Supplemental Information

Supplemental Methods & Materials

Generation of D2R-OE Mice

Briefly, mice expressing the human D2 receptor under control of the tet-operator (tet-O_D2R mice) were crossed to mice expressing the tetracycline transactivator (tTA) transgene under the calcium/calmodulin-dependent kinase IIα promoter (CamKIIa-tTA mice) (1), offspring were used for behavioral and molecular analysis. To control for genetic background, we followed the recommendations made by the Banbury conference on genetic background in mutant mice (2). Namely, that mutations be maintained in congenic lines, and that mutants be analyzed in a defined hybrid (and preferably F1) genetic background. Therefore tetO-D2R mice were maintained on a congenic C57BL/6J background and CamKIIa-tTA mice were maintained on a congenic 129S6/SvEvTac background. Crossing these lines resulted in F1 offspring including double transgenic mice which carry both the TetO-D2 and CamKIIa-tTA transgenes and which express the D2R transgene specifically in the striatum (D2R-OE mice). Littermate mice carrying only one of the transgenes, or neither transgene, were combined and used as controls. Mice were genotyped by triplex polymerase chain reaction (PCR) using primers specific for tTA, tet-O and a fragment of the endogenous D1 receptor gene (to provide a positive control for the PCR). To specifically test the effect of transgenic D2R overexpression we compared double transgenic mice to control mice that included single-transgenic and wild-type littermates. To regulate tet-O-driven gene expression, mice were fed doxycycline-supplemented chow (40 mg/kg; Bioserv, Frenchtown, NJ) beginning at 12 weeks of age. Behavioral

experiments or gene expression analysis were commenced after a minimum of 2 weeks of doxycycline chow. To motivate mice to earn rewards in the operant tasks, food was restricted to 1 h daily access in the home cage after testing (regular maintenance mouse chow of the same chow supplemented with doxycycline). Water was available ad libitum.

Mice were housed, bred and tested according to the local IACUC guidelines. They were maintained on a 12 hour light/dark cycle and tested during the light phase.

Behavior Testing

Apparatus

The operant chambers (Med-Associates, St. Albans, VT; model env-307w) had internal dimensions $22^{½}$ x $18^{½}$ x $12^{½}$ and were located in a light- and sound- attenuating cabinet equipped with an exhaust fan, which provided 72 dB background white noise. Each chamber was equipped with a feeder trough that was centered on one wall of the chamber. A reward of one drop of evaporated milk could be provided by raising a dipper. An infrared photocell detector was used to record head entries into the trough. A retractable lever was mounted on the same wall as the feeder trough. The chambers were illuminated throughout all sessions with a houselight (Med Associates #1820) located at the top of the chamber. An audio speaker was positioned 8.5 cm from the floor on the wall opposite the feeder trough. The speaker delivered a brief tone (90 db, 2500 Hz, 200 ms) to signal when the liquid dipper was raised.

Procedures

One session was run each day 5 days per week. Independent groups of mice were used for each experiment. The table below (Table S1) provides an outline of the training and testing procedures used for each experiment. A detailed explanation, as well as the number of sessions

used is provided in the supplementary text. The numbers of mice used for each experiment and condition are also provided in the table, as well as in the figure legends in the results section.

Table S1. Outline of training and testing sessions

	Progressive Ratio Increments	**Progressive Interval**	**Chronic Haloperidol**	**Pilot SB242084**	**Acute SB242084**
Control mice	*No dox n = 8* *Dox =9*	*n = 6*	*Vehicle n = 8* *0.1 mg/kg n = 6* *0.25 mg/kg n = 5*	*n = 6*	*Vehicle n = 8* *0.75 mg/kg n = 8*
D2R-OE mice	*No dox n =7* *Dox n =6*	*n = 4*	*Vehicle n = 5* *0.1 mg/kg n = 7* *0.25 mg/kg n = 6*	*n = 6*	*Vehicle n =7* *0.75 mg/kg n = 8*
	Dipper Training	Dipper Training	Dipper Training	Dipper Training	Dipper Training
	↓	↓	↓	↓	↓
	Modified Lever Press Training	Standard Lever Press Training	Standard Lever Press Training	Standard Lever Press Training	Standard Lever Press Training
	↓	↓	↓	↓	↓
	Variable Ratio/Random Ratio	Variable Ratio/Random Ratio	Fixed Interval (4s, 8s, 16s, 24s)	Fixed Interval (4s, 8s, 16s, 24s)	Fixed Interval (4s, 8s, 16s, 24s)
	↓	↓	↓		↓
	Progressive Ratio (+1, +2, +5, +10)	Progressive Interval (x2)	Progressive Ratio (X2)		Progressive Ratio (X2)

Procedure detail

Dipper training. All mice were first trained to consume the liquid reward from the dipper located inside the feeder trough. Mice were placed inside the chambers with the dipper in the raised position, providing access to a drop of evaporated milk. The dipper was retracted 10 s after the first head entry into the feeder trough. A variable intertrial-interval (ITI) ensued,

followed by a new trial identical to the first. The session ended after 30 min or 20 dipper presentations. On the following day, mice received another session similar to the first, except that the dipper retraction was response-independent. On each trial the dipper was raised for 8 s and then lowered whether or not mice had made a head entry. Sessions like this continued until a mouse made head entries during at least 20 of 30 dipper presentations in one session. In this and all other segments of the experiment, sessions occurred once per day, 5 days per week.

Standard lever press training. Mice were required to press a lever to earn the liquid reward. For the first lever press training session, mice were placed in the chamber for 8 h. At the beginning of the session the lever was extended into the chamber, and lever presses were reinforced on a continuous reinforcement (CRF) schedule. In this and all subsequent sessions, the reward consisted of raising the dipper for 5 s. To familiarize mice with the retraction and extension of the lever, after the twentieth reinforcement, the lever was retracted. After a variable delay (average 30 seconds), the lever was extended, and the cycle repeated. If a mouse did not earn 100 reinforcements in the session, it repeated the procedure the next day. Two days after the first successful 8 h lever press training session, mice received a shorter CRF training session. The session began with the lever extended. The lever was retracted after every two reinforcements and then re-extended after a variable ITI (average 30 seconds). The session ended when the mouse earned 60 reinforcements, or one hour elapsed. Mice continued receiving sessions like this until they earned 40 rewards in one session. Mice then moved to either fixed interval (FI) training (haloperidol and SB24280 experiments) or one, 120-minute session of variable ratio/random ratio (VR/RR) training (progressive interval experiment). In the latter case, after running on a VR-2 schedule for 20 reinforcements, the training program switched to an RR-5 schedule for 30 reinforcements. The RR section of the program was capped at 20 presses per

reward, and the lever was extended for the entirety of the session. For mice that received VR/RR training, testing on either progressive interval (PI) or progressive ratio (PR) schedules began the following day.

Modified lever press training. Mice which were tested in the PR increment experiment underwent modified lever press training consisting of two phases. The first phase consisted of three sessions, each containing 60 trials. In each trial the lever was presented after a variable ITI averaging 30 s. A lever press during the first 6 s of lever presentation resulted in immediate reward. After the lever had been extended for 6 s without a press, the dipper was raised for 8 s. In both cases, the lever was retracted at the moment the dipper was raised. In the second phase of lever press training, mice received three days of the shorter CRF training session as described above. After the third day, the mice moved on to VR/RR training for one day, and then began PR testing.

FI training. For all PRx2 (see below) experiments the mice were first trained on a fixed interval schedule in order to be consistent with the training used in our earlier PRx2 experiments (3). In FI training, lever presses were not reinforced until after a fixed interval (timed relative to the lever extension) had elapsed. Mice began on FI-4s schedule, meaning that the first lever press occurring more than 4 s after lever extension was reinforced. Each reinforcement was followed by a variable ITI (mean = 30 s, range = 110 s), during which the lever remained retracted, and then a new trial, signaled by the extension of the lever. When a mouse earned at least 40 rewards in one session, the FI duration was extended in the next session. The FI durations were 4 s, 8 s, 16 s, and 24 s. When a mouse reached the criterion of 40 rewards in one session on the FI-24s it was moved to PR training.

PR testing. PR training was used to assess the amount of effort a mouse was willing to expend to obtain a reward. On each trial, the lever was extended and after the mouse made a criterion number of lever presses a reward was delivered. For the majority of the experiments presented here, the criterion was set at 2 lever presses for the first trial and then doubled with each successive trial (PRx2), such that the second trial required 4 lever presses, the third trial 8 presses and so forth. Mice were tested one session a day for between 2 and 5 days. To see if the genotype difference would be consistent across different PR schedules, the increment in output required after each reward was varied in the PR increments experiment (Figure 1), i.e on the PR+5 schedule the first reward was available after 5 lever presses, the next after 10, the next after 15, etc. The mice were tested on each PR schedule for 5 days, and the order of schedules was counterbalanced across subjects, i.e. where some mice were switched to a more difficult schedule, others were switched to an easier one. For all PR schedules, the session ended after 2 h or after 3 min had elapsed without a lever press. We chose to end the session after 3 minutes of non-responding because in a previous study in which the testing session continued for two hours, post hoc analysis determined that excluding any responses made after 3 minutes of responding did not change the animals' breakpoint.

PI testing. In this task, the delay between reward presentations was doubled after each reward (2 s, 4 s, 8 s, 16 s, 32 s, etc…). So long as the mouse made at least one response the next scheduled reward was presented at the appropriate time. The session continued until no response had been made for 3 minutes or 2 hours had elapsed. The mice were tested for 5 consecutive days and the average performance of each mouse on the last 3 days was analyzed.

Gene Expression Analysis

RNA isolation

For both gene chip and real time PCR experiments, total RNA was extracted from the striata or prefrontal cortex (PFC) of 5 individual D2R-OE and 5 Control littermate mice using trizol and purified using the RNAeasy clean-up kit (Qiagen, Hilden, Germany).

Gene chip analysis

Total RNA from each of the 10 animals was amplified and transcribed into cRNA using the MessageAmp II Biotin Enhanced Kit (Ambion, Austin, TX) and hybridized with 10 Affymetrix 430A2.0 microarrays in the Gene Chip Facility of the Columbia Genome Center. The raw data was analyzed using dChip (http://www.biostat.harvard.edu/complab/dchip/) and ErmineG (http://bioinformatics.ubc.ca/ermineJ/).

Quantitative real time reverse transcription-PCR (RT-PCR)

The same RNA isolated for the gene chip experiments was used for quantitative real time RT-PCR as described previously (4). After treatment with DNAse1, purification with the RNAeasy kit (Qiagen), cDNA was then synthesized using the Invitrogen Superscript II First Strand Synthesis System (Invitrogen, Carlsbad, CA). Real time PCR was carried out in 50 ul total reaction volumes using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and 10 μM of each primer. Primer sequences used included:

5-HT2A:GGCGATTCTGCCTGAGACTAAA+CATGAGATCCAAAACGAGGAGG

5-HT2C:ACAAAAAGCCTCCTGTTCGAC+TGCCAGGCTCTGTGTCATTA

GAPDH:TGCAGTGGCAAAGTGGAGATT+TTGAATTTGCCGTGAGTGGA

(GAPDH was used as reference in each experiment for normalization). Reactions were run in triplicate on a Biorad DNA engine with chromo4 real time detection system, using the following

program 2' 50^{o}C, 10' 95^{o}C, (15" 95^{o}C, 1' 60^{o}C) x 40 cycle + Dissociation curve. Opticon monitor software was used to determine C(t) and the Mean Normalized Expression (MNE) levels were calculated using the equation $MNE = CT_{reference}^{Ereference} / CT_{target}^{Etarget}$ for details see (5).

Oligo in situ hybridization

Single-label *in situ* hybridization was performed using an antisense oligonucleotide specific to the endogenous 5-HT2C receptor gene: The oligonucleotide sequence used was: GACTGCTAAATTGGGTCCTATAGATCGAGGTACCATAATTAAGA.

Immunofluorescence

Mice were deeply anesthetized and perfused transcardially with first 5 ml PBS then 45 ml of 4% paraformaldehyde in PBS. Brains were post-fixed 4 h then cryoprotected in 30% sucrose for 48 h at 4ºC, frozen in isopentane and stored at −70ºC until sectioning. Twenty μm coronal sections of the striatum were cut and stained free floating by first washing with PBS, then incubated (15 mins) in 20 mM sodium acetate and washed again with PBS. The sections were then incubated in a blocking serum [1.5% heat inactivated fetal bovine serum for 1 h at RT], then incubated for 24 hours at 4ºC with the commercially available goat polyclonal anti-5-HT2CR antibody (1:100; sc-15081, Santa Cruz Biotechnology, Santa Cruz, CA) in the same blocking solution. Sections were then incubated for 90 min at RT with donkey anti-goat Alexafluor 647, used to 1:1000 (Molecular Probes, Eugene, OR). After washing with PBS, sections were stained with DAPI (4′,6-diamidino-2-phenylindole, Sigma Aldrich, St. Louis, MO), washed again, mounted onto Superfrost Plus slides (VWR, Arlington Heights, IL) and coverslipped using FluorSave mounting medium (Calbiochem, San Diego, CA) and stored protected from light at 4ºC until viewing.

For analysis, sections were viewed using a laser scanning confocal microscope (Olympus Fluoview FV1000) and images captured using Fluoview software. Imaging sequentially for Alexafluor 647 and DAPI with a 60 X oil objective confirmed specificity of the staining in cell bodies and extension into neuronal processes, comparable to staining previously reported with this antibody (data not shown). A 20 X objective was then used to capture an image of the Alexafluor 647 stain from each of 3-5 sections of the striatum from 5 mice of each genotype. For each image the same size area was captured using the same laser power and image acquisition settings. The images were imported into ImageJ software (http://rsb.info.nih.gov/ij/) and average intensities were calculated for the dorsal and ventral striatum for each animal.

Supplemental Results

Physical and Neurological Examination

A cohort of 14 D2R-OE female mice and 18 control female littermates aged 4-5 months were examined as described (6, 7). Each mouse was observed in a standard cage for 3 minutes; during this time no abnormal spontaneous behaviors were observed for any mouse. Posture and fur quality appeared normal and all mice appeared well groomed. Four out of 18 control and 2 out of 14 D2R-OE mice had no whiskers, presumably due to barbering by cage mates (n.b., mice are housed in mixed genotype groups). Reflexes were then tested in the following way: while freely moving in the cage, a cotton-tipped applicator was introduced into the cage and all animals responded normally by briefly sniffing the applicator then ignoring it. The cage was then shaken side to side and up and down, all mice responded normally by extending all legs to balance. Mice were then placed on their backs and all mice responded normally by instantly righting themselves. While freely moving in the cage, the whiskers (of mice which had whiskers) were lightly touched with a cotton-tipped applicator and all mice responded normally by stopping whisker movements then turning their head toward the applicator. Mice were then held by the scruff and the applicator used to approach the eye then ear, all mice responded normally by eye blinking but no ear twitch was observed for any animal. Mice were then placed on a "cliff" edge (a box 6 inches wide, 6 inches long and 12 inches high), and all mice were observed to reach their head over the edge but not fall off. Neuromuscular strength was measured by placing the mice on a wire cage lid, then turning it upside down 5 inches above the bench. All mice remained on the wire top for the maximum 60 second period without falling off. Gait analysis was performed by analyzing footprint patterns by dipping the hindpaws in non-toxic blue ink and

the forepaws in red food coloring and placing the animal on a piece of paper underneath a 55 cm long open topped tunnel with walls 10 cm apart and 15 cm high. The start of the open tunnel was brightly lit and at the end of the tunnel was a darkened box to encourage the mice to walk along the paper. The average stride length was recorded for each mouse (including left and right feet) and no significant difference between D2R-OE and control mice was found (average hindpaw stride (mm) +/- SEM Control = 70.5 +/- 0.7, D2R-OE = 71.2 +/- 1.3: t-test P = 0.69; average forepaw stride (mm) +/- SEM control = 71.3 +/- 0.8, D2R-OE = 70.7 +/- 1.3: t-test P = 0.73). The average base width between left and right foot prints was also measured and found to be normal (average hindpaw width (mm) +/- SEM Control = 26.8 +/- 1.3, D2R-OE = 26.7 +/- 0.5: t-test P = 0.77; average forepaw width (mm) +/- SEM Control = 15.3 +/- 0.3, D2R-OE = 15.1 +/- 0.6: t-test P = 0.79). We previously measured locomotor activity in a novel arena, acoustic startle reflex and prepulse inhibition of the acoustic startle reflex and reported all measures to be normal (4).

Bodyweight and chow intake was recorded in a separate cohort of mice 10 D2R-OE and 12 control mice which were housed in pairs of the same genotype, regular home chow placed in a petri dish on the floor of the cage. Chow consumption was recorded over 3 days and the average amount consumed per mouse per day calculated. The bodyweight of the same mice was recorded at the same time of day for three consecutive days and an average bodyweight for each mouse calculated.

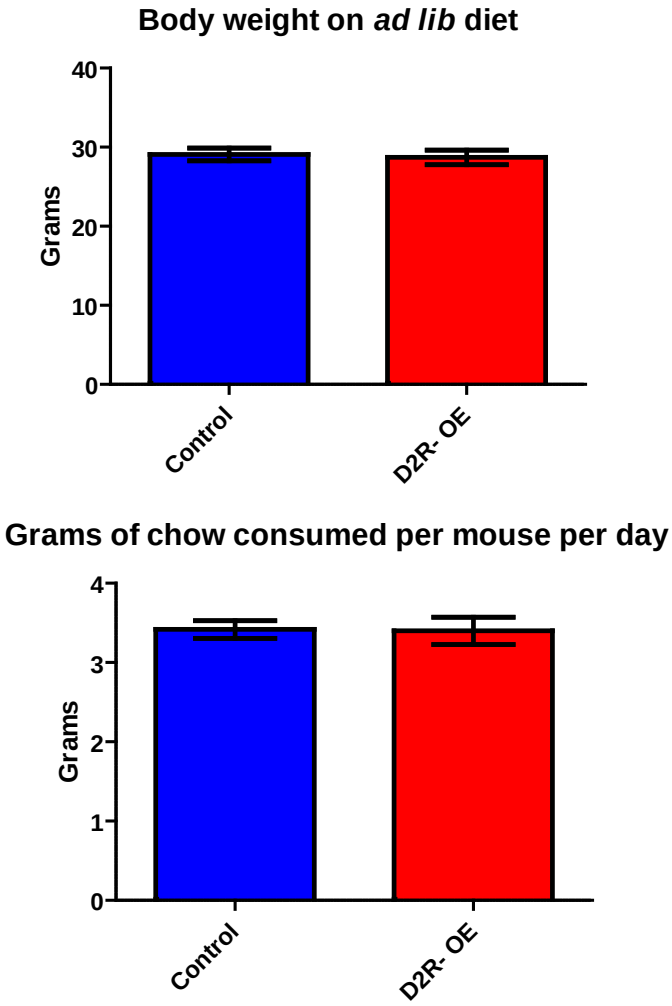

Figure S1. Top: Body weight on an *ad lib* diet was not significantly different between D2R-OE mice and Controls. D2R-OE Mean ± SEM = 28.70 ± 0.9315, N = 10; Control Mean ± SEM = 29.08 ± 0.8115, N = 12. **Bottom:** Grams of chow consumed per mouse per day when paired in genotype matched pairs was not significantly different between D2R-OE mice and Controls. D2R-OE Mean ± SEM 3.400 ± 0.1716, N = 5 pairs; Control Mean ± SEM 3.417 ± 0.1118, N = 6 pairs.

Performance on a Progressive Interval Schedule

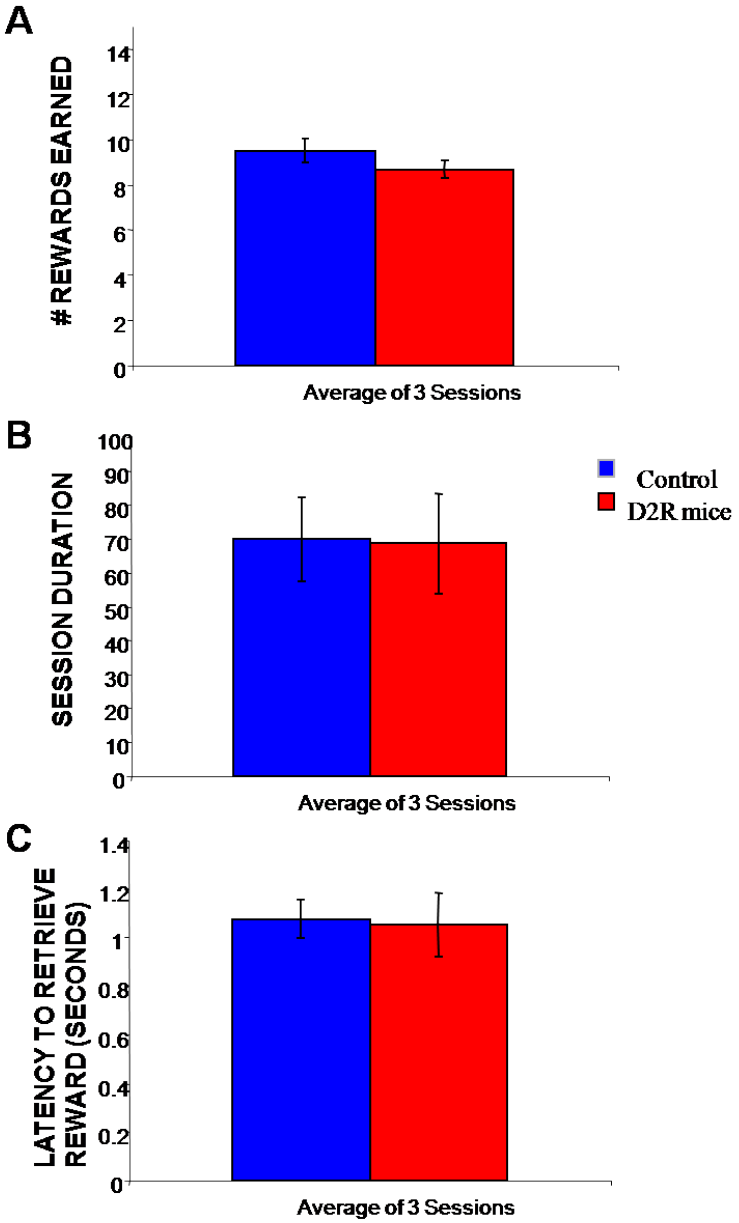

Figure S2. Performance on a progressive interval schedule was unaffected in D2R-OE mice, indicating that the incentive motivation deficit in D2R-OE mice is not due to a decrease in tolerance for delay to reward. There was no effect of genotype on number of reinforcers earned **(A)** D2R-OE avg = 8.66 +/- 0.41, Control avg = 9.5 +/- 0.53, t-test p = 0.29, the session duration (**B)** D2R-OE avg 68.52 min +/- 14.65, Control avg = 69.86 min +/- 12.41, t-test p = 0.95 or the mean latency to retrieve rewards (**C)** D2R-OE avg 1.053 s +/- 0.13, Control avg = 1.077 +/- 0.08, t-test p = 0.87 (Control n = 6, D2R-OE n = 4).

Expression of 5-HT Receptors in Striatum of D2R-OE and Control Mice

Table S2. Relative expression of 5-HT receptor expression in striatum of D2R-OE and Control mice. Fold change = D2R-OE/ Control, p = Students t-test. n = 5 mice per group.

	Gene on (no Dox)			Gene off (Dox)	
	p-value	Fold change		p-value	fold change
Serotonin transporter	0.64	1.02		0.30	2.52
5HT-1A Receptor†	0.56	0.98		0.02	1.34
5HT-1A Receptor†	0.63	0.98		0.55	1.13
5HT-1B Receptor	0.27	0.97		0.42	1.11
5HT-1D Receptor	0.93	1.00		0.08	1.10
5HT-1F Receptor	0.62	1.03		0.98	1.01
5HT-2A Receptor*	ND	ND		ND	ND
5HT-2B Receptor	0.26	0.94		0.24	1.25
5HT-2C Receptor	0.02	1.38		0.57	1.16
5HT-3A Receptor	0.18	1.02		0.55	1.12
5HT-3B Receptor	0.92	1.00		0.31	1.53
5HT-4 Receptor	0.10	0.94		0.56	0.79
5HT-5A Receptor	0.20	0.96		0.86	1.02
5HT-5B Receptor	0.32	0.97		0.50	0.88
5HT-6 Receptor	0.55	1.03		0.83	1.03
5HT-7 Receptor	0.02	0.94		0.82	0.94

† These two sets of data result from independent probes set on the gene chip which correspond to unique (non-overlapping) segments of the non coding 3'portion of the 5-HT1A receptor gene.

* The 5HT-2A receptor gene was not represented on the chip, but qRT-PCR analysis revealed no difference in expression of the transcript across genotype (Mean Normalized Expression [MNE] values: Control/D2R-OE MNE = 0.034 +- 0.007/0.027 +/- 0.005, p = 0.48). n = 5 mice per group.

The 5HT-2C receptor gene is highlighted as the only serotonin receptor gene which is significantly altered in D2R-OE mice and normalized when the D2R transgene is switched off.

5-HT Receptor Expression in the Frontal Cortex of D2R-OE Mice

Table S3. 5HT- receptor expression in the frontal cortex of D2R-OE mice. Fold change = D2R-OE/ Control, p = Students t-test. n = 5 mice per group.

Transcript	P value	Fold change
Serotonin transporter	0.83	0.98
5HT-1A Receptor†	0.25	0.92
5HT-1A Receptor†	0.86	1.03
5HT-1B Receptor	0.12	0.95
5HT-1D Receptor	0.23	1.20
5HT-1F Receptor	0.46	1.05
5HT-2A Receptor*	ND	ND
5HT-2B Receptor	0.12	1.1
5HT-2C Receptor	0.84	1.04
5HT-3A Receptor	0.18	0.91
5HT-3B Receptor	0.56	0.86
5HT-4 Receptor	0.06	1.41
5HT-5A Receptor	0.73	1.03
5HT-5B Receptor	0.68	1.04
5HT-6 Receptor	0.76	1.01
5HT-7 Receptor	0.34	0.85

† These two sets of data result from independent probes set on the gene chip which correspond to unique (non overlapping) segments of the non coding 3'portion of the 5HT1A receptor.

* The 5HT-2A receptor gene was not represented on the chip.

Pilot experiment for SB24280

Before testing the effect of the 5-HT2c antagonist SB24280 on the performance of control and D2R-OE mice in the progressive ratio task, we tested the drug at three different concentrations on the fixed interval schedule in a within subjects design experiment. Six mice of each genotype were tested once a day Tuesday to Friday over a period of 3 weeks. In each week mice received injection of one concentration of SB24280 on two days and vehicle injections on the other 2 days; the order of doses was varied and counterbalanced across genotype. Figure S3 shows the effect of each dose of SB on the rate of lever pressing in the FI 24 test. None of the

doses administered significantly affected press rates for either genotypic group. An examination of individual subject data indicated that for 11 of the 12 subjects, response rate was an inverted U-shaped function of dose. For these 11 subjects, maximal responding was observed at either the 0.5 or 1.0 mg/kg dose. We therefore chose to use 0.75 mg/kg in the progressive ratio experiment.

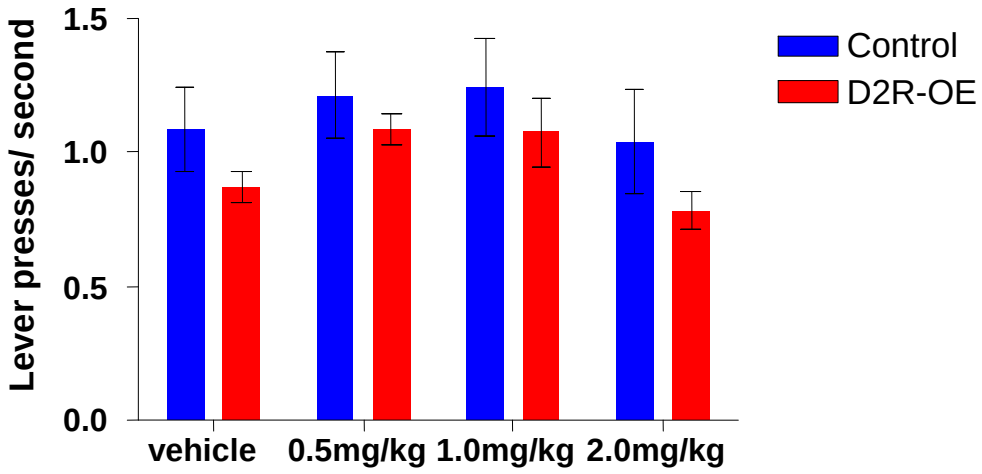

Figure S3. The effect of 3 doses of SB24280 on rate of lever pressing in the fixed interval task. Two way ANOVA showed that there was no overall effect of drug, and no interaction between drug and genotype.

Supplemental References

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