main effects of delay ($F_{(5, 70)} = 0.6677$, $p = 0.6492$), genotype ($F_{(1, 14)} = 0.4012$, $p = 0.5367$), or delay x genotype interactions were found ($F_{(5, 70)} = 0.9462$, $p = 0.4569$). **C.** Press latency in free trials for control and D2R-knockout mice (related to Fig. 4D) broken down by large and small rewards. No significant main effects of genotype ($F_{(1, 14)} = 0.1323$, $p = 0.7215$), delay ($F_{(5, 70)} = 2.162$, $p = 0.0681$), or delay x genotype interactions ($F_{(5, 70)} = 0.5748$, $p = 0.719$) were found in the small choice. **D.** In the large choice, no significant main effects of genotype ($F_{(1, 13)} = 6.54 \times 10^{-6}$, $p = 0.998$), delay ($F_{(5, 63)} = 0.5050$, $p = 0.7714$), or delay x genotype interactions ($F_{(5, 63)} = 1.020$, $p = 0.4137$) were observed.

SUPPLEMENTARY MATERIALS AND METHODS

Operant Apparatus

Mice were run in sixteen operant chambers (model ENV-307w, Med-Associates, St. Albans, VT) each inside of a light- and sound-attenuating cabinet. The chamber interior (22 × 18 × 13 cm) was equipped with a liquid dipper resting in a feeder trough that administered a drop (13-15 µL) of undiluted evaporated milk (Nestlé Carnation) when raised. The feeder trough was centered on one wall in the chamber, and head entries into this trough were measured via an infrared photocell detector. Retractable levers were located on either side of the feeder trough, and each had an LED light above it. The flooring of the chamber consisted of metal rods placed 0.87-cm apart. An audio speaker (ENV-324W), located inside the operant chamber on the wall opposite the dipper and levers, was used to deliver tone stimulus (90 dB, 2500 Hz). The chamber was illuminated by a house light mounted on the wall opposite the trough during all sessions. The experimental protocols were controlled via Med-PC computer interface and Med-PC IV or V software. Behavioral events were recorded with a temporal resolution of 10 ms.

Dipper and lever press training.

For operant behavior experiments, mice were food-restricted and maintained at 85-90% of their baseline body weight; water was available *ad libitum*. For the first dipper training session, 20 dipper presentations were separated by a variable inter-trial interval (ITI, mean 45 s) and ended after 20 rewards were retrieved or after 30 min had elapsed, whichever occurred first. Dipper remained in raised position until a head entry was made into the food port, and was lowered 10 s later. Criterion consisted of the mouse making head entries during 20 dipper presentations in one session. In the second training session, the dipper was lowered 8 s after being raised, and the criterion was achieved when mice made head entries during 30 of 30 dipper presentations. Lever press training was done using a fixed ratio-1 (FR-1) schedule, where each press led to 1

reward. Levers were retracted after each reinforcer and were presented again after a variable ITI (average 30 s). Dippers were raised for 5 s. FR-1 training was done in 2 sessions per day (one for each lever; order alternated each day). The session ended following 20 reinforcers earned, or after 30 min. Sessions were repeated daily until all mice earned 20 reinforcers on each lever. Next, to determine initial lever preference for discounting experiments, mice were presented with both levers in single sessions over 3 days, with both levers rewarded on a FR-1 schedule. Sessions ended after a total of 30 reinforcers earned or after 30 min, whichever occurred first. A mouse's initially preferred lever was determined as the one with the greater total number of presses after the 3 days. This lever was assigned the small reward (1 dipper) in subsequent discounting experiments, whereas the less preferred lever was assigned the large reward (3 milk dippers given in succession).

Temporal Discrimination and Peak Interval

Following initial dipper training, mice received FR-1 lever training, in which a reward was given immediately following a lever press that occurred within the first 30 s of lever presentation or if no press was made for 30 s. In both cases, the lever retracted upon dipper presentation. Trials occurred on a variable ITI (mean = 30 s). Criterion was met for these FR-1/FT-30 sessions when mice earned at least 30 rewards by lever pressing in two consecutive sessions. For discrimination training, half of the mice were trained to press the left lever following a 2 s ("short") tone and the right lever following an 8 s ("long") tone. This rule was reversed for the remaining mice. The first training day involved the presentation of a single tone duration and its corresponding lever. The other tone-lever pair was presented the next day. The next two sessions used single lever presentation but randomly interspersed trials of each type. Trials were randomized by picking from a list without replacement such that no more than 4 trials of the same type occurred in a row. For the next three days, 50% of the trials randomly presented

either the 2 s or 8 s tone followed by extension of both levers (choice response trials), while the other 50% of trials presented single duration-single lever pairings (forced response trials). Mice were then trained on a 75% choice response session for 3 days, such that 75% of trials consisted of choice response trials and 25% of trials presented single duration-single lever pairings. In the final phase of sessions, all the trials were choice response trials. The first 5 sessions of this type included correction trials in which an incorrect response elicited the re-presentation of the same tone duration in the following trial. The remaining 10 sessions did not include this correction. For all choice response sessions, reward was given only when the correct lever was pressed. The levers were retracted following reward presentation or an incorrect lever press. Not pressing within 10 s of the end of tones led to lever retraction and no reward. In a subsequent experiment, the duration of the short tone was increased from 2 to 6 s and the long tone was increased from 8 to 24 s. Original lever assignments were maintained. Sixteen additional sessions of this type were conducted, and mice ran one session per day throughout all experiments.

Peak Interval Training

Following the temporal discrimination task, the same mice received one additional FR-1 session before starting fixed interval training. Here, trials began with lever extension. Lever presses were only rewarded if they occurred after a fixed interval following lever extension. Each reinforcement was followed by a variable ITI (mean = 30 s) during which the lever remained retracted. The FI durations were 4, 8, 16, and 24 s. When a mouse earned at least 40 rewards in one session, the FI duration was extended in the next session. Sessions ended after either 60 min or 40 rewards. In peak interval training, a target interval of 24 s was used as described [1]. Half of the mice in each group were randomly assigned the left lever and half were assigned the right lever, irrespective of lever assignments in the preceding duration discrimination task. Each training session consisted of FI-24 and peak trials. In peak trials, the lever was extended for 72-

96 s, but lever presses were not rewarded. Initially, mice were presented with a random combination of 48 FI-24 s trials and 12 peak trials. Once they earned 40 rewards, sessions then consisted of 36 FI-24 s trials and 24 peak trials. All trials were randomly selected from an array without replacement. Sessions ended after 90 min or when mice completed 60 trials. Mice received 15 peak interval sessions.

Open Field

A separate cohort of D2R- and EGFP-expressing mice was tested in open field boxes (42 cm long x 42 cm wide) equipped with infrared photobeams to measure locomotor activity (Med Associates, St. Albans, VT). Data were acquired using Kinder Scientific Motor Monitor software (Poway, CA). Total distance traveled as well as distance in 5-min bins over 90 minutes were measured. A separate cohort of CIN-D2KO and control mice was tested at a different site using different open field chambers as above (26 cm long x 15 cm wide), and video-tracking software (Anymaze).

Histology and image analysis

Mice were transcardially perfused with ice-cold 4% paraformaldehyde (Sigma, St. Louis, MO) in PBS under ketamine/xylazine anesthesia. Brains were harvested, postfixed overnight, and washed in PBS. Coronal sections were cut at 30-µm using a Leica VT2000 vibratome (Richmond, VA). Sections were incubated for 2 h at room temperature in blocking solution (10% fetal bovine serum, 0.5% bovine serum albumin in 0.5% TBS-Triton X-100) and then labeled overnight at 4ºC with primary antibodies against GFP (chicken, 1:1000, AB13970 Abcam, Cambridge, MA) and ChAT (goat. 1:100, AB144P Millipore, Burlington, MA). Sections were incubated at room temperature with the corresponding fluorescent secondary antibodies for 2 h. Sections were then mounted and coverslipped on slides with Vectashield containing containing DAPI (Vector, Burlingame, CA). Slides were imaged using a Nikon Eclipse Ti-E epifluorescence

microscope or Leica TCS SP8 laser-scanning confocal microscope before being processed with NIH Image J software. Ten brain sections spanning the rostral-caudal extent of the NAc were stained and imaged. Using ImageJ software, sections were individually aligned to corresponding atlas planes (Franklin and Paxinos, 3rd edition) using the BigWarp plug-in. Immunolabeled cells were manually counted within atlas-defined regions of interest outlining the NAc core, NAc shell, and rostral aspect of the CPu in both hemispheres, and presented as the percentage of total ChAT-positive cells that were GFP-positive.

REFERENCES

1. Drew MR, Simpson EH, Kellendonk C, Herzberg WG, Lipatova O, Fairhurst S, et al. Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. J Neurosci. 2007;27(29):7731-9.