Supplementary Materials and Methods

Immunohistochemistry and light microscopy

Mice were anesthetized with pentobarbital and then perfused intracardially using 0.2M phosphate buffered saline (PBS) followed by 4% formaldehyde. Brains were removed and post-fixed overnight. Then, the brains were transferred to PBS. The brain BLAQ protocol was used as described previously to process 40µm thick coronal brain sections [1]. The sections were washed in PBST (PBS, 0.2% Triton X-200) for 1hr and then rinsed with deionized water (diH2O). To quench aldehyde induced catecholamine fluorescence, sections were incubated in 0.5% sodium borohydride twice for 10 min each. Afterwards, sections were rinsed with diH2O, followed by the incubation of 0.1% filtered Sudan Black B solution twice for 15 min each. The sections were then washed in PBS twice for 30 min each, blocked overnight with 5% bovine serum albumin-PBST at 4ºC, and incubated with primary antibodies, Rabbit Anti-Drd2 (1:500; Cat No. D2R-Rb-Af750; Frontier Biosciences, Japan) for 72hrs at 4ºC. The incubation of the primary antibody was followed by four washes of PBST for 1hr each. The secondary antibody, Alexa 488 donkey anti-rabbit (1:500; Cat No. A-21206; RRID: AB-2535792; Thermo Fisher Scientific, USA), to visualize dopamine D2 receptor expression was incubated overnight at 4ºC. Following four 1hr washes in PBST, sections were mounted on slides and cover slipped with Fluoromount G (Electron Microscopy Sciences, USA). Images were taken at 20x 0.8 NA objective using an Axiovert 200 microscope equipped with GFP filter, AxioVision software (Zeiss, Germany), and Axiocam MR fluorescence camera.

Progressive Ratio Test

Following instrumental conditioning, mice were tested for 1 day on a progressive ratio schedule. To obtain successive food rewards within a 1hr cutoff, mice had to press a lever progressively increasing the number of presses (10, 20, 25, 32, 40, 50, 62…) [2]. The total number of lever presses and the breakpoint reached were measured. The breakpoint is defined as the last series in which a food reward was obtained.

Food pellet preference test

The food pellet preference test was performed 1 week after instrumental training, once mice had regained their initial body weight. Mice were given a free choice of either standard chow or food pellet-reward (used in instrumental training) for 12hrs during the dark cycle, the most active period. Mice were singly housed during testing. Food was weighed before and after
testing. Food pellet-reward preference was determined as the ratio between the food pellet-reward consumption over the total amount of food consumed.

**Open field test**
An open field test was run on a group of experimentally naïve mice to corroborate previous findings of locomotor impairment in iMSN-Drd2KO mice [3]. Mice were allowed to acclimate to the testing room overnight in their home cages. The following day, mice were placed in Noldus PhenoTypers (Noldus IT, Netherlands) and allowed to explore for 15 min. Body position was video tracked via EthoVision (Version 11, Noldus IT) and used to calculate animal speed in 30 sec bins.

**Forced swim test**
To confirm that iMSN-Drd2KO mice were capable of normal swimming behavior, a subset of mice underwent a forced swim test 24 hr after completion of the water T-maze protocol. The L-shaped walls were removed from the T-maze apparatus, and mice were placed in the center of the chamber and allowed to swim for 10 min. Body position was video tracked via EthoVision (Version 11, Noldus IT) and used to calculate animal speed and immobility in 30 sec bins.
Supplementary Figures

**Supplementary Figure 1: Selective deletion of striatal D2 receptors in iMSN-Drd2KO mice.**

Drd2$^{loxP/loxP}$ coronal hemisections show immunofluorescence for D2R (GFP fluorescence) in the dorsal striatum (Str) and midbrain substantia nigra (SN). However, D2R immunofluorescence was reduced in the DS of the iMSN-Drd2KO mouse hemisection, with similar receptor expression in the SN relative to Drd2$^{loxP/loxP}$.

**Supplementary Figure 2: Inter-press interval for instrumental learning task.**

(A) The Drd2$^{loxP/loxP}$ mice decreased their inter-press interval (IPI; n = 7, t-test, p < 0.05) on an FR1
schedule, with no change in IPI on FR5 (t-test, p > 0.05). (B) There were no significant differences in the IPI of the iMSN-Drd2KO mice on either an FR1 (n = 6, t-test, p > 0.05) or FR5 (t-test, p > 0.05) training paradigm. Error bars = SEM. * p < 0.05

**Supplementary Figure 3: Progressive ratio test.** There is a trend towards decreased lever presses (Left, t-test, p = 0.051) and lower breakpoint (Right, t-test, p > 0.05) during the progressive ratio test in the iMSN-Drd2KO (n = 6) compared to controls (Drd2loxP/loxP, n = 7). Error bars = SEM.
Supplementary Figure 4: Both the iMSNs-Drd2KO and Drd2<sup>loxP/loxP</sup> mice prefer reward pellets over standard chow. Reward preference corrected for bodyweight in the iMSN-Drd2KO (n = 6) and controls (Drd2<sup>loxP/loxP</sup>, n = 7).

Supplementary Figure 5: iMSN-Drd2KO mice show task specific motor deficits. Averaged speed for the Drd2<sup>loxP/loxP</sup> (gray bar) and iMSN-Drd2KO (orange bar) mice in the (A) open field (n = 5 per genotypes, t-test, p < 0.05) and (B) forced swim tests (n = 4-5 per genotypes, t-test, p > 0.05). Error bars = SEM. * p < 0.05
Supplementary Figure 6: No impairment in the time to reach platform in T-maze once swimming was initiated. iMSN-Drd2KO mice did not differ from controls in the time it took to reach the platform during the acquisition training (RM ANOVA main effect of genotypes $F_{1,13} = 1.373$, $p = 0.26$) and reversal learning (RM ANOVA main effect of genotypes $F_{1,13} = 0.1253$, $p = 0.73$). Error bars = SEM.
Supplementary Figure 7: Head entries during the acquisition of Pavlovian conditioning and extinction. (A,B) Averaged head entry responding during the CS+/CS- Pavlovian conditioning in the Drd2loxP/loxP (n = 11, gray circles, RM ANOVA main effect of cue presentations $F_{1,20} = 7.977, p = 0.011$) and iMSN-Drd2KO (n = 10, orange circles, RM ANOVA main effect of cue presentations $F_{1,18} = 4.136, p = 0.057$) mice. During extinction, head entries increased during CS+ compared to CS- responding in both genotypes (Drd2loxP/loxP, $t$-test, $p < 0.0001$; iMSN-Drd2KO, $t$-test, $p < 0.001$). Error bars = SEM.

Supplementary Figure 8: Frequency of consumption across training days. (A, B) Consumption frequency (licks/minute) was higher across training sessions for CS+ compared to the CS- responding for the Drd2loxP/loxP (n = 11; gray circles) and iMSN-Drd2KO (n = 10; orange circles) mice (Drd2loxP/loxP, RM ANOVA main effect of training $F_{14,280} = 10.29, p < 0.0001$, main
effect of cue presentations $F_{1,20} = 239.4$, $p < 0.0001$, training x cue interaction, $F_{14,280} = 12.11$, $p < 0.0001$; iMSN-Drd2KO, main effect of training $F_{5.627,101.3} = 5.12$, $p = 0.0002$, main effect of cue presentations $F_{1,18} = 124.4$, $p < 0.0001$, training x cue interaction, $F_{14,252} = 6.11$, $p < 0.0001$). The Drd2loxP/loxP mice consumed CS+ associated sucrose faster than the iMSN-Drd2KO mice (RM ANOVA main effect of genotypes $F_{19,266} = 14.23$, $p < 0.0001$, Drd2loxP/loxP $222.9 \pm 12.69$; iMSN-Drd2KO $175.0 \pm 9.55$, $t$-test $p = 0.0023$).

Supplementary Figure 9: Response latency to onset of First CS+ presentation of training day. Although there was a significant effect of training (RM ANOVA main effect of training $F_{15,285} = 1.785$, $p = 0.036$), there was not a significant difference in the CS+ response latency between the Drd2loxP/loxP ($n = 11$, gray circles) and iMSN-Drd2KO ($n = 10$, orange circles) mice (RM ANOVA main effect of genotypes $F_{1,19} = 0.81$, $p = 0.38$).
References

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