ARTICLE   OPEN

Dopamine D2 receptor signaling on iMSNs is required for initiation and vigor of learned actions

Shana M. Augustin[1], Gabriel C. Loewinger[1,2], Timothy J. O'Neal[3,4], Alexxai V. Kravitz[3,5] and David M. Lovinger[1]

Striatal dopamine D2 receptors (D2Rs) are important for motor output. Selective deletion of D2Rs from indirect pathway-projecting medium spiny neurons (iMSNs) impairs locomotor activities in a task-specific manner. However, the role of D2Rs in the initiation of motor actions in reward seeking and taking is not fully understood, and there is little information about how receptors contribute under different task demands and with different outcome types. The iMSN-D2Rs modulate neuronal activity and synaptic transmission, exerting control on circuit functions that may play distinct roles in action learning and performance. Selective deletion of D2Rs on iMSNs resulted in slower action initiation and response rate in an instrumental conditioning task, but only when performance demand was increased. The iMSN-Drd2KO mice were also slower to initiate swimming in a T-maze procedural learning task but were unimpaired in cognitive function and behavioral flexibility. In contrast, in a Pavlovian discrimination learning task, iMSN-Drd2KO mice exhibited normal acquisition and extinction of rewarded responding. The iMSN-Drd2KO mice showed performance deficits at all phases of rotarod skill learning. These findings reveal that dopamine modulation through iMSN-D2Rs influences the ability to self-initiate actions, as well as the willingness and/or vigor with which these responses are performed. However, these receptors seem to have little influence on simple associative learning or on stimulus-driven responding. The loss of normal D2R roles may contribute to disorders in which impaired dopamine signaling leads to hypokinesia or impaired initiation of specific voluntary actions.

INTRODUCTION

The striatum contributes to decision making, voluntary movement, action selection, behavioral flexibility, and dynamic updating during learning [1–6]. Among these functions, the striatum supports specific aspects of action learning and selection, with roles in learning, including Pavlovian and instrumental conditioning [7–9]. To perform these functions, the striatum integrates information from cortical, limbic, and thalamic afferents with dopaminergic inputs that convey reward and salience signals to facilitate actions that maximize desired outcomes [5, 10–12]. Dopamine contributes to general locomotor activation and hedonic functions, as well as executive functions involved in decision making and precise temporal action control [13–19]. Parkinson's disease (PD) patients suffer severe deficits in movement control and voluntary action initiation due to the loss of nigrostriatal dopamine [14, 20–22]. Interestingly, PD patients can often perform movements in response to environmental or physical stimuli [23–25], indicating that nigrostriatal dopamine most strongly influences voluntary movement initiation and performance.

Dopamine modulation of striatal neuronal activity and synaptic transmission influences action initiation and termination [19, 26–29] and may affect action maintenance and bias future action selection [30, 31]. Reduced dopaminergic tone and/or signaling results in

motor deficits [32, 33] and reduced effortful choices in decision making tasks [17, 34, 35], suggesting that dopamine plays a critical role in modulating the strength of voluntary actions. Dopamine modulates striatal neuronal function via activation of different receptor subtypes, including the $G_{i/o}$-linked D2Rs [36, 37]. Global D2R deletion results in the reduction, slowing, and decreased initiation of movement [38, 39]. Pharmacological studies suggest D2R roles in effort-based decision making and flexibility in the face of increasing task demand [16, 17, 34, 40–45], and the dopamine system is implicated in the temporal control of action sequences. The specific striatal cellular loci of the D2Rs mediating these behaviors is not known. Also, little is known about striatal D2R roles in behavioral responses to conditioned and unconditioned environmental stimuli. Striatal D2Rs are expressed strongly by the indirect pathway-projecting medium spiny neurons (iMSNs) and cholinergic interneurons [46, 47], where they modulate neuronal activity as well as local and distal neurotransmitter release. However, D2Rs also modulate neurotransmitter release at cortical and dopaminergic afferents in striatum [15, 46, 48]. Targeted deletion of iMSN-D2Rs results in the reduction and decreased speed of motor output in a task-specific manner [49], and thus these reductions cannot simply be explained by generalized motor impairments. Recent evidence suggests that iMSN-D2Rs can modulate effort and energy expenditure during food foraging

Dopamine D2 receptor signaling on iMSNs is required for initiation and...
SM Augustin et al.

2088

[50]. This suggests that iMSN-D2Rs may signal moment-by-moment changes in phasic dopamine induced by reward predictors and/or increased task demand [51].

Despite these findings, more information is needed to differentiate iMSN-D2R contributions to action initiation and vigor in specific tasks from their role in general locomotion. For example, it remains unclear whether iMSN-D2Rs are needed for proper initiation of self-initiated versus stimulus-signaled actions. In addition, there is little information as to whether iMSN-D2R roles in action control vary with positively versus negatively reinforced actions and are related to the effort requirement of the task. Finally, it is not clear if iMSN-D2Rs contribute to associative learning. To address these questions, we used mice in which D2Rs were constitutively removed from iMSNs to investigate the receptor role in action learning, initiation, and execution in self-initiated instrumental and maze learning tasks and stimulus-generated Pavlovian conditioning. Our findings indicate distinct roles for iMSN-D2Rs in action initiation in self-initiated tasks independent of task outcome, despite learning being intact. However, slower action initiation was only evident under conditions where increased effort was required for task completion in the instrumental learning paradigm. In contrast, iMSN-D2Rs appear to contribute very little to acquisition and extinction during simple Pavlovian conditioning. Our findings highlight the functional heterogeneity in iMSN-D2R modulation of action control based on factors involved in action initiation and task effort requirements.

MATERIALS AND METHODS
Animals
All experiments and animal care were performed in compliance with the National Institutes of Health Care and Use of Animals guidelines and approved by the Institutional Animal Care and Use Committees of the National Institute on Alcohol Abuse and Alcoholism and the National Institute of Diabetes and Digestive and Kidney Diseases. Adora2a Cre and Drd2$^{loxP/loxP}$ were purchased from MMRRC (036158-UCD) and Jackson laboratory (020631), respectively. Drd2$^{loxP/loxP}$ mice, which have a floxed exon 2 dopamine D2 (Drd2) allele, were bred with Drd2$^{loxP/loxP}$ Adora2a Cre mice to generate iMSN-Drd2KO mice as previously described [49, 52, 53]. Mice were group-housed and maintained on a 12-h light/dark schedule in a temperature and humidity-controlled environment, and given free access to food and water, unless otherwise stated. All experiments were done during the light cycle, except the food preference test. Mice (9–15 weeks) of both sexes were used.

Operant conditioning chambers
Mice were trained in Med Associates (VT, USA) operant chambers, enclosed in sound-attenuating and light-resistant ventilated boxes. Each chamber contained two retractable levers on either side of the food receptacle, in which a food pellet (Bio-Serv, NJ, USA; formula F0071) or 20 μl of 20% sucrose solution was delivered. The food receptacle was equipped with a lickometer to measure consumption.

Instrumental self-paced lever-press training
Mice were food restricted to ~90% of their initial body weight for the entire experiment. Mice were first trained on a random interval (~60 s) schedule for food delivery. The following day, mice began training with a single lever (left or right counterbalanced for genotype and extended throughout the session) on a fixed-ratio schedule of 1 (FR1) for 5 days followed by a fixed-ratio schedule of 5 (FR5) for 9 days. Training sessions ended after 60 min or 30 rewards were earned, whichever occurred first. Note that no predictive or discriminative stimuli were included in training, and thus the training and performance was self-initiated and self-paced.

Water T-maze test
Mice were tested for short-term spatial and working memory using a water T-maze [54], which consisted of two L-shaped pieces of opaque white acrylic (18.5-cm long, 25-cm tall) placed in a bin (42 × 23.5 cm) filled with 23 °C water, ~13 cm deep. The water was made opaque with nontoxic white acrylic paint to prevent mice from seeing the hidden platform, and the T-maze was encircled by white curtains to prevent mice from using contextual cues to navigate. Prior to training, mice were placed in the T-maze and allowed to explore for 60 s. The first arm each mouse entered was recorded, and the platform was placed in the opposite arm. The following day, acquisition trials began (10 trials/day), where mice were placed at the base of the T-maze and allowed to swim to the platform (5 × 5 × 12 cm). Each trial lasted 60 s, or until mice remained on the platform for 5 s. Trials were considered successful if a mouse located the platform within 60 s, or otherwise were considered failures. Mice failing to reach the platform within 60 s were gently nudged towards the platform and allowed to sit in place for 10 s before returning to their cage. All mice were dried off and returned to home cages between trials, with an intertrial interval (ITI) of 10 min. Acquisition sessions continued until 80% of the daily trials were successful for 4 consecutive days, at which point individual mice proceeded to reversal learning. Reversal learning trials (10 trials/day) were run identically to acquisition trials, but the location of the hidden platform was reversed for each mouse. Reversal sessions continued until 80% of trials were successful for 2 consecutive days. All trials were video recorded and analyzed using EthoVision XT tracking system (Noldus, Netherlands). Errors were counted if the mice (1) entered the incorrect arm or (2) left the correct arm without locating the platform.

Pavlovian training
Mice were trained in the operant chambers previously described on a cue-induced licking Pavlovian task. The mice were food restricted to ~90% of their initial body weight for the entire experiment. Mice were first trained to approach/consume sucrose on a 90 s random delivery schedule. Next, mice were trained for 15 daily sessions by pairing presentation of a conditioned stimulus (CS+) with sucrose delivery.

A non-reward-paired conditioned stimulus (CS−) was also presented. Each training session (~1 h) consisted of 24 trials (12 reinforced CS+/12 non-reinforced CS−) of a 20-s presentation of either a 3-kHz tone or white noise (70 dB) presented on a pseudorandom ITI of 90 s with 12-s step increments. For half the mice, the tone was the CS+ and the white noise was the CS−, while the remaining mice received the opposite contingency. For CS+ presentation, the sucrose was delivered twice during two of the first three 5-s epoch in a pseudorandom sequence determined by the computer. Sucrose was not delivered during the last 5-s epoch to ensure that there was enough time for sucrose consumption during the CS+ presentation. Sucrose delivered during the first 5-s epoch was delayed 3 s. Trials were not initiated by the mice, nor were the sucrose deliveries contingent on their behavior. After 15 conditioning sessions, an extinction test was given that was similar to the conditioning sessions except for the absence of sucrose delivery during the CS+. Licks and head entries into the sucrose port during the CS+, CS−, and ITI during the Pavlovian sessions and extinction test were measured to assess learning.

Accelerating rotarod
Mice were habituated to a static rotarod (Med Associates), then, trained for 7 days with four trials per day on an accelerating rotarod (4–40 rpm in 300 s). The ITI was 5 min. The latency to fall was recorded. The trial was terminated at 300 s or if the mouse held onto the rod for one complete rotation.

Dopamine D2 receptor signaling on iMSNs is required for initiation and...
SM Augustin et al.

2089

Statistical analysis

Statistical tests were performed in GraphPad Prism, using repeated-measure ANOVA with the post hoc Sidak's test for multiple factors comparison and student's t tests when appropriate. Results were considered significant if $p < 0.05$. Averaged data are presented as mean ± SEM.

RESULTS

iMSN-D2R deletion "slows" lever press responding at higher cost, and impairs action initiation

Mice carrying a loxP-flanked Drd2 gene were bred with Adora2A Cre$^{+/-}$ mice to generate iMSN-selective D2R knockout mice (iMSN-Drd2KO) and the appropriate littermate controls (Drd2$^{loxP/loxP}$). Our group and others have shown that this breeding scheme results in 80–90% reduction in striatal Drd2 mRNA expression [49, 53]. In striatum, there was decreased striatal D2R immunoreactivity as would be expected for loss of the major cellular source of D2Rs in this region. No immunoreactivity reduction was observed in the midbrain, an area that expresses high levels of D2R but not Adora2A Cre [55] (Fig. S1).

We trained the iMSN-Drd2KO and littermate Drd2$^{loxP/loxP}$ mice to press a lever for food reward on fixed-ratio schedules, FR1 and FR5. However, as the cost of reward increased under the FR5 schedule, the iMSN-Drd2KO mice pressed less (Fig. 1a, RM ANOVA, main effect of genotypes $F_{1,11} = 6.78$, $p = 0.02$; FR5 training × genotype interaction $F_{8,88} = 1.26$, $p = 0.28$) and at a slower rate, for food reward (Fig. 1b, RM ANOVA, main effect of genotypes $F_{1,11} = 11.24$, $p = 0.006$; FR5 training × genotype interaction $F_{8,88} = 1.24$, $p = 0.29$). There was a significant escalation of lever-press rate on the FR5 schedule in the Drd2$^{loxP/loxP}$ later in training compared to the start of FR5 (t-test, $p < 0.05$ Day 8, $p > 0.05$ Day 2–7, 9). This level of escalation was not seen in the iMSN-Drd2KO mice (t-test, Day 2–9 $p > 0.05$). To further understand the effects of iMSN-D2R signaling on the initiation of lever pressing, we quantified the latency to initiate the first press. There was a negligible increase in the latency to press the lever on the FR1 schedule in iMSN-Drd2KO mice relative to Drd2$^{loxP/loxP}$ mice (Fig. 1c, t-test, $p > 0.05$). The deficit was more pronounced and was significant on the FR5 schedule (Fig. 1c; t-test, $p < 0.0001$). On the FR1 schedule, there was no difference in total lever presses (Fig. 1a, RM ANOVA main effect of genotypes $F_{1,11} = 0.01$, $p = 0.92$; FR1 training × genotype interaction $F_{4,44} = 0.18$, $p = 0.95$) but a small interaction for lever press responding across training (Fig. 1b, RM main effect of genotypes $F_{1,11} = 1.07$, $p = 0.32$; FR1 training × genotype interaction $F_{4,44} = 3.09$, $p = 0.03$) between genotypes. The latency to initiate lever pressing decreased with training in the Drd2$^{loxP/loxP}$ on the FR1 (Fig. 1d, FR1 t-test, $p < 0.05$) and not on the FR5 schedule (Fig. 1d, FR5 t-test, $p > 0.05$). The iMSN-Drd2KO mice decreased their latency to initiate lever-pressing behavior on the FR1 (Fig. 1e, t-test, $p < 0.01$), but not on the FR5 schedule (Fig. 1e, t-test, $p > 0. 05$). On Day 1 of FR1 training, the iMSN-Drd2KO mice took longer to initiate lever pressing compared to Drd2$^{loxP/loxP}$ (Fig. 1d, e, t-test, $p < 0.05$). Across multiple days of FR training, the Drd2$^{loxP/loxP}$ mice significantly decreased their latency to first lever press on the last day of FR5 training compared to the beginning of FR1 training (Fig. 1d, t-test, $p < 0.01$), suggesting that exploratory behavior contribution is minimal and/or significantly reduced after multiple training days. A similar pattern is seen in the iMSN-Drd2KO mice, with a significant decrease in the latency to initiate lever-pressing behavior from the first day of FR1 compared to the last day of FR5 training (Fig. 1e, t-test, $p < 0.01$). In the iMSN-Drd2KO mice, latency to first press was similar to that observed at the end of FR1 training and did not decrease further during FR5 training (Fig. 1e, t-test, $p > 0.05$), remaining higher than in Drd2$^{loxP/loxP}$ mice. We also examined the latency to press following reward retrieval (Fig. 1f, g). The iMSN-Drd2KO mice took more time to initiate a new lever-pressing sequence on the

FR5 schedule (Fig. 1g, RM ANOVA, main effect of genotypes $F_{1,11} = 11.12$, $p = 0.0067$), and this delayed initiation persisted with no improvement during training (RM ANOVA main effect of FR5 training $F_{8,88} = 1.17$, $p = 0.33$). There was no significant difference between groups during FR1 training (Fig. 1f, RM ANOVA main effect of genotypes $F_{1,11} = 2.55$, $p = 0.14$), although the latency was a bit longer on the last few days of FR1 training. There was a signficant effect of training (RM ANOVA main effect of FR1 training $F_{4,44} = 4.58$, $p = 0.004$). The iMSN-Drd2KO mice also had longer inter-press intervals on the FR5 (Fig. 1h, t-test, $p < 0.0001$), but not the FR1 schedule (Fig. 1h, t-test, $p > 0.05$). The length of time to complete a five-press sequence on the FR5 schedule was increased in the iMSN-Drd2KO mice (Fig. 1i, t-test, $p < 0.0001$). Also, the iMSN-Drd2KO mice completed fewer FR5 sequences than the Drd2$^{loxP/loxP}$ mice (Fig. 1j, t-test, $p < 0.001$). On the FR1 schedule, all mice earned maximum reward pellets per session (Fig. 1k, t-test, $p > 0.05$). However, iMSN-Drd2KO mice decreased the number of pellets earned on the higher cost FR5 schedule compared to Drd2$^{loxP/loxP}$ mice (Fig. 1k, t-test, $p < 0.0001$).

To ensure that differences in lever-press behavior were not driven by reduced preference, a food pellet preference test was administered. Both genotypes preferred food reward over standard chow to a similar degree in this test (Fig. S4, t-test, $p > 0.05$). As an additional measure of learning, the latencies between the lever press and reward retrieval were compared between genotypes. As training progressed and the animals learned the contingency, one would expect a decrease in the reward retrieval time. Indeed, the latency to retrieve reward following lever press(es) decreased with training (Fig. 1l, RM ANOVA main effect of training $F_{3,30} = 11.13$, $p < 0.0001$). There was no significant difference in the latencies to retrieve reward after lever press(es) between genotypes (Fig. 1l, RM ANOVA training × genotype interaction $F_{3,30} = 2.78$, $p = 0.06$). These results indicate that iMSN-D2Rs play a critical role in determining the pace of self-initiated responding in an effort-dependent manner.

Action initiation is impaired in a spatial memory task in iMSN-Drd2KO mice

It was previously demonstrated that iMSN-Drd2KO mice can learn specific behaviors, suggesting that iMSN-D2Rs contribute to task-specific deficits and not generalized motor deficits [49]. Those authors also noted that iMSN-Drd2KO mice showed no deficits in the forced swim assay, despite a profound bradykinesia in the open field. Consistently, iMSN-Drd2KO mice showed no deficits in the forced swim test (Fig. S5). We took advantage of this to avoid the confound of the motor impairment on tests of learning and tested the spatial learning ability of these mice using a water submerged T-maze test (Fig. 2a). During the acquisition phase, the iMSN-Drd2KO mice took longer to reach the escape platform across the training days (Fig. 2b, c, RM ANOVA main effect of day $F_{2,26} = 8.71$, $p = 0.0013$, main effect of genotype $F_{1,13} = 22.28$, $p = 0.0004$, day × genotype interaction, $F_{2,26} = 9.88$, $p = 0.0006$). However, the iMSN-Drd2KO mice did not make more errors than the Drd2$^{loxP/loxP}$ mice (Fig. 2d, RM ANOVA main effect of genotype $F_{1,13} = 1.08$, $p = 0.32$). Rather, the iMSN-Drd2KO mice took longer to initiate each trial than Drd2$^{loxP/loxP}$ (Fig. 2e, RM ANOVA main effect of genotype $F_{1,13} = 29.42$, $p = 0.0001$). Once swimming was initiated, the iMSN-Drd2KO mice completed the trials in similar time as Drd2$^{loxP/loxP}$ (Fig. S6), confirming that the deficit in task performance was not due to a motor impairment or learning impairment, but was specific to the initiation of swimming at the start of the trial. This delay in action initiation worsened with increased training (RM ANOVA main effect of day $F_{2,26} = 20.07$, $p < 0.0001$, day × genotype interaction, $F_{2,26} = 15.08$, $p < 0.0001$). Mice were then trained in a reversal version of the task in which the location of the rewarded arm was switched. During reversal, a similar pattern emerged, where iMSN-Drd2KO mice took longer to reach the new escape platform than Drd2$^{loxP/loxP}$ mice (Fig. 2f, RM ANOVA main effect of genotype $F_{1,13} = 32.19$, $p < 0.0001$). The
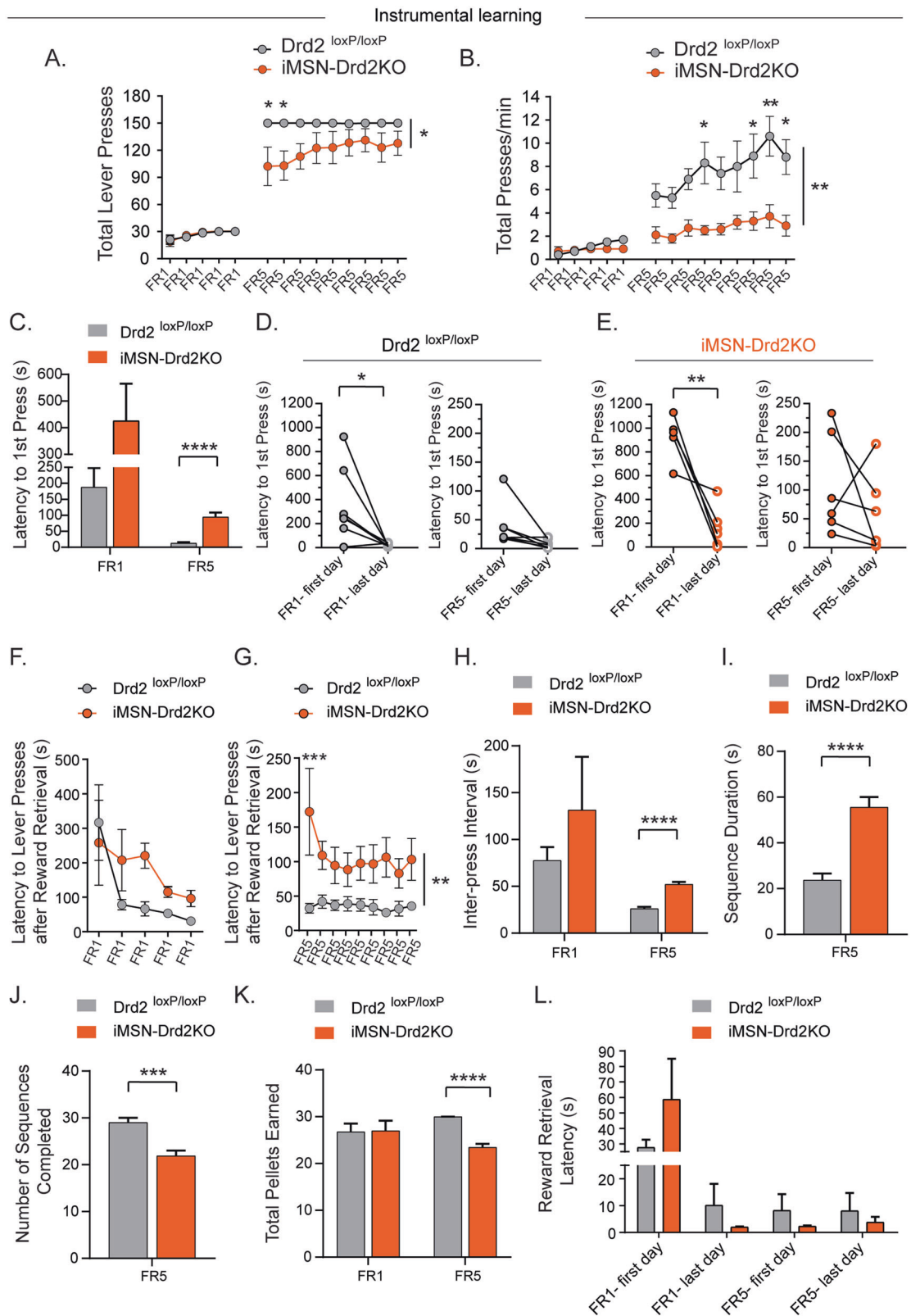
Dopamine D2 receptor signaling on iMSNs is required for initiation and…
SM Augustin et al.

2090

Instrumental learning

Fig. 1 **iMSN-D2R deletion produces deficits in instrumental lever-press performance. a** iMSN-Drd2KO ($n = 6$; orange circles) mice displayed decreased lever presses compared to Drd2$^{loxP/loxP}$ ($n = 7$, gray circles) mice in the FR5 component of an escalating fixed-ratio scheduled task. **b** The rate of responding was also decreased during FR5 learning and performance in the iMSN-Drd2KO mice. **c–e** iMSN-Drd2KO mice had increased latency to initiate lever-pressing behavior at the beginning of each session and **f, g** after reward retrieval. **h** The iMSN-Drd2KO mice had longer inter-press interval, **i, j** took longer to complete five sequential presses and completed fewer sequences than controls on an FR5 schedule. **k** iMSN-Drd2KO mice earned fewer pellets on the FR5 schedule. **l** However, the iMSN showed similar reward retrieval times after lever press(es). Data expressed as mean ± SEM. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, including post hoc tests.

Dopamine D2 receptor signaling on iMSNs is required for initiation and…
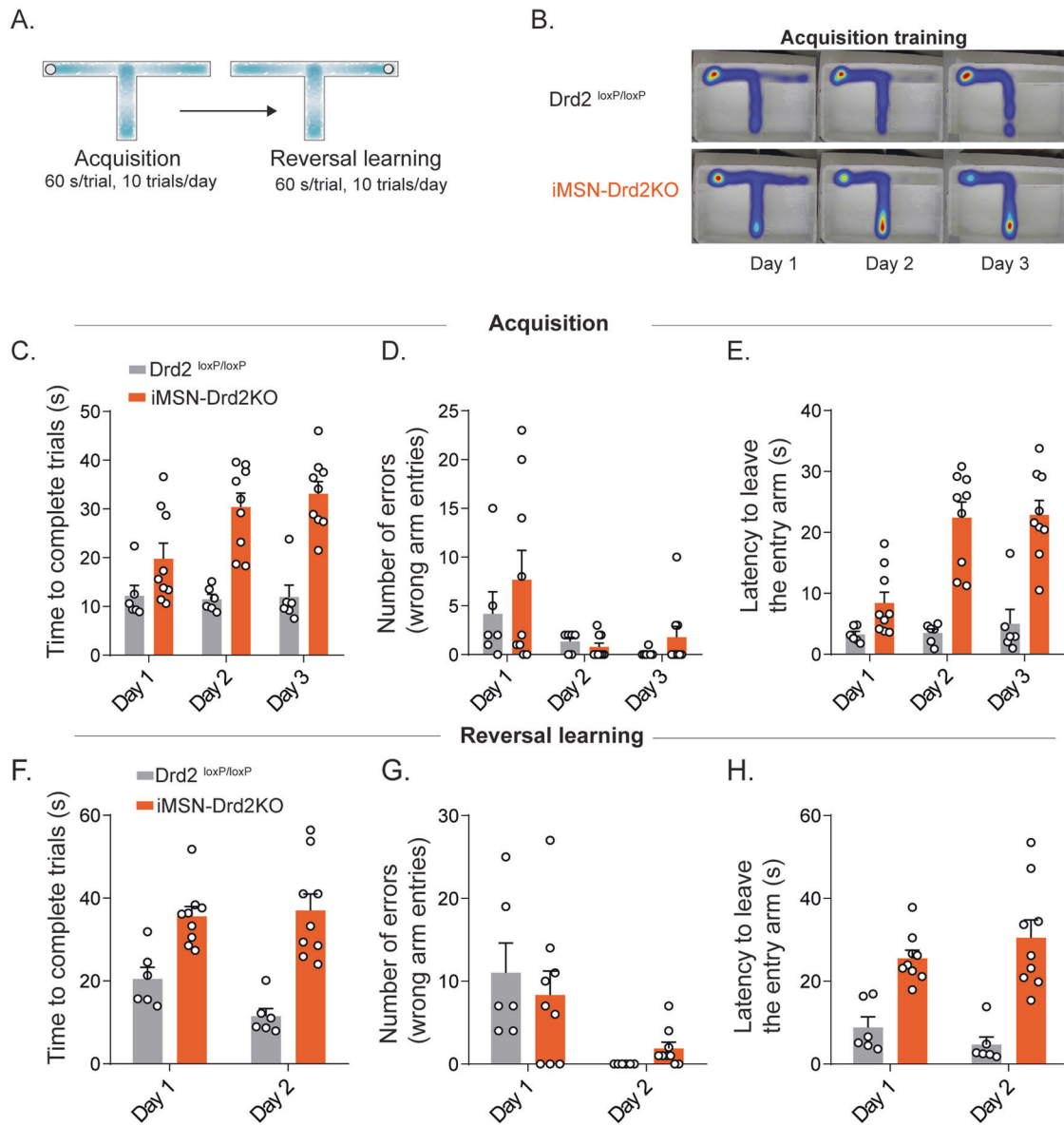SM Augustin et al.

2091

Fig. 2   iMSN-Drd2KO mice show delayed action initiation in a cognitive task. a Schematic diagram of the water T-maze. **b** Representative heat maps across days during the acquisition (initial) training of both genotypes. **c** Average time to complete trials across training days for the Drd2$^{loxP/loxP}$ (gray bar; $n = 6$) and iMSN-Drd2KO (orange bar; $n = 9$) mice during the acquisition phase. **d** Total number of errors. **e** Latency to leave entry (start) arm. **f** Average time to complete trials during reversal learning training. **g** Total number of errors and **h** latency to leave entry position to find new escape platform. Data displayed as ±SEM.

iMSN-Drd2KO mice did not improve across days (RM ANOVA main effect of day $F_{1,13} = 1.94$, $p = 0.1875$), but the iMSN-Drd2KO mice learned similarly to Drd2$^{loxP/loxP}$ (Fig. 2g, RM ANOVA; main effect of genotype $F_{1,13} = 0.02$, $p = 0.8839$). The iMSN-Drd2KO mice displayed a similar deficit as seen during initial learning, in which these mice took longer to initiate swimming to the new location (Fig. 2h, RM ANOVA; main effect of genotype $F_{1,13} = 32.19$, $p < 0.0001$). Collectively, the results indicate that action initiation in the iMSN-Drd2KO is impaired, while learning and cognitive flexibility are intact.

Pavlovian acquisition and extinction learning are intact in iMSN-Drd2KO mice
To investigate whether the response/action initiation slowing seen in self-initiated tasks was associated with task demand or conditioning paradigm, we trained iMSN-Drd2KO mice in a Pavlovian paradigm, in which cue presentation signaled sucrose

availability. Very little movement was required in this task. Mice were trained on an auditory CS task with two 20-s cues, one of which predicted reward delivery (CS+) and the other did not (CS−) (Fig. 3a). Both genotypes learned to discriminate cue presentations. Consumption of CS+-paired sucrose reward proved to be a more reliable measurement of learning than head entries (Fig. S7). Total consumption during the CS+ increased with training compared to a lower level of stable CS− responding in both genotypes (Fig. 3b, c, Drd2$^{loxP/loxP}$, RM ANOVA main effect of training $F_{3.91,78.21} = 10.29$, $p < 0.0001$, main effect of cue presentation $F_{1,20} = 239.5$, $p < 0.0001$; training × 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 presentation $F_{1,18} = 124.4$, $p < 0.0001$; training × cue interaction, $F_{14,252} = 6.11$, $p < 0.0001$) during Pavlovian acquisition. Total CS+-associated reward consumption per session and frequency of consumption were higher in Drd2$^{loxP/loxP}$ mice than in iMSN-Drd2KO (Fig. 3b, c, total consumption: Drd2$^{loxP/loxP}$ 891 ± 50.74, iMSN-Drd2KO

Dopamine D2 receptor signaling on iMSNs is required for initiation and...
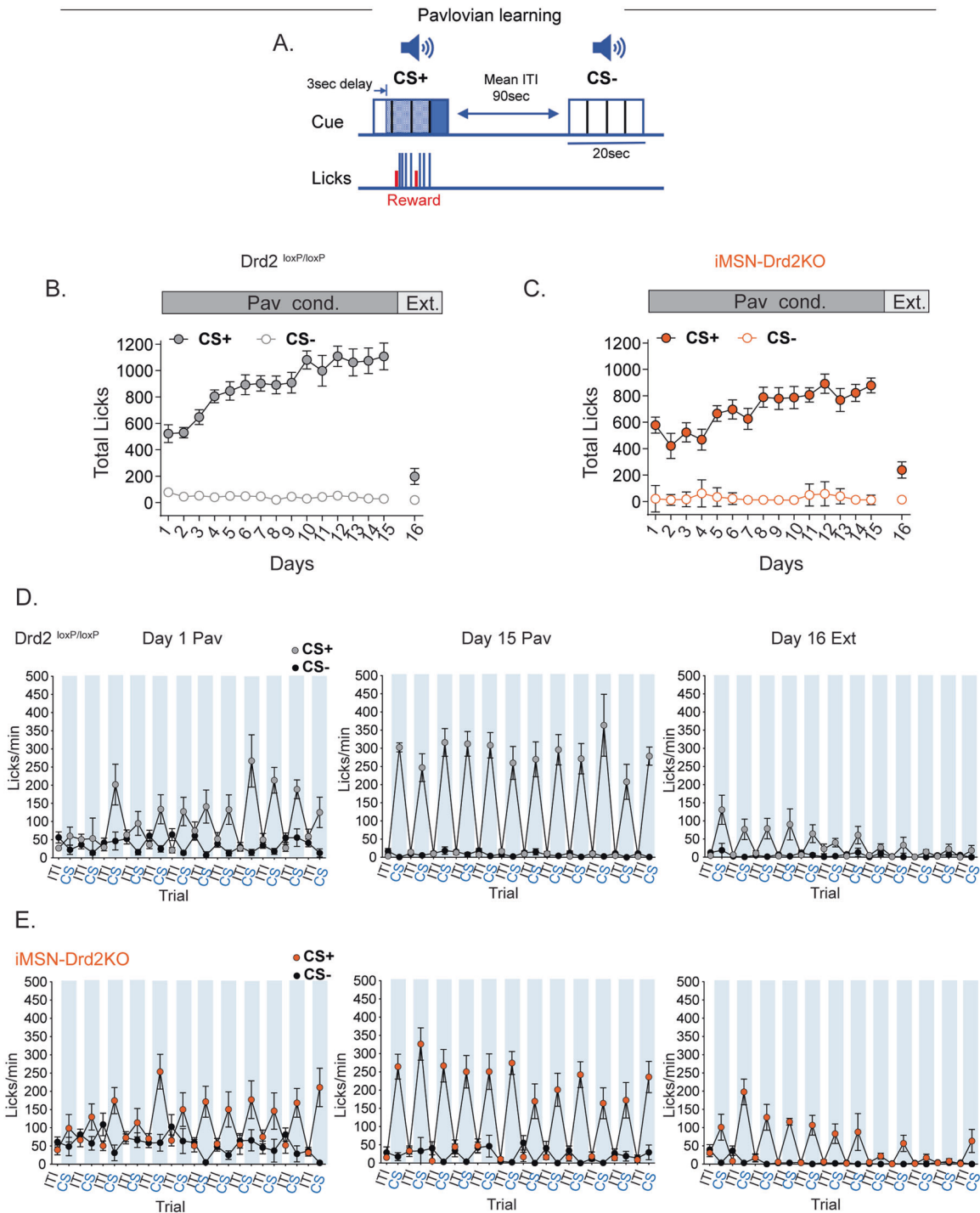SM Augustin et al.

2092

Fig. 3 iMSN-D2R deletion does not impair the Pavlovian acquisition and extinction learning. a CS task schematic diagram. CS+ cue presentation signaled sucrose reward availability. Only during the patterned light blue area (Epoch 1–3) was sucrose delivered twice during CS+ presentation. Sucrose was not delivered during the last epoch, dark blue area. No sucrose deliveries were made during CS− cue presentation. Licks were recorded during the CS+, CS−, and ITI trial periods. **b, c** Total licks across training session for CS+ and CS− for the Drd2$^{loxP/loxP}$ ($n = 11$; gray circles) and iMSN-Drd2KO ($n = 10$; orange circles) mice. **d, e** Average licks per minute during CS presentations (blue vertical bars), CS+ and CS-, and the ITI trial periods (white vertical bars- black circles) for day 1 and 15 of Pavlovian conditioning (Pav) and extinction-day 16 (Ext) in both genotypes. Data shown as ±SEM. ITI = intertrial interval.

700.2 ± 38.19; t-test, $p < 0.01$, RM ANOVA main effect of training $F_{19,266} = 14.23$, $p < 0.0001$; consumption frequency: see Fig. S8). There was no genotype difference in the time it took mice to approach the food receptacle and initiate licking (Fig. S9, RM ANOVA main effect of genotype $F_{1,19} = 0.81$, $p = 0.38$), indicating no slowed movement in this low-motor-demand task. Distinct patterns

of CS+ responding emerged after 15 days of conditioning compared to Day 1 in both genotypes (Fig. 3d, e). Even on Day 1, both genotypes increased their licks/min during CS+ versus CS− trials (Drd2$^{loxP/loxP}$, RM ANOVA main effect of cue presentation $F_{1,20} = 42.36$, $p < 0.0001$; iMSN-Drd2KO, RM ANOVA main effect of cue presentation $F_{1,18} = 40.87$, $p < 0.0001$). There was no significant
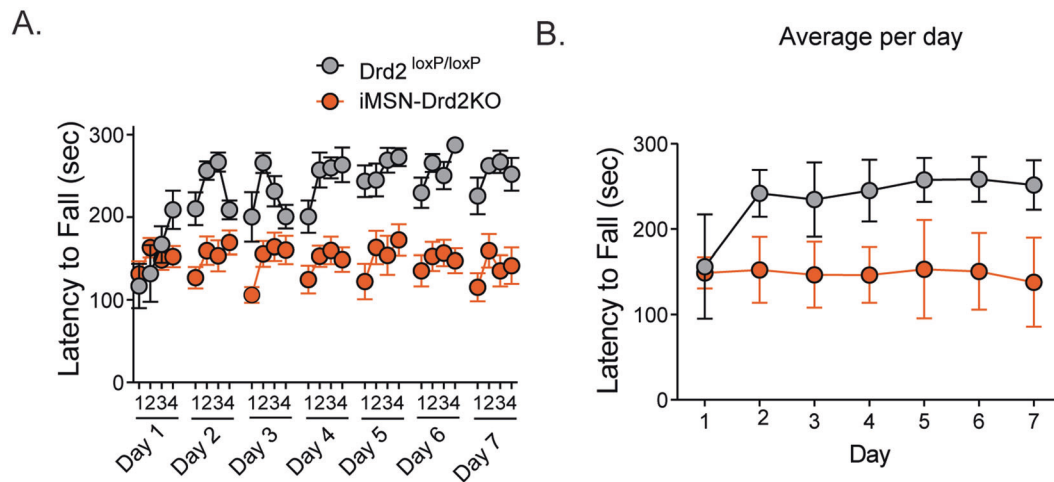
Dopamine D2 receptor signaling on iMSNs is required for initiation and...
SM Augustin et al.

2093

Fig. 4 Targeted deletion of iMSN-D2Rs impairs skill learning. Latency to fall from the accelerating rotarod in the Drd2$^{loxP/loxP}$ ($n = 8$, gray circles) and iMSN-Drd2KO ($n = 9$, orange circles) mice within trials (**a**) and averaged trials (**a**) across days. Error bars = SEM.

difference in CS+-associated licks/min early (trials 1–5) compared to late in training (trials 8–12) on Day 1 in the iMSN-Drd2KO (early 154.1 ± 27.93; late 170.5 ± 11.55, t-test, $p > 0.05$) and Drd2$^{loxP/loxP}$ (early 108.8 ± 27.33; late 185.5 ± 26.33, t-test, $p > 0.05$) mice. In addition, CS+-associated responding was similar between genotypes across the trials (Drd2$^{loxP/loxP}$, 145.0 ± 18.23; iMSN-Drd2KO, 162.0 ± 12.05, t-test, $p > 0.05$). On Day 15, consummatory rates were higher during the CS+ (Fig. 3d, e; blue region) compared to Day 1 in both the iMSN-Drd2KO (234.8 ± 14.23, t-test, $p = 0.002$) and Drd2$^{loxP/loxP}$ (285.9 ± 11.47, t-test, $p < 0.0001$) mice. Although the data indicate that iMSN-Drd2KO mice have learned the contingency associated with CSs, the frequency of consummatory behavioral responding was lower in the iMSN-Drd2KO mice on Day 15 compared to Drd2$^{loxP/loxP}$ (t-test, $p < 0.05$).

Since both genotypes discriminated between the CS+ and CS−, we examined extinction learning to differentiate conditioning from habitual responding. During extinction, conditioned sucrose seeking was measured by presenting the CS+/CS− without reward delivery. There was a significant reduction in the total CS+-associated consumption during extinction on Day 16 testing compared to the last day of Pavlovian conditioning (Day 15) in both genotypes (Fig. 3d, e; Drd2$^{loxP/loxP}$, 54.77 ± 10.17, t-test, $p < 0.0001$; iMSN-Drd2KO, 82.36 ± 15.58, t-test, $p < 0.0001$), and this reduction was similar across genotypes (t-test, $p > 0.05$). Overall, there was a decrease in the frequency to consume, and consumption during extinction in response to the former CS+, suggesting that the iMSN-D2R deletion may not play a role in Pavlovian extinction.

Skill learning is impaired in iMSN-Drd2KO mice
To determine if iMSN-Drd2KO mice show altered skill learning, we examined the performance of Drd2$^{loxP/loxP}$ and iMSN-Drd2KO mice on the accelerating rotarod. As published [49], the iMSN-Drd2KO mice showed impaired performance on the rotarod compared to Drd2$^{loxP/loxP}$ mice (Fig. 4a, RM ANOVA; main effect of trial latency $F_{27,405} = 5.35$, $p < 0.0001$; main effect of genotype $F_{1,15} = 41.67$, $p < 0.0001$, trial latency × genotype interaction, $F_{27,405} = 3.440$, $p < 0.0001$). The iMSN-Drd2KO mice showed decreased latency to fall on all trials across training (Fig. 4b, RM ANOVA; main effect of genotype $F_{1,6} = 40.01$, $p = 0.0007$). These findings indicate that iMSN-D2Rs may be important in action learning, including fine motor control.

DISCUSSION
Among the proposed roles for striatal dopamine and dopamine receptors is control of action learning and performance. We

sought to determine if iMSN-D2Rs contribute to these behaviors using several tasks that involve different actions, with different task demands and outcomes. The tasks were chosen based on striatal involvement, as well as roles for dopamine, in instrumental and Pavlovian learning, and variants of the water maze task. Use of the separate tasks allowed us to determine if impairments were specific to behaviors driven by positive reinforcement (instrumental and Pavlovian conditioning) versus negative reinforcement (water maze). We specifically chose self-paced instrumental conditioning as this task requires animals to initiate action sequences without the aid of predictive stimuli, and thus might be especially susceptible to impairment of mechanisms involved in controlling effort. Self-initiated actions are also impaired in PD patients and dopamine-depleted animals and thus we could also determine if iMSN-D2Rs might have roles that are disrupted in these depleted states. Our findings, and previous studies [49, 50, 52] indicate that the major role of iMSN-D2Rs is in action vigor and initiation, and this role becomes more prominent with greater task demand requirements.

In the self-paced instrumental task, the observation that lever-pressing was altered under the FR5, but not the FR1 schedule, indicates that iMSN-D2Rs are not required for learning or performing a new action but are recruited when the effort required to obtain reward is increased. These findings may also indicate that iMSN-D2Rs are necessary for sustaining repetitive actions. The increased latency to initiate lever-press sequences in the instrumental task and swimming toward the goal in the water maze task also supports a role for iMSN-D2Rs in action initiation. This idea aligns with the findings in open-field experiments where the mice show less movement initiation and overall movement. Thus, these mice show hypokinesis, "slowness" and decreased action initiation regardless of the motivational aspects of the task.

Our findings may also be consistent with a recent study indicating altered responding in a home cage progressive ratio (PR) task [50], indicating that iMSN-D2Rs can override "thrifty" behaviors [50]. However, we did not observe reduced responding or breakpoint in a single-session PR task (Fig. S3). The differences in the PR tasks likely account for the different effects of iMSN-D2R knockout. In the Mourra et al. study, mice obtained all their food via the PR task in their home cage. The PR contingency was reset if mice failed to make the requisite number of responses, allowing them to obtain food at a lower response cost after the reset. This appears to be the case in the iMSN-Drd2KO mice. In the present study, the single-session PR task ended when mice failed to make the requisite responses within 1 h. The task demand in this PR design requires eventual high effort in both groups of mice,

perhaps obscuring small differences in response rate under very-high-demand circumstances. The findings from Mourra et al. and the present study have the common feature that responding is slower under conditions in which animals experience a higher-demand task. In our FR5 paradigm, this experience is likely evident early in training, as mice increase their pressing rate above FR1 levels during Day 1 of FR5 training. In the home cage, continuous PR task used by Mourra et al. [50], the increased demand and alternative resetting strategy become evident only after break-points have been achieved. Nonetheless, in both tasks animals decrease response rate when faced with the greater effort requirement. The view that this represents thriftier behavior makes sense in the context of responding for food reward, although the strategy is less than optimal in the FR5 task as iMSN-Drd2KO mice never receive as many rewards as controls even after several days of FR5 training. Thus, loss of signaling by this receptor can result in suboptimal thrift. Alternatively, receptor loss may simply set an effort (i.e., response rate) barrier beyond which animals cannot work, and this will result in suboptimal action production in a task with set criteria, but not when animals have the option to revert to an easier path to reward. It should also be noted that slowed responding and action initiation extends beyond the case when mice are seeking food, as receptor loss clearly slows actions driven, at least in part, by negative reinforcement in the water T-maze task. Overall, our findings indicate that the iMSN-D2Rs are required to invigorate self-initiated behavior in a variety of settings, consistent with past pharmacological studies that could not target a specific cellular D2R population [16].

The response rate decrease in the iMSN-Drd2KO mice in the instrumental task indicates that the receptor regulates the vigor with which animals carry out a newly learned behavior. The observation that slower action initiation was only seen with increased task demand indicates that iMSN-D2Rs may contribute to neuronal signals involved in anticipation of the effort necessary to complete a task [56, 57], as well as the action execution itself. Indeed, the findings of Mourra and coworkers indicate that iMSN-Drd2KO mice restart PR tasks at higher rates than controls (i.e., show lower breakpoint), and this resetting appears to be enhanced with increasing PR task demand [50]. This finding is consistent with iMSN-D2R involvement in enhancing the effort needed for task completion once requirements are understood. In fact, some iMSN-Drd2KO mice showed large increases in the IPI on the last day of FR5 training, which may be indicative of "giving up" (Fig. S2). The observation that iMSN-Drd2KO mice earn fewer rewards when the cost is increased might suggest a reduction in the motivation to work for food reward. However, we didn't observe reduced breakpoint in a single-session PR task (Fig. S3). Thus, it is the decrease in effort (i.e., response rate) under higher task demand that accounts for altered performance.

Mice lacking iMSN-D2Rs showed little impairment in either learning or performance in the Pavlovian paradigm despite the proposed roles of dopamine in reward-based learning [58]. Studies of PD patients and dopamine-depleted animals [22, 59–64] indicate that loss of dopaminergic transmission impairs self-initiated actions while actions associated with specific environmental events are spared. The lack of performance deficits in the Pavlovian task is consistent with a role for dopamine in self-initiated, well-learned actions. However, the lack of impairment of learning in the iMSN-Drd2KO mice is somewhat surprising given the evidence that iMSNs encode and regulate reward and aversion-based responses [65–67]. In addition, the activity of dopaminergic neurons provides a well-known reward prediction error signal [10], iMSNs respond to outcome presentation in Pavlovian conditioning [67], CS-induced dopamine increases are observed following Pavlovian conditioning [68], and dopaminergic neuron activation produces Pavlovian learning by providing both incentive value and movement invigoration signals [69, 70]. Our

findings indicate that the iMSN-D2Rs have no role in the former process, and only a limited role in the latter function for a well-learned CS-driven behavior. This finding also agrees with the observations of Kelly et al. [39]. There is evidence that different dopaminergic inputs and striatal subregions have larger roles in different types of conditioning, with the ventral tegmental-nucleus accumbens (NAc) systems playing a prominent role in Pavlovian associations and the dorsal striatum (DS) controlling instrumental action learning and production [5, 58, 71–73]. A recent study indicated that D2Rs act to refine discrimination learning in a Pavlovian task via a role in plasticity at synapses onto iMSN [73]. There appeared to be no D2R role in acquisition or extinction, consistent with our findings. It must be noted that these investigators did not explicitly examine the role of iMSN-D2Rs in this learning deficit, and it is known that D2Rs on cholinergic neurons can influence plasticity at iMSN synapses [53, 74]. We must emphasize that our findings cannot be extrapolated to indicate no role for iMSN-Drd2KOs in associative learning. We have simply provided evidence that basic instrumental and Pavlovian associations, as well as Pavlovian extinction, are intact with receptor loss.

The deficits in the rotarod task might suggest a learning-related role for iMSN D2 receptors in agreement with published findings [49] that mice lacking these receptors do not improve their performance over several days of training in this skill. However, this task requires constant physical exertion and thus it is difficult to disentangle learning from effort. Notably, we did not observe a deficit in rotarod performance in the iMSN-Drd2KO mice on the first training day, only on subsequent days, consistent with the observation in other tasks that the performance of these mice plateaus at some level of task demand. In contrast, a previous study found a deficit in initial rotarod performance [39]. However, this is most likely due to the procedures used, as this group provided mice with experience at a fixed speed prior to training on the accelerating version of the task, giving the wild-type mice opportunity to improve, while we only habituated mice to an immobile rod prior to training. The findings in the rotarod task support the idea that iMSN-D2Rs regulate the effort that mice will expend in a movement-based task. However, it would be interesting to examine performance in a skill-learning task in which learning can more easily be distinguished from performance.

The most prominent physiological consequence of the loss of iMSN-D2Rs is removal of the suppression of GABA release at synapses in the Globus Pallidus external segment (GPe) and at local collaterals in striatum. The net effect on striatal output of this loss will be increased indirect pathway output relative to the direct pathway due to enhanced GABAergic inhibition in GPe and increased collateral inhibition of direct pathway MSNs (dMSNs) [49]. These mechanisms have been implicated in bradykinesia observed in the iMSN-Drd2KO mice [49]. However, iMSN-D2Rs have other cellular roles, including inhibition of adenylyl cyclase/cAMP production. Activation of cAMP signaling increases iMSN activity [75], and thus the loss of D2 suppression of signaling would likely increase iMSN/indirect pathway output. However, Lemos and coworkers observed decreased activity of both d- and iMSNs in the iMSN-Drd2KO mice [49]. Thus, the changes in striatal activity with iMSN-D2R loss are more complicated than predicted by changes in cAMP actions, and likely involve greater collateral inhibition of the dMSNs. Nonetheless, the decreased GPe neuronal activity in these mice is consistent with increased indirect pathway function [49]. The D2 inhibition of cAMP signaling also contributes to short-term modulation and plasticity at glutamatergic synapses that result in decreased activation of iMSNs [48, 53, 76–79]. Losing this iMSN-D2R modulation and plasticity would also enhance indirect pathway output. The iMSN-D2R influence on LTD at glutamatergic synapses may contribute to action learning and performance. However, our findings indicate that simple associative learning was not altered in the iMSN-Drd2KO mice,

Dopamine D2 receptor signaling on iMSNs is required for initiation and…
SM Augustin et al.

2095

suggesting that this mechanism has minimal overall influence on task learning, or that LTD has a larger influence on action initiation/performance or more complex associations such as those studied by Iino et al. [73]. iMSN-D2Rs may have important roles in patterning and specificity of striatal output needed for optimal action performance both in the short-term and with extended experience. While more work is needed to assess iMSN activity and GABA release in vivo following iMSN-D2R loss, the available data indicate that receptor loss will increase indirect pathway output resulting in impaired action initiation perhaps involving impaired ability to select actions.

We did not examine if iMSN-D2Rs in different striatal subregions have different roles. Knockdown of D2Rs in NAc mimicked the open-field locomotion phenotype but not rotarod deficits in the study by Lemos and coworkers [49], suggesting that regional differences are important for performance of different actions. In addition, NAc dopamine signaling has been shown to be required for effort exertion when behavioral task demands are high [80], but may not be needed when demands are low [81–83]. Accumbens D2Rs may facilitate this willingness to work during high cost responding [84]. While it is likely that the slowing and initiation deficits seen in the iMSN-Drd2KO mice under higher task demand in our self-paced instrumental task involve the DS given the role of this subregion in instrumental learning [5, 72], we cannot rule out NAc contributions.

Our findings are consistent with a growing literature [85–88] indicating that one of the major roles of striatal dopamine is to invigorate performance of self-initiated actions. This stimulation of effort can be adaptive when faster or more concerted effort is advantageous to minimize exposure (e.g., during foraging behavior) or is needed for successful task completion. Interestingly, the iMSN-D2Rs appear to be an important molecular component for this action invigoration. However, the deficits in action initiation observed with complete loss of striatal dopamine are more severe than those seen in these mice [89, 90]. Thus, other dopamine-activated processes are important for self-initiated movement. Identifying these mechanisms and how they interact with the iMSN-D2Rs will be important for understanding the severe akinesia in disorders such as in PD.

AUTHOR CONTRIBUTIONS
SMA, AVK, and DML conceived the project. TJO performed T-maze experiment, analyzed data, and wrote the associated sections. SMA performed and analyzed all the other experiments. GCL aided in the analysis of behavior and the experimental design of Pavlovian experiment. SMA and DML wrote the manuscript with the input of AVK.

ADDITIONAL INFORMATION
Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41386-020-00799-1).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES
1. Balleine BW, Liljeholm M, Ostlund SB. The integrative function of the basal ganglia in instrumental conditioning. Behav Brain Res. 2009;199:43–52.
2. Graybiel AM, Aosaki T, Flaherty AW, Kimura M. The basal ganglia and adaptive motor control. Science. 1994;265:1826–31.
3. Packard MG, Knowlton BJ. Learning and memory functions of the Basal Ganglia. Annu Rev Neurosci. 2002;25:563–93.
4. Wilson CJ. Basal ganglia. In: Shepherd GM, editor. New York: Oxford University; 2004. p. 361–413.
5. Yin HH, Knowlton BJ. The role of the basal ganglia in habit formation. Nat Rev Neurosci. 2006;7:464–76.
6. Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci. 1990;13:266–71.
7. Balleine BW, Delgado MR, Hikosaka O. The role of the dorsal striatum in reward and decision-making. J Neurosci. 2007;27:8161–5.
8. Balleine BW, Dickinson A. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology. 1998;37:407–19.
9. Liljeholm M, O'Doherty JP. Contributions of the striatum to learning, motivation, and performance: an associative account. Trends Cogn Sci. 2012;16:467–75.
10. Schultz W. Multiple dopamine functions at different time courses. Annu Rev Neurosci. 2007;30:259–88.
11. Cohen MX, Frank MJ. Neurocomputational models of basal ganglia function in learning, memory and choice. Behav Brain Res. 2009;199:141–56.
12. Howe MW, Dombeck DA. Rapid signalling in distinct dopaminergic axons during locomotion and reward. Nature. 2016;535:505–10.
13. Redgrave P, Rodriguez M, Smith Y, Rodriguez-Oroz MC, Lehericy S, Bergman H, et al. Goal-directed and habitual control in the basal ganglia: implications for Parkinson's disease. Nat Rev Neurosci. 2010;11:760–72.
14. Mink JW. The Basal Ganglia and involuntary movements: impaired inhibition of competing motor patterns. Arch Neurol. 2003;60:1365–8.
15. Bello EP, Mateo Y, Gelman DM, Noain D, Shin JH, Low MJ, et al. Cocaine super-sensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. Nat Neurosci. 2011;14:1033–8.
16. Salamone JD, Correa M, Farrar A, Mingote SM. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology. 2007;191:461–82.
17. Bardgett ME, Depenbrock M, Downs N, Points M, Green L. Dopamine modulates effort-based decision making in rats. Behav Neurosci. 2009;123:242–51.
18. De Corte BJ, Wagner LM, Matell MS, Narayanan NS. Striatal dopamine and the temporal control of behavior. Behav Brain Res. 2019;356:375–79.
19. Jin X, Costa RM. Start/stop signals emerge in nigrostriatal circuits during sequence learning. Nature. 2010;466:457–62.
20. Bloxham CA, Mindel TA, Frith CD. Initiation and execution of predictable and unpredictable movements in Parkinson's disease. Brain. 1984;107:371–84.
21. Dujardin K, Laurent B. Dysfunction of the human memory systems: role of the dopaminergic transmission. Curr Opin Neurol. 2003;16 Suppl 2:S11–6.
22. Hallett M. Parkinson revisited: pathophysiology of motor signs. Adv Neurol. 2003;91:19–28.
23. Martin JP. The basal ganglia and posture. J.B. Lippincott, Philadelphia; 1967.
24. Rubinstein TC, Giladi N, Hausdorff JM. The power of cueing to circumvent dopamine deficits: a review of physical therapy treatment of gait disturbances in Parkinson's disease. Mov Disord. 2002;17:1148–60.
25. Thaut MH, McIntosh GC, Rice RR, Miller RA, Rathbun J, Brault JM. Rhythmic auditory stimulation in gait training for Parkinson's disease patients. Mov Disord. 1996;11:193–200.
26. Jin X, Tecuapetla F, Costa RM. Basal ganglia subcircuits distinctively encode the parsing and concatenation of action sequences. Nat Neurosci. 2014;17:423–30.
27. Jin X, Costa RM. Shaping action sequences in basal ganglia circuits. Curr Opin Neurobiol. 2015;33:188–96.
28. Martiros N, Burgess AA, Graybiel AM. Inversely active striatal projection neurons and interneurons selectively delimit useful behavioral sequences. Curr Biol. 2018;28:560–73 e5.
29. Cui G, Jun SB, Jin X, Pham MD, Vogel SS, Lovinger DM, et al. Concurrent activation of striatal direct and indirect pathways during action initiation. Nature. 2013;494:238–42.
30. Howard CD, Li H, Geddes CE, Jin X. Dynamic nigrostriatal dopamine biases action selection. Neuron. 2017;93:1436–50.e8.
31. Samejima K, Ueda Y, Doya K, Kimura M. Representation of action-specific reward values in the striatum. Science. 2005;310:1337–40.
32. Palmiter RD. Dopamine signaling in the dorsal striatum is essential for motivated behaviors: lessons from dopamine-deficient mice. Ann N Y Acad Sci. 2008;1129:35–46.

Dopamine D2 receptor signaling on iMSNs is required for initiation and...
SM Augustin et al.

2096

33. Surmeier DJ, Graves SM, Shen W. Dopaminergic modulation of striatal networks in health and Parkinson's disease. Curr Opin Neurobiol. 2014;29:109–17.

34. Salamone JD, Cousins MS, Bucher S. Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. Behav Brain Res. 1994;65:221–9.

35. Cousins MS, Atherton A, Turner L, Salamone JD. Nucleus accumbens dopamine depletions alter relative response allocation in a T-maze cost/benefit task. Behav Brain Res. 1996;74:189–97.

36. Jiang M, Spicher K, Boulay G, Wang Y, Birnbaumer L. Most central nervous system D2 dopamine receptors are coupled to their effectors by Go. Proc Natl Acad Sci USA. 2001;98:3577–82.

37. Gurevich EV, Gainetdinov RR, Gurevich VV. G protein-coupled receptor kinases as regulators of dopamine receptor functions. Pharm Res. 2016;111:1–16.

38. Baik JH, Picetti R, Saiardi A, Thiriet G, Dierich A, Depaulis A, et al. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature. 1995; 377:424–8.

39. Kelly MA, Rubinstein M, Phillips TJ, Lessov CN, Burkhart-Kasch S, Zhang G, et al. Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. J Neurosci. 1998;18:3470–9.

40. Denk F, Walton ME, Jennings KA, Sharp T, Rushworth MF, Bannerman DM. Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort. Psychopharmacology. 2005;179:587–96.

41. Wise RA. Dopamine and reward: the anhedonia hypothesis 30 years on. Neurotox Res. 2008;14:169–83.

42. Nicola SM. The flexible approach hypothesis: unification of effort and cue-responding hypotheses for the role of nucleus accumbens dopamine in the activation of reward-seeking behavior. J Neurosci. 2010;30:16585–600.

43. Collins AG, Frank MJ. Opponent actor learning (OpAL): modeling interactive effects of striatal dopamine on reinforcement learning and choice incentive. Psychol Rev. 2014;121:337–66.

44. Floresco SB, St Onge JR, Ghods-Sharifi S, Winstanley CA. Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. Cogn Affect Behav Neurosci. 2008;8:375–89.

45. Hamid AA, Pettibone JR, Mabrouk OS, Hetrick VL, Schmidt R, Vander Weele CM, et al. Mesolimbic dopamine signals the value of work. Nat Neurosci. 2016;19:117–26.

46. Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, et al. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science. 1990;250:1429–32.

47. Centonze D, Grande C, Usiello A, Gubellini P, Erbs E, Martin AB, et al. Receptor subtypes involved in the presynaptic and postsynaptic actions of dopamine on striatal interneurons. J Neurosci. 2003;23:6245–54.

48. Bamford NS, Zhang H, Schmitz Y, Wu NP, Cepeda C, Levine MS, et al. Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. Neuron. 2004;42:653–63.

49. Lemos JC, Friend DM, Kaplan AR, Shin JH, Rubinstein M, Kravitz AV, et al. Enhanced GABA transmission drives bradykinesia following loss of dopamine d2 receptor signaling. Neuron. 2016;90:824–38.

50. Mourra D, Gnazzo F, Cobos S, Beeler JA. Striatal dopamine D2 receptors regulate cost sensitivity and behavioral thrift. Neuroscience. 2020;425:134–45.

51. Schultz W. Predictive reward signal of dopamine neurons. J Neurophysiol. 1998;80:1–27.

52. Friend DM, Devarakonda K, O'Neal TJ, Skirzewski M, Papazoglou I, Kaplan AR, et al. Basal Ganglia dysfunction contributes to physical inactivity in obesity. Cell Metab. 2017;25:312–21.

53. Augustin SM, Chancey JH, Lovinger DM. Dual dopaminergic regulation of corticostriatal plasticity by cholinergic interneurons and indirect pathway medium spiny neurons. Cell Rep. 2018;24:2883–93.

54. Guariglia SR, Chadman KK. Water T-maze: a useful assay for determination of repetitive behaviors in mice. J Neurosci Methods. 2013;220:24–9.

55. Reyes S, Cottam V, Kirik D, Double KL, Halliday GM. Variability in neuronal expression of dopamine receptors and transporters in the substantia nigra. Mov Disord. 2013;28:1351–9.

56. LeSauter J, Balsam PD, Simpson EH, Silver R. Overexpression of striatal D2 receptors reduces motivation thereby decreasing food anticipatory activity. Eur J Neurosci. 2020;51:71–81.

57. Filla I, Bailey MR, Schipani E, Winiger V, Mezias C, Balsam PD, et al. Striatal dopamine D2 receptors regulate effort but not value-based decision making and alter the dopaminergic encoding of cost. Neuropsychopharmacology. 2018;43:2180–89.

58. Keiflin R, Pribut HJ, Shah NB, Janak PH. Ventral tegmental dopamine neurons participate in reward identity predictions. Curr Biol. 2019;29:93–103.e3.

59. Pullman SL, Watts RL, Juncos JL, Chase TN, Sanes JN. Dopaminergic effects on simple and choice reaction time performance in Parkinson's disease. Neurology. 1988;38:249–54.

60. Brown VJ, Schwarz U, Bowman EM, Fuhr P, Robinson DL, Hallett M. Dopamine dependent reaction time deficits in patients with Parkinson's disease are task specific. Neuropsychologia. 1993;31:459–69.

61. van Hilten JJ, van Eerd AA, Wagemans EA, Middelkoop HA, Roos RA. Bradykinesia and hypokinesia in Parkinson's disease: what's in a name? J Neural Transm. 1998;105:229–37.

62. Rafal RD, Friedman JH, Lannon MC. Preparation of manual movements in hemiparkinsonism. J Neurol Neurosurg Psychiatry. 1989;52:399–402.

63. Olsson M, Nikkhah G, Bentlage C, Bjorklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J Neurosci. 1995;15:3863–75.

64. Jordan N, Sagar HJ, Cooper JA. A component analysis of the generation and release of isometric force in Parkinson's disease. J Neurol Neurosurg Psychiatry. 1992;55:572–6.

65. Kravitz AV, Kreitzer AC. Striatal mechanisms underlying movement, reinforcement, and punishment. Physiology. 2012;27:167–77.

66. Tai LH, Lee AM, Benavidez N, Bonci A, Wilbrecht L. Transient stimulation of distinct subpopulations of striatal neurons mimics changes in action value. Nat Neurosci. 2012;15:1281–9.

67. Shin JH, Kim D, Jung MW. Differential coding of reward and movement information in the dorsomedial striatal direct and indirect pathways. Nat Commun. 2018;9:404.

68. Sun F, Zeng J, Jing M, Zhou J, Feng J, Owen SF, et al. A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. Cell. 2018;174:481–96.e19.

69. Saunders BT, Richard JM, Margolis EB, Janak PH. Dopamine neurons create Pavlovian conditioned stimuli with circuit-defined motivational properties. Nat Neurosci. 2018;21:1072–83.

70. Salamone JD, Correa M. The mysterious motivational functions of mesolimbic dopamine. Neuron. 2012;76:470–85.

71. Day JJ, Carelli RM. The nucleus accumbens and Pavlovian reward learning. Neuroscientist. 2007;13:148–59.

72. O'Doherty J, Dayan P, Schultz J, Deichmann R, Friston K, Dolan RJ. Dissociable roles of ventral and dorsal striatum in instrumental conditioning. Science. 2004;304:452–4.

73. Iino Y, Sawada T, Yamaguchi K, Tajiri M, Ishii S, Kasai H, et al. Dopamine D2 receptors in discrimination learning and spine enlargement. Nature. 2020; 579:555–60.

74. Augustin SM, Lovinger DM. Functional relevance of endocannabinoid-dependent synaptic plasticity in the central nervous system. ACS Chem Neurosci. 2018;9: 2146–61.

75. Bouabid S, Zhou FM. Cyclic AMP-producing chemogenetic activation of indirect pathway striatal projection neurons and the downstream effects on the globus pallidus and subthalamic nucleus in freely moving mice. J Neurochem. 2018; 145:436–48.

76. Lerner TN, Kreitzer AC. RGS4 is required for dopaminergic control of striatal LTD and susceptibility to parkinsonian motor deficits. Neuron. 2012; 73:347–59.

77. Wang Z, Kai L, Day M, Ronesi J, Yin HH, Ding J, et al. Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. Neuron. 2006;50:443–52.

78. Yin HH, Lovinger DM. Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. Proc Natl Acad Sci USA. 2006;103:8251–6.

79. Augustin SM, Beeler JA, McGehee DS, Zhuang X. Cyclic AMP and afferent activity govern bidirectional synaptic plasticity in striatopallidal neurons. J Neurosci. 2014;34:6692–9.

80. Salamone JD, Pardo M, Yohn SE, Lopez-Cruz L, San Miguel N, Correa M. Mesolimbic dopamine and the regulation of motivated behavior. In: Simpson E, Balsam P, editors. Cham: Springe; 2015.

81. Salamone JD, Wisniecki A, Carlson BB, Correa M. Nucleus accumbens dopamine depletions make animals highly sensitive to high fixed ratio requirements but do not impair primary food reinforcement. Neuroscience. 2001; 105:863–70.

82. Aberman JE, Ward SJ, Salamone JD. Effects of dopamine antagonists and accumbens dopamine depletions on time-constrained progressive-ratio performance. Pharm Biochem Behav. 1998;61:341–8.

83. Ishiwari K, Weber SM, Mingote S, Correa M, Salamone JD. Accumbens dopamine and the regulation of effort in food-seeking behavior: modulation of work output by different ratio or force requirements. Behav Brain Res. 2004; 151:83–91.

84. Gallo EF, Meszaros J, Sherman JD, Chohan MO, Teboul E, Choi CS, et al. Accumbens dopamine D2 receptors increase motivation by decreasing inhibitory transmission to the ventral pallidum. Nat Commun. 2018;9:1086.

Dopamine D2 receptor signaling on iMSNs is required for initiation and…
SM Augustin et al.

2097

85. Wassum KM, Ostlund SB, Balleine BW, Maidment NT. Differential dependence of Pavlovian incentive motivation and instrumental incentive learning processes on dopamine signaling. Learn Mem. 2011;18:475–83.

86. Smith KS, Berridge KC, Aldridge JW. Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. Proc Natl Acad Sci USA. 2011;108:E255–64.

87. Robbins TW, Everitt BJ. Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol. 1996;6:228–36.

88. Berridge KC. From prediction error to incentive salience: mesolimbic computation of reward motivation. Eur J Neurosci. 2012;35:1124–43.

89. Sakai K, Gash DM. Effect of bilateral 6-OHDA lesions of the substantia nigra on locomotor activity in the rat. Brain Res. 1994;633:144–50.

90. Duty S, Jenner P. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. Br J Pharmacol. 2011; 164:1357–91.