

Striatal projection neurons coexpressing dopamine D1 and D2 receptors modulate the motor function of D1- and D2-SPNs

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The role of the striatum in motor control is commonly assumed to be mediated by the two striatal efferent pathways characterized by striatal projection neurons (SPNs) expressing dopamine (DA) D1 receptors or D2 receptors (D1-SPNs and D2-SPNs, respectively), without regard to SPNs coexpressing both receptors (D1/D2-SPNs). Here we developed an approach to target these hybrid SPNs in mice and demonstrate that, although these SPNs are less abundant, they have a major role in guiding the motor function of the other two populations. D1/D2-SPNs project exclusively to the external globus pallidus and have specific electrophysiological features with distinctive integration of DA signals. Gain- and loss-of-function experiments indicate that D1/D2-SPNs potentiate the prokinetic and antikinetic functions of D1-SPNs and D2-SPNs, respectively, and restrain the integrated motor response to psychostimulants. Overall, our findings demonstrate the essential role of this population of D1/D2-coexpressing neurons in orchestrating the fine-tuning of DA regulation in thalamo-cortico-striatal loops.

The thalamo-cortico-striatal loops are essential cerebral modules for the initiation, integration and regulation of motor, cognitive and emotional processes^{1–4}. The functional specificity and precision of their actions are finely tuned through the complex design of striatal organization^{5–7}. Striatal control of cortical excitation and inhibition requires two distinct output pathways^{8,9}: a direct pathway from the cortex to the striatum to the entopeduncular nucleus (EPN) and substantia nigra pars reticulata (SNr) that facilitates motor cortical activity and an indirect pathway from the cortex to the striatum to the external globus pallidus (GPe) to the subthalamic nucleus to the EPN/SNr that inhibits it. The direct pathway involves dopamine (DA)

D1 receptor (D1R)-expressing striatal projection neurons (SPNs) (D1-SPNs), whereas the indirect pathway involves DA D2R-expressing SPNs (D2-SPNs)¹⁰. This organization underscores why DA is such an essential neurotransmitter not only in motor control¹¹ but also in the reward system and cognitive processes¹². Because DA oppositely modulates the activity of D1-SPNs in the direct pathway and the activity of D2-SPNs in the indirect pathway, it is often postulated that DA has a synergistic effect promoting locomotion⁶. This general description is supported by findings that the motor response to cocaine administration is suppressed or blunted in *Drd1a*-knockout (*Drd1a* KO) mice¹³ and SPN-*Drd2* KO mice¹⁴, respectively. Although these findings support the

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classical model that attributes a unique and homogeneous role to DA in the striatum, growing evidence indicates several levels of genetic and functional heterogeneity within the rodent striatal system; as a result, the modulatory actions of DA and its impact on striatal output are still the subjects of intense investigation. In contrast to the early and highly influential model of the basal ganglia circuits (that is, the classical model) that posits opposite motor roles for the two SPN populations, it has been shown that optogenetic activation of D1- and D2-SPNs produces different responses in the SNr and cortical neurons, with stimulation of each pathway eliciting both excitation and inhibition^{15–17}. Moreover, it has also been shown that the two populations are jointly active during a motor task and work together to produce voluntary movements^{18–21}. Finally, single-cell molecular analysis of striatal neurons reveals more complex heterogeneity and identifies several distinct clusters of SPNs^{22–27}. Notably, in addition to the presence of D1-SPNs and D2-SPNs, the existence of hybrid SPNs that express both D1Rs and D2Rs has previously been identified by indirect approaches^{28–33}, but their roles and functions remain unknown. Using targeted approaches, we demonstrate here that this population of D1/D2-SPNs forms a distinct striatal output crucial for motor control. This third pathway and its modulation by DA operates in conjunction with the canonical D1- and D2-SPN pathways to influence motor functions.

SPNs coexpressing D1/D2 constitute a specific population

Previous single-cell and transgenic studies have provided only indirect and sometimes controversial indications of D1/D2-SPN distribution^{28–32}. Here we used direct and highly sensitive double-probe fluorescent in situ hybridization (sdFISH) throughout the striatum to demonstrate the native coexpression of *Drd1a* and *Drd2*. Unbiased automated quantitative analysis showed that cells coexpressing *Drd1a* and *Drd2* accounted for 6–7% of all DA receptor-expressing cells in both the dorsal and ventral parts of the striatum, across its rostro-caudal extent (Fig. 1a,b and Extended Data Fig. 1a,b).

To target this population of D1/D2-SPNs selectively, we used an intersectional approach³⁴ (Fig. 1c) by generating a novel *D1^{FlpO}* driver line expressing flippase O (FlpO) recombinase in D1-SPNs (Extended Data Fig. 1c,d). *D1^{FlpO}* mice were crossed with the *A2a^{Cre}* driver line expressing Cre recombinase exclusively in D2-SPNs, thereby excluding cholinergic interneurons³⁵ (Extended Data Fig. 1e). Using this approach, we were able to target D1/D2-SPNs, D1-SPNs and D2-SPNs selectively. This relies on the presence of Cre (C) and/or FlpO (F): Con/Fon, Coff/Fon and Con/Foff (Fig. 1c and Extended Data Fig. 1f). We first used enhanced yellow fluorescent protein (eYFP) expression with INTRASECT (INTRonic Recombinase Sites Enabling Combinatorial Targeting) vectors to validate the proper recombination of the constructs in mice expressing both Cre and FlpO and in mice expressing only Cre or only FlpO (Extended Data Fig. 1f). No leaking recombination of the Con/Fon, Coff/Fon and Con/Foff vectors was observed (Extended Data Fig. 1f). We subsequently quantified the cell densities observed for each transfected SPN subpopulation (Extended Data Fig. 1g,h). With this approach, we found that of all SPNs in the dorsal striatum, $16.5 \pm 1.4\%$ were D1/D2-SPNs, $45.7 \pm 2.8\%$ were D2-SPNs and $37.8 \pm 0.5\%$ were D1-SPNs. The lower percentages obtained using sdFISH are most likely due to underdetection. Next, we similarly expressed channelrhodopsin 2 (ChR2) fused to eYFP to trace neuronal pathways (Fig. 1d and Extended Data Fig. 1f). We found that D1/D2-SPNs have a single projection pathway to the GPe, dissociating them from a direct pathway to the EPN/SNr.

This mapping indicates that D1/D2-SPNs give rise to an additional indirect pathway, which may propagate a different DA-mediated signal to downstream basal ganglia nuclei. Furthermore, in terms of their cell morphology, this population is distinguished by a more ramified proximal arborization than D1-SPNs and a larger soma area than D2-SPNs (Fig. 1e–g).

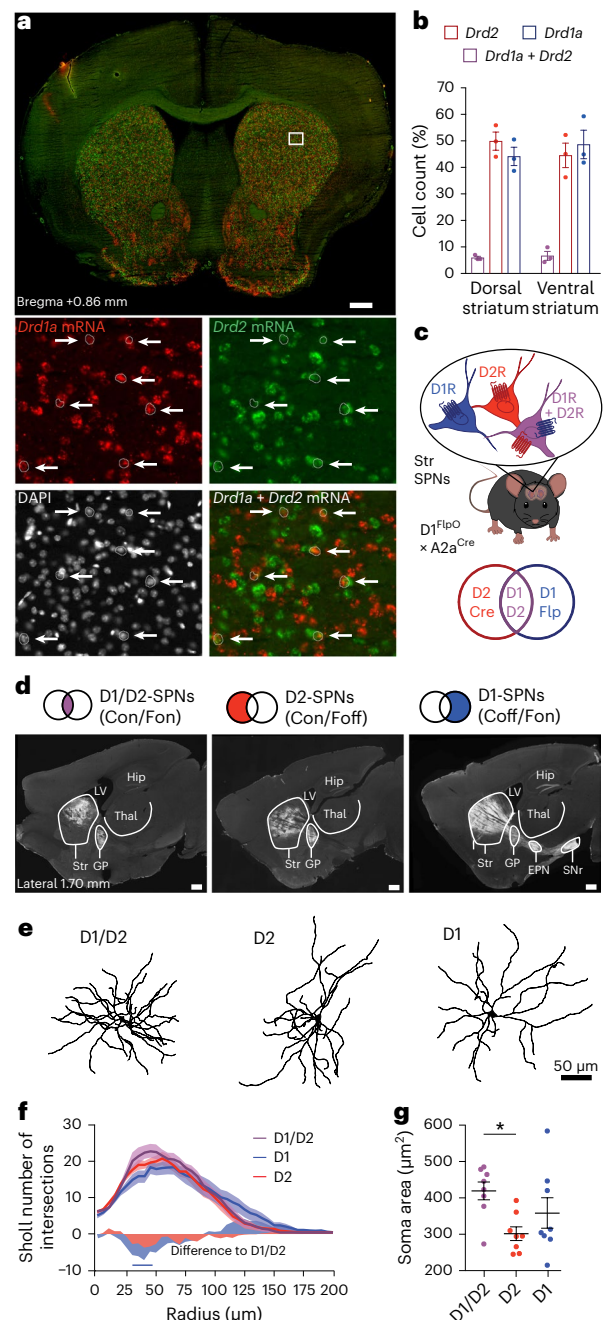


Fig. 1 | Morphologically distinct D1/D2-coexpressing SPNs establish an additional efferent striatal pathway. a, Representative coronal section of FISH illustrating *Drd1a*/*Drd2* coexpression (arrows) in the striatum (scale: top image, 1 mm; bottom zoomed images, 20 μ m). **b**, Percentage of *Drd1a*-, *Drd2*- and *Drd1a*/*Drd2*-positive neurons over cells expressing either *Drd1a* or *Drd2* in the dorsal and ventral striatum ($n = 3$ mice). **c**, Schematic illustrating the INTRASECT targeting strategy applied to *D1^{FlpO}/A2a^{Cre}* mice (schematic illustrations were created with BioRender.com). **d**, Representative parasagittal sections illustrating the projection patterns of D1/D2-, D2- and D1-SPNs obtained from *D1^{FlpO}/A2a^{Cre}* mice injected with the respective INTRASECT AAV constructs (scale: 500 μ m). Str, striatum; GP, globus pallidus; Thal, thalamus; Hip, hippocampus; LV, lateral ventricle. **e**, Reconstruction of the dendritic arborization of a representative D1/D2-SPN (left), D2-SPN (middle) and D1-SPN (right). **f**, **g**, Sholl analysis (**f**) and soma area (**g**) of reconstructed SPNs (D1-SPNs, $n = 8$; D2-SPNs, $n = 8$; D1/D2-SPNs, $n = 8$) (in **f**, the horizontal blue line indicates two-sided t -test Bonferroni-corrected $P < 0.05$ between D1/D2 and D1; in **g**, one-way analysis of variance (ANOVA) followed by two-sided Tukey's tests: $*P = 0.029$). Data are presented as mean values $\pm$ s.e.m. Detailed statistics are shown in Supplementary Table 1. Source data are provided as a source data file.

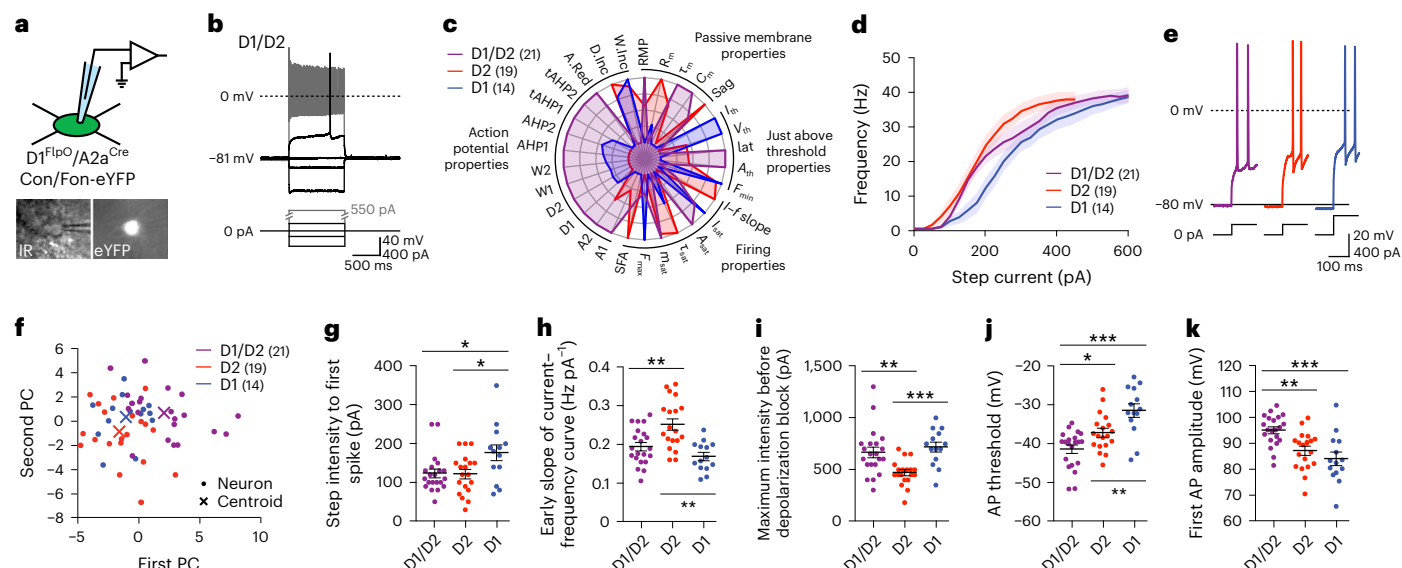


Fig. 2 | D1/D2-SPNs differ through unique action potential properties. **a**, Diagram of patch-clamp recordings in a YFP-labeled D1/D2-SPN (top) and representative photomicrographs under infrared (IR) illumination (bottom left) and fluorescent illumination (bottom right). **b**, Typical voltage response of the same D1/D2-SPN as in **a** in response to hyperpolarizing and depolarizing current steps. **c**, Radar plot revealing differences between the three SPN types across all electrophysiological properties extracted (see ‘Recording analysis’ in Methods for the definitions of the electrophysiological parameters). Means for each population are normalized in the range 0–1. **d**, Average current–frequency relationship for the three SPN subpopulations. **e**, Representative examples of action potential response to depolarizing current in one D1/D2-SPN, one D1-SPN

and one D2-SPN. **f**, Two-dimensional projection of the set of neurons recorded (circles) and the populations’ centroids (cross) following principal component (PC) analysis into the first- and second-order components. **g–k**, Quantification of the minimal step current to discharge (**g**), early slope of the current–frequency curve (**h**), current intensity to discharge saturation (**i**), action potential (AP) threshold (**j**) and amplitude of the first action potential (**k**) in D1/D2-SPNs ($n = 21$ cells from nine mice), D1-SPNs ($n = 14$ cells from five mice) and D2-SPNs ($n = 19$ cells from five mice) (one-way ANOVA followed by two-sided Tukey’s tests: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Data are presented as mean values $\pm$ s.e.m. Detailed statistics are shown in Supplementary Table 1. Source data are provided as a source data file.

D1/D2-SPNs display distinct electrophysiological features

Based on their morphological specificity, we next examined the electrophysiological properties of D1/D2-SPNs in comparison to the two classical populations. Thirty electrophysiological parameters were extracted from whole-cell current-clamp recordings describing passive membrane properties, firing properties, action potential properties and threshold properties (Fig. 2a–c and Extended Data Table 1). As previously reported^{36–38}, D1-SPNs and D2-SPNs differed with respect to many features (Fig. 2c–k and Extended Data Fig. 2a–c). We found some mixed properties for D1/D2-SPNs; the maximum intensity before the depolarization block and the current–frequency early slope are similar to those of D1-SPNs (Fig. 2h,i), whereas the step intensity to the first action potential is similar to that of D2-SPNs (Fig. 2g and Extended Data Table 1). More essentially, D1/D2-SPNs exhibit a unique combination of action potential properties, such as a lower action potential threshold and a larger action potential amplitude (Fig. 2e,j,k and Extended Data Fig. 2). Taken together, the anatomical, morphological and electrophysiological results support the existence of a third functional SPN population.

D1/D2-SPNs drive immobility and the motor functions of other SPNs

To identify the motor function of this third SPN population, we performed *in vivo* optogenetic stimulations of D1/D2-SPNs in the dorsomedial part of the striatum (Fig. 3 and Supplementary Fig. 1). These experiments took place at the start of the dark photoperiod under dim light, when mice are most active (Supplementary Fig. 2). We assessed not only the effect of activating D1/D2-SPNs (purple; Fig. 3a,b) or D2-SPNs alone (red) or D1-SPNs alone (dark blue) but also the effect of activating D1/D2-SPNs together with either D2-SPNs (pink, D2 + D1/D2-SPNs) or D1-SPNs (light blue, D1 + D1/D2-SPNs). The sixth experimental group comprised D1/D2^{eYFP} control mice (gray).

The conditions D2 + D1/D2-SPNs and D1 + D1/D2-SPNs correspond to what was defined as ‘indirect pathway SPNs’ and ‘direct pathway SPNs’, respectively, in previous studies. Thus, using optogenetic stimulation as a gain-of-function approach enabled us to isolate the functional contribution of each SPN subpopulation and to reexamine the respective roles of exclusive D2- and D1-SPNs.

Unilateral activation of the indirect pathway has been shown to produce ipsiversive rotational behavior, whereas activation of the direct pathway results in contraversive rotations³⁹. Consistent with their GPe-targeted projection pattern, unilateral stimulation of D1/D2-SPNs also resulted in ipsilateral rotations, similar to that of D2-SPNs or D2 + D1/D2-SPNs, whereas stimulation of D1-SPNs or D1 + D1/D2-SPNs resulted in the opposite rotatory behavior (Fig. 3c). These effects directly confirm that our intersectional optogenetic strategy is effective in all three populations.

We next examined the impact of bilateral stimulation on free-running mice in a square open field (Fig. 3d). A comparative analysis of three different stimulation patterns with pulsed light and a constant 30-s illumination (Extended Data Fig. 3, Supplementary Fig. 1 and Supplementary Note) revealed that the most reliable optogenetic paradigm is a constant illumination, which allows for sustained neuronal depolarization with SPNs firing at an unconstrained frequency dictated by the intrinsic properties of SPNs and the summation of excitatory and inhibitory currents (Supplementary Fig. 1f–i and Supplementary Note). Motor behavior was classified into three states based on the speed of the mouse’s center of mass: locomotion, small movements and immobility (Fig. 3d). On average, wild-type (WT) mice spent $44 \pm 0.7\%$ of their time in locomotion, $40 \pm 0.8\%$ in small movements and $16 \pm 0.6\%$ in immobility (Fig. 3d). Optogenetic activation of each SPN population significantly reorganized this motor behavior distribution during the active (dark) photoperiod (Fig. 3d,e and Supplementary Table 2); these effects could be largely different during

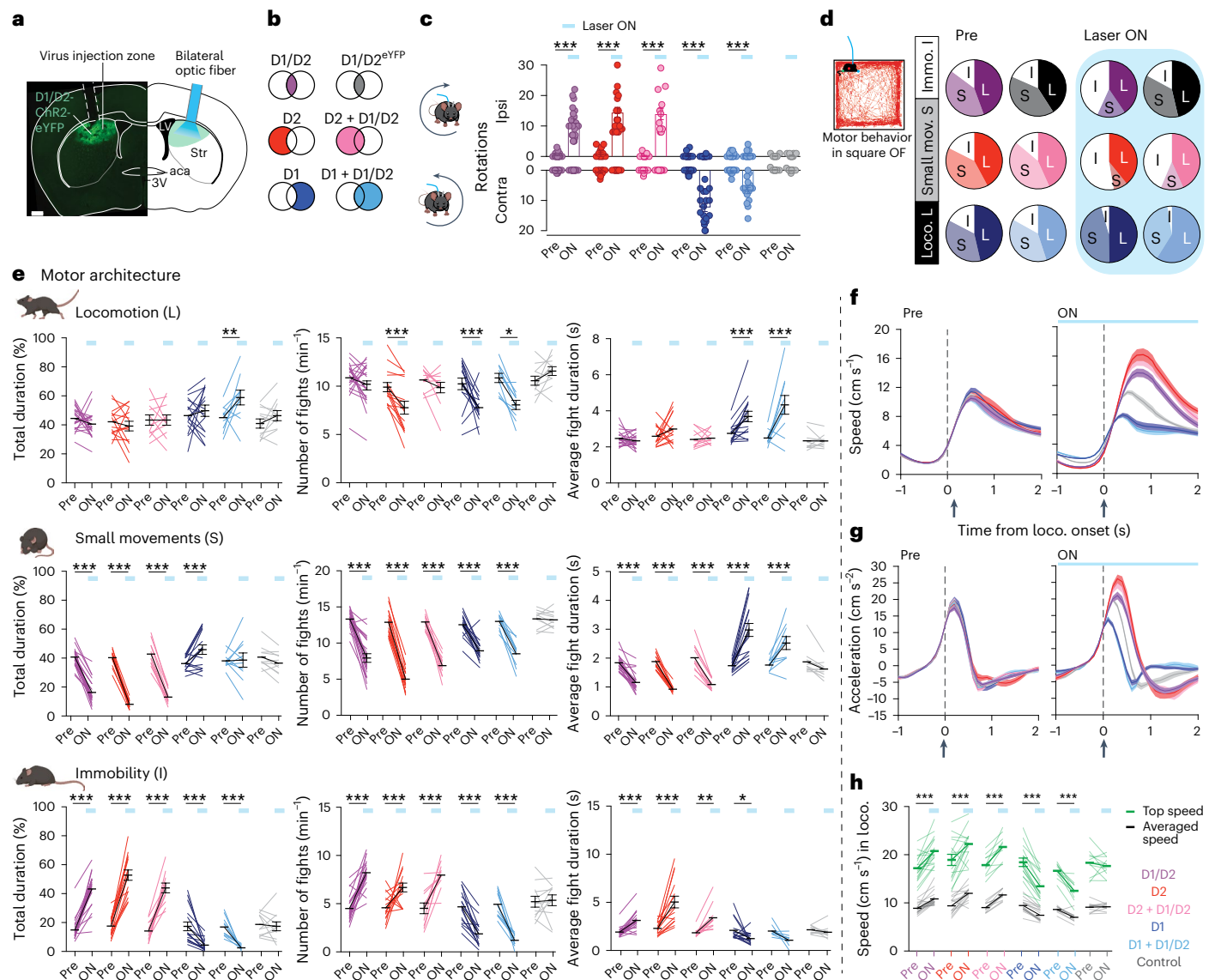


Fig. 3 | D1/D2-SPNs promote immobility and control the effects of D1- and D2-SPNs on locomotion. **a**, Coronal section and corresponding atlas plane depicting optic fiber placement targeting the dorsal striatum and ChR2 expression in D1/D2-SPNs in D1^{FlpO}/A2a^{Cre} mice (scale: 500 μ m). Str, striatum; 3V, third ventricle; aca, anterior commissure area; LV, lateral ventricle. **b**, Schematic representation of the experimental groups. **c**, Rotational behavior elicited in mice by unilateral continuous illumination (60 s) of ChR2-expressing D1/D2-SPNs ($n = 21$), D2-SPNs ($n = 16$), D2 + D1/D2-SPNs ($n = 13$), D1-SPNs ($n = 18$), D1 + D1/D2-SPNs ($n = 16$) and control D1/D2^{eYFP}-SPNs ($n = 11$) (two-way ANOVA followed by two-sided Tukey's test, baseline (Pre) versus light on (ON): *** $P < 0.001$) (schematic illustrations were created with BioRender.com). **d**, Distribution and proportions (%) of the three motor states (L, locomotion; S, small movements; I, immobility) defined in free-running mice exploring an open field (OF) during the baseline (Pre) and stimulation (Laser ON) periods (D1/D2: $n = 21$, D2: $n = 15$, D2 + D1/D2: $n = 11$, D1: $n = 16$, D1 + D1/D2: $n = 10$, control D1/D2^{eYFP}: $n = 11$) (two-way ANOVA followed by two-sided Tukey's test, baseline versus light on: *** $P < 0.001$) (schematic illustrations were created with BioRender.com). **e**, Effect of bilateral optogenetic stimulation (10 $\times$ 30-s trials with 90 s between trials)

on motor architecture in the open field evaluated during locomotion (top row), small movements (middle row) and immobility (bottom row) by the total time spent in each state (left column), the number of episodes per minute in each state (middle column) and the mean duration of these episodes (right column) (D1/D2: $n = 21$, D2: $n = 15$, D2 + D1/D2: $n = 11$, D1: $n = 16$, D1 + D1/D2: $n = 10$, control D1/D2^{eYFP}: $n = 11$) (two-way ANOVA followed by two-sided Tukey's test, baseline versus light on: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (schematic illustrations were created with BioRender.com). **f–h**, Effect of laser stimulation in each group on the averaged temporal evolutions of mouse speed (f) and acceleration (g) around locomotion onset and on averaged speed (black) and top speed (ninth decile, green) during locomotion episodes (h) (D1/D2: $n = 21$, D2: $n = 15$, D2 + D1/D2: $n = 11$, D1: $n = 16$, D1 + D1/D2: $n = 10$, control D1/D2^{eYFP}: $n = 11$) (two-way ANOVA followed by two-sided Tukey's test, baseline versus light on: *** $P < 0.001$). Data are presented as mean values $\pm$ s.e.m. Detailed statistics are shown in Supplementary Table 1. Source data are provided as a source data file.

the inactive (light) photoperiod (Supplementary Fig. 2 and Supplementary Note). Of note, the activation of D1/D2-SPNs, despite being a smaller population, led to a strong increase in immobility coupled with a marked inhibition of small movements. This pattern was the same as that observed in response to stimulation of the indirect pathway by exclusive activation of D2-SPNs or mixed coactivation of D2 + D1/D2-SPNs (Fig. 3d,e). These stimulations had no significant effect on

the total time spent in locomotion (Fig. 3d,e). However, there was a notable difference in the microstructure of the locomotion state induced by exclusive stimulation of D2-SPNs versus that induced by coactivation of D2 + D1/D2-SPNs: stimulation of exclusively D2-SPNs significantly reduced the number of locomotor episodes while lengthening the mean duration of these episodes, albeit nonsignificantly (Extended Data Fig. 4a). By contrast, stimulation of D1/D2-SPNs or

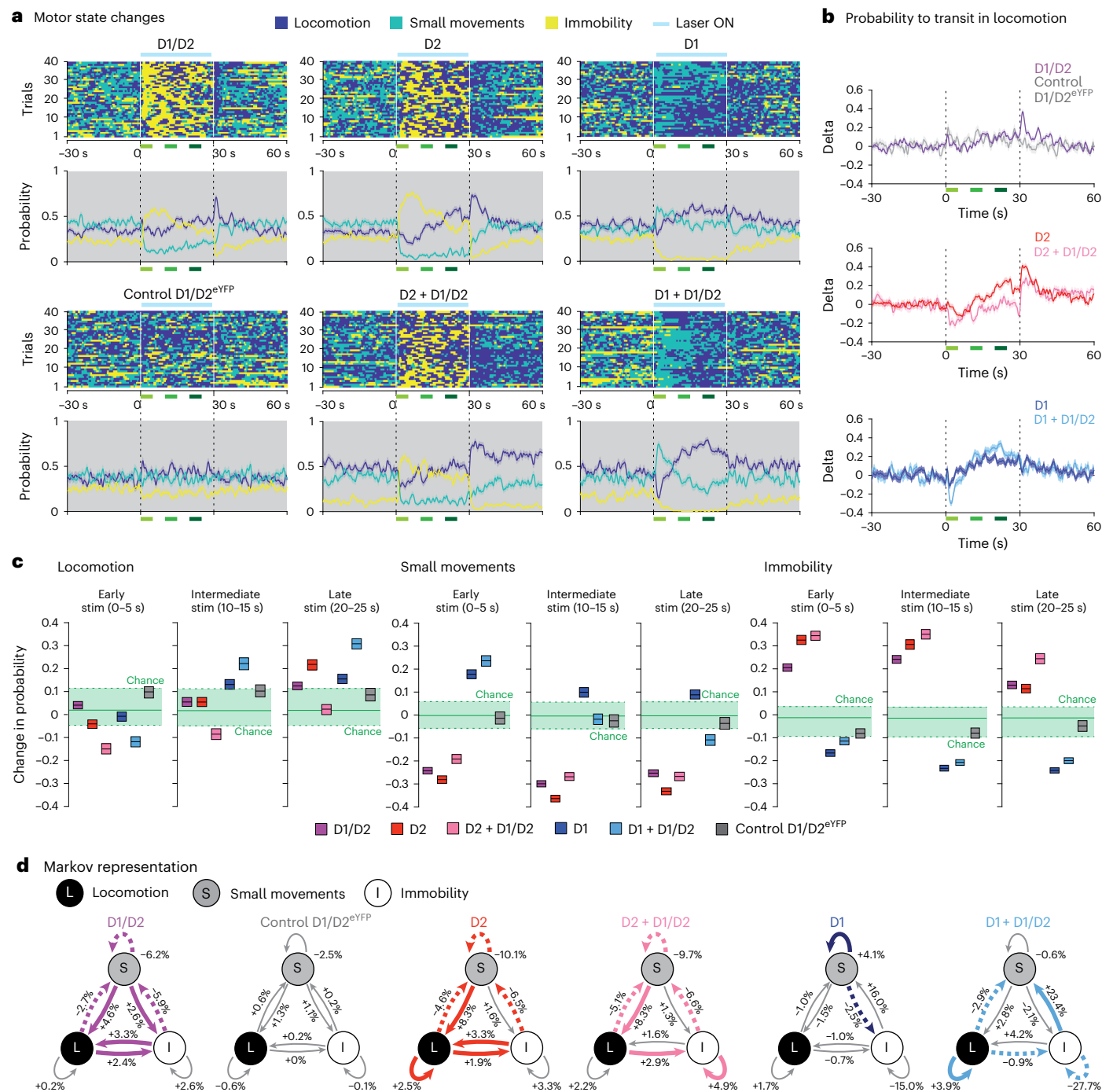


Fig. 4 | D1/D2-SPN coactivation modulates the temporal dynamics of motor changes elicited by D1- or D2-SPN stimulation. **a**, Motor states for ten stimulation trials in four representative mice of each genotype (top) and corresponding temporal evolution of the probability (bottom) to observe locomotion, small movements and immobility before, during and after laser stimulation (solid line, mean; shading, bootstrap 95% confidence interval (CI)). Blue bar, laser stimulation. **b**, Temporal evolution of changes in probabilities to observe locomotion centered on prestimulation values (solid line, mean; shading, bootstrap 95% CI). **c**, Changes in the occurrence probability of locomotion (left), small movements (middle) and immobility (right) induced by laser stimulation during the early (0–5 s; light green bars in **a** and **b**), intermediate

(10–15 s; green bars in **a** and **b**) and late (20–25 s; dark green bars in **a** and **b**) periods of the photostimulation (colored shading, bootstrap 95% CI for each experimental group; light green, bootstrap 95% CI for chance). **d**, Changes induced by laser stimulation in conditional transition probabilities between the three motor states for each group during the late (20–25 s) photostimulation period, evaluated using a Markov chain. Values indicate the average change in transition probability, and solid and dashed colored lines highlight significant increases and decreases, respectively, during stimulation as compared to the baseline period. Detailed statistics are shown in Supplementary Table 1. Source data are provided as a source data file.

mixed D2 + D1/D2-SPNs induced no change in these parameters. This suggests that the coactivation of D1/D2-SPNs can block some prolocomotor D2-SPN-mediated effects. When D1/D2-SPNs and D2-SPNs are costimulated, immobility is facilitated and small movement is

inhibited, producing a clear antikinetic effect of indirect pathway activation (Supplementary Note). In addition, we observed significant differences between exclusive stimulation of D1-SPNs and coactivation of D1 + D1/D2-SPNs. As previously reported^{39,40}, stimulation of mixed

D1 + D1/D2-SPNs induced a significant increase in the time spent in locomotion, by a lengthening of locomotor episodes and an almost complete abolition of the immobility state (Fig. 3e and Extended Data Fig. 4a). By contrast, exclusive stimulation of D1-SPNs resulted in a large increase in the time spent in small movements at the expense of immobility (Fig. 3e and Extended Data Fig. 4a). This suggests that coactivation of D1/D2-SPNs with D1-SPNs allows for a net prolocomotor effect of the direct pathway.

To refine our examination of the locomotion state in response to optogenetic stimulation during the dark photoperiod, we carried out analyses focusing on this specific motor state of the animals when the stimulation is ongoing. We analyzed the speed and acceleration temporal profiles specifically at the onset of a locomotion state (Fig. 3f,g and Extended Data Fig. 4e,f) and the average speed during locomotion episodes (Fig. 3h, Extended Data Fig. 4d and Supplementary Note). In this context, stimulating D1/D2-SPNs alone, D2-SPNs alone or D2 + D1/D2-SPNs together increased both speed and acceleration when animals were engaged in locomotion. Conversely, stimulation of D1-SPNs alone or stimulation of D1 + D1/D2-SPNs reduced both speed and acceleration.

It has been shown that the indirect and direct pathways can promote aversive and rewarding effects, respectively^{41–43}. Therefore, we performed optogenetic real-time place preference experiments in which laser stimulation was controlled by the entry and exit of the animals from a specific zone in the open field (Extended Data Fig. 5). We found that the stimulated zone was preferred during D1-SPN (exclusive and mixed) activation and avoided during D1/D2-SPN and D2-SPN (exclusive and mixed) stimulation (Extended Data Fig. 5a). We also examined the speed profiles around locomotion episode onsets occurring within and outside the stimulated zone (Extended Data Fig. 5b). Consistent with our previous observations (Fig. 3f), we observed a decrease in speed during D1-SPN stimulation and an increase in speed during D1/D2-SPN or D2-SPN stimulation within the stimulated zone.

Overall, these results reveal effective and opposite control over locomotion exerted by D1/D2-SPNs, depending on whether they are coactivated with D1-SPNs or D2-SPNs. These findings also highlight the more nuanced motor effects of exclusive stimulation of D1- and D2-SPNs alone, leading us to reexamine their prokinetic and antikinetic roles, respectively.

D1/D2-SPNs reshape the functional identity of D1- and D2-SPNs

We examined the temporal evolution of the probability to observe each of the motor states around each optogenetic stimulation event: before stimulation, during three periods under light stimulation (early, 0–5 s; intermediate, 10–15 s; and late, 20–25 s) and after stimulation (ten trials per mouse; Fig. 4a–c).

D1/D2-SPN stimulation produced a consistent increase in immobility at the expense of small movements, without any notable alteration in locomotion (Fig. 4a–c and Extended Data Fig. 6). These analyses also revealed major differences between exclusive D1-SPN stimulation and mixed D1 + D1/D2-SPN stimulation (Fig. 4a–c). Immediately at the onset of stimulation (Fig. 4a–c), the coactivation of D1 + D1/D2-SPNs produced a decrease in locomotion, which quickly reversed into a large increase in locomotion. This effect on locomotion was mirrored by the temporal evolution of small movements (Fig. 4a–c and Extended Data Fig. 6) and the stabilization of the ongoing small-movement episodes when D1 + D1/D2-SPN stimulation started during a small-movement episode (Extended Data Fig. 7b). Conversely, activation of D1-SPNs alone produced an increase in locomotion only after 20 s, and small movements were increased throughout the whole duration of stimulation (Fig. 4a–c and Extended Data Fig. 6). For both D1-SPN stimulation and D1 + D1/D2-SPN stimulation, the inhibition of immobility followed a similar temporal evolution, immediate and maintained throughout the stimulation. These locomotion-promoting effects

during simultaneous stimulation of D1- and D1/D2-SPNs were consistent with the stabilization of the locomotor state underpinned by a lengthening of locomotor episodes and were not observed in the exclusive stimulation of D1-SPNs alone (Extended Data Fig. 4a). By contrast, exclusive D1-SPN stimulation resulted mainly in the induction of small movements, which was prevented by the coactivation with D1/D2-SPNs (Extended Data Fig. 4c).

To extend this characterization of motor state changes, we applied a Markov chain model to assess how the dynamic organization of motor states is affected by each SPN stimulation (Fig. 4d and Extended Data Fig. 7a). Conditional transition probabilities indicated that activation of D1-SPNs alone facilitates small movements by inhibiting exit probabilities from this state; this convergence to small movements was less strong when D1-SPNs were coactivated with D1/D2-SPNs because transitions from locomotion to small movements were inhibited and maintenance of locomotion was strengthened (Fig. 4d and Extended Data Fig. 7a). Altogether, these findings indicate that coactivation of D1/D2-SPNs is required to trigger the prokinetic effects associated with stimulation of the classical direct pathway.

These comparative analyses also identified differences in the exclusive and mixed D2-SPN populations, illustrated by a divergence of effects on state changes to the locomotor state depending on whether or not D1/D2-SPNs are coactivated (Fig. 4a–c). Immediately after the onset of stimulation, immobility was quickly and persistently facilitated in both cases (Fig. 4a–c and Extended Data Fig. 7a), an observation reinforced by the stabilization of immobility episodes when stimulation started during immobility (Extended Data Fig. 7b). Divergence occurred later during the stimulation of exclusive D2-SPNs, when the probability of being in the locomotor state was increased (Fig. 4a–c), whereas such an effect was prevented by costimulation of D1/D2-SPNs (Fig. 4a–c). During most of the stimulation period, D2 + D1/D2-SPN stimulation inhibited locomotion. This effect faded away late during the stimulation (Fig. 4a–c). Consistent with this, computing the conditional transition probabilities under D2-SPN and D2 + D1/D2-SPN activations indicated an increased probability of remaining in the locomotor state only under D2-SPN stimulation (Fig. 4d and Extended Data Fig. 7a). These results suggest that coactivation of D1/D2-SPNs alongside D2-SPNs blocks a late prokinetic effect induced by exclusive stimulation of D2-SPNs and thus further facilitates the antikinetic role of indirect pathway activation.

DA exerts a unique effect on D1/D2-SPN activity

Given the fundamental role of DA in the modulation of locomotion in the striatum, we explored its actions and functions on D1/D2-SPNs. We first evaluated *ex vivo* the effects of DA bath application on SPN activity (Fig. 5 and Extended Data Fig. 8). We confirmed that at depolarized membrane potentials, DA depolarizes D1-SPNs and hyperpolarizes D2-SPNs (Fig. 5c and Extended Data Fig. 8b). Consistent with this, it increased D1-SPN excitability and decreased D2-SPN excitability, as indicated by substantial changes in the firing response to depolarizing current pulses, membrane resistance and the current–frequency relationship (Fig. 5d,e and Extended Data Fig. 8c–e). In addition, we confirmed a decrease in the action potential threshold in both populations in response to DA (Fig. 5f); the ramp current to the action potential threshold was significantly decreased in D1-SPNs, whereas it was unchanged in D2-SPNs (Fig. 5g).

For D1/D2-SPNs, we found DA-induced depolarization in a D1R activation-dependent manner (Fig. 5a–c and Extended Data Fig. 8c,h), but there was no change in excitability or firing (Fig. 5d,e and Extended Data Fig. 8a–e). This lack of effect might be related to the peculiar D1/D2-SPN action potential properties described above (Fig. 2). Indeed, in sharp contrast to the two other SPN populations, DA increased the action potential threshold in D1/D2-SPNs (Fig. 5b,f), which was abolished in the presence of either D1R or D2R antagonists (Extended Data Fig. 8k–m). The ramp current to the action potential threshold

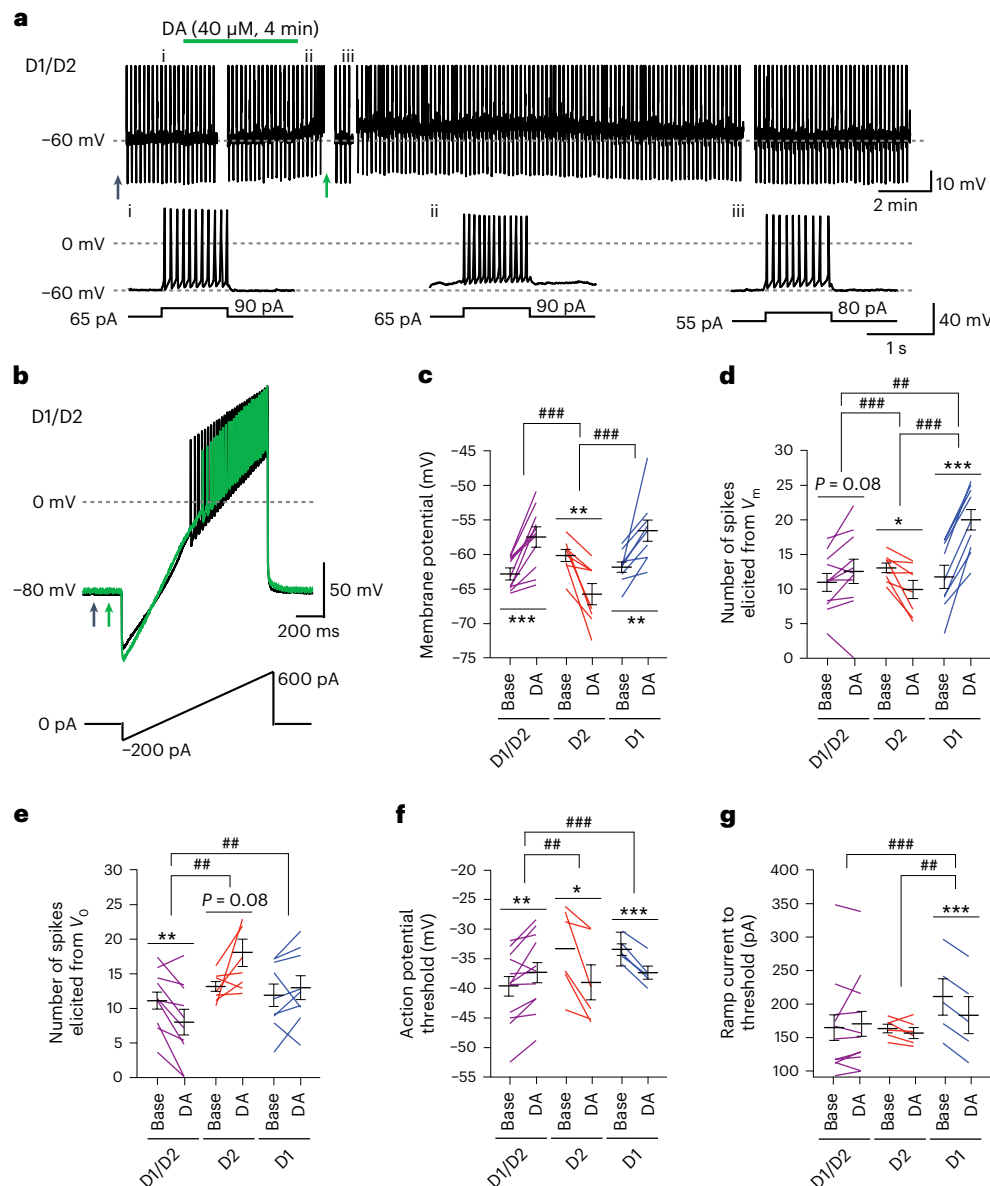


Fig. 5 | Coexpression of D1 and D2 cancels DA-induced changes in excitability.

a, Effect of bath-applied DA (40 μ M, 4 min) on membrane potential and evoked firing activity in one D1/D2-SPN (top) and highlights on voltage traces recorded in response to 25-pA depolarizing current steps at times indicated by i, ii and iii (bottom). **b**, Voltage response in one D1/D2-SPN to ramp current injection before (black) and after (green) DA application at times indicated by the arrows in **a**. **c–e**, Quantification of DA-induced changes in membrane potential (**c**) and in the number of elicited spikes by a depolarizing pulse from the current membrane potential (V_m) (**d**) and from the initial membrane potential (V_0) (**e**) in D1-SPNs ($n = 9$ cells from four mice), D2-SPNs ($n = 8$ (**c**, **d**) or 7 (**e**) cells from four mice)

and D1/D2-SPNs ($n = 11$ (**c**, **d**) or 10 (**e**) cells from four mice). **f**, **g**, DA-induced effects on the action potential threshold (**f**) and the minimal ramp current to elicit discharge (**g**) in D1-SPNs ($n = 5$ cells from four mice), D2-SPNs ($n = 6$ cells from four mice) and D1/D2-SPNs ($n = 12$ cells from four mice) (two-way ANOVA followed by Bonferroni-corrected two-sided paired t -tests between baseline and after DA application (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) and two-sided t -tests comparing the magnitude of DA effect between SPN subpopulations (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)). Data are presented as mean values $\pm$ s.e.m. Detailed statistics are shown in Supplementary Table 1. Source data are provided as a source data file.

remained unchanged (Fig. 5g). These observations highlight the unique role of D1/D2-SPNs, which differentially integrate DA signals potentially through the simultaneous activation of D1Rs and D2Rs.

Loss of D1/D2 coexpression impairs the in vivo DA response

To investigate the role of DA signal integration in D1/D2-SPNs in vivo, we generated a model in which the *Drd1a* gene was deleted selectively in D2-SPNs (D1^{CKO}/D2 mice). As a result, D1^{CKO}/D2 mice have a true engineered two striatal output pathway system, with a direct D1-SPN output and a single indirect striatal output consisting of neurons that express

only D2Rs (Fig. 6a,b). In this model, the D1/D2 output was, therefore, suppressed. We found that this had two clear consequences.

First, D1^{CKO}/D2 mice displayed significantly higher spontaneous locomotor activity (Fig. 6c and Extended Data Fig. 9a), characterized by more time spent in the locomotion state and less immobility than control littermates (D1^{WT}/D2). These effects resulted from a lengthening of locomotion episodes and a decreased frequency of entries into immobility in D1^{CKO}/D2 mice (Fig. 6c and Extended Data Fig. 9a). This difference in locomotion between D1^{CKO}/D2 and D1^{WT}/D2 mice was maintained when the animals received acute doses of psychostimulants: 1, 5 or 10 mg kg^{–1} cocaine (Fig. 6d) and 1 mg kg^{–1} amphetamine

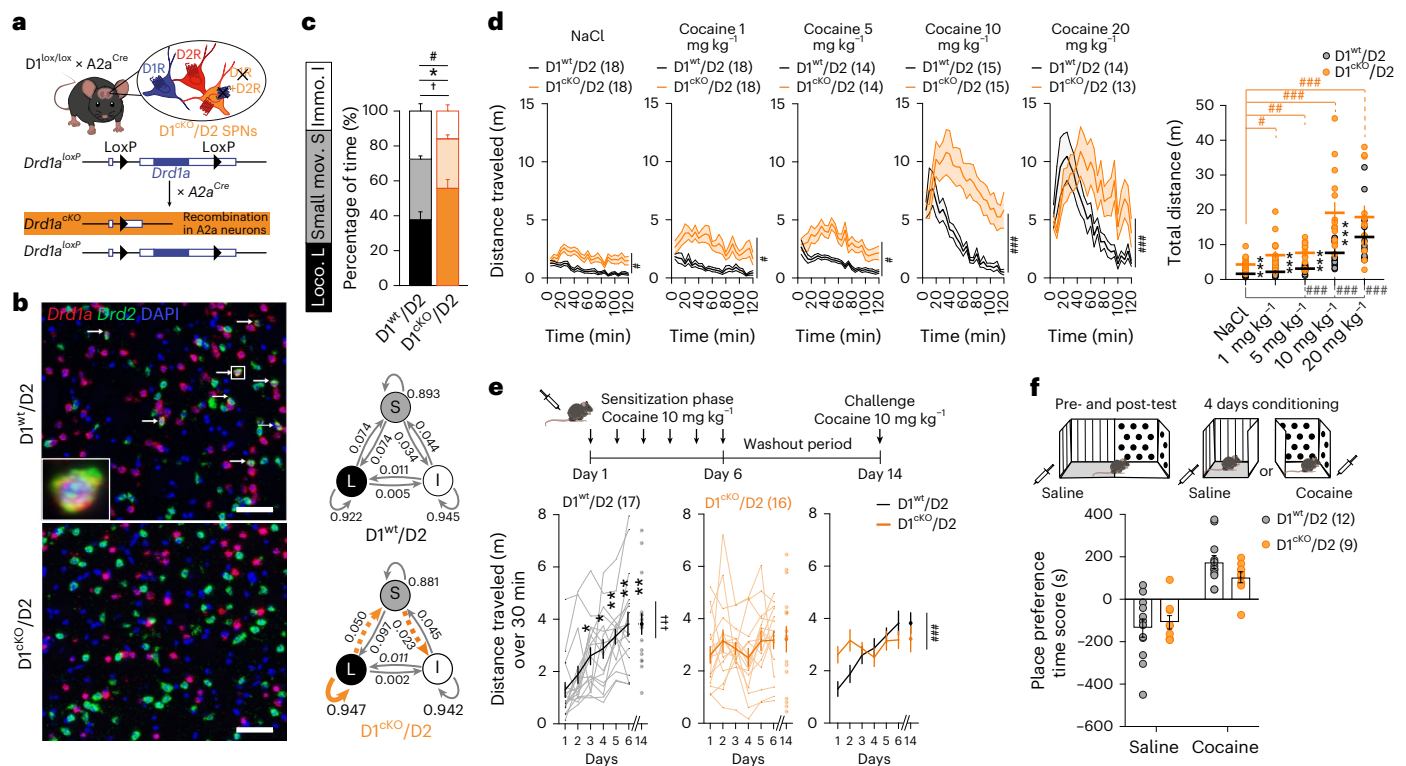


Fig. 6 | D1/D2-SPNs are necessary for proper in vivo integration of DA signals.

a, Diagram illustrating the generation of $D1^{cKO}/D2$ mice (schematic illustrations were created with BioRender.com). **b**, Photomicrographs from RNAscope in $D1^{wt}/D2$ and $D1^{cKO}/D2$ mice illustrating the suppression of DRD1 and DRD2 mRNA coexpression. Arrows show double-positive cells in a representative $D1^{wt}/D2$ mouse, with higher magnification on one of these cells in the square. Scale: 50 μ m. **c**, Comparison of spontaneous motor behavior in an open field between $D1^{wt}/D2$ mice ($n = 12$) and $D1^{cKO}/D2$ mice ($n = 15$) evaluated through the time spent in each motor state (top; two-sided t -tests, $D1^{wt}/D2$ versus $D1^{cKO}/D2$: for locomotion, $^{\dagger}P < 0.05$; for small movements, $^*P < 0.05$; for immobility, $^{\#}P < 0.05$) and the conditional transition probabilities between each motor state (bottom; for the plot corresponding to $D1^{cKO}/D2$ mice, orange solid and dashed lines, respectively, indicate significantly higher or lower probabilities than in $D1^{wt}/D2$ mice). **d**, Temporal distribution of the prolocomotor effect of acute cocaine injection (left plots; two-way ANOVA, genotype $\times$ time interaction; NaCl, $F(23,782) = 1.60$, $^{\#}P < 0.05$; 1 mg kg^{-1} , $F(23,782) = 1.74$, $^{\#}P < 0.05$; 5 mg kg^{-1} , $F(23,782) = 1.79$, $^{\#}P < 0.05$; 10 mg kg^{-1} , $F(23,782) = 4.56$, $^{***}P < 0.001$; 20 mg kg^{-1} , $F(23,782) = 5.39$, $^{***}P < 0.001$)

and comparison of the dose-response relationships of the locomotor effect of cocaine in $D1^{wt}/D2$ mice (NaCl, $n = 18$; 1 mg kg^{-1} , $n = 18$; 5 mg kg^{-1} , $n = 14$; 10 mg kg^{-1} , $n = 15$; 20 mg kg^{-1} , $n = 14$) and $D1^{cKO}/D2$ mice (NaCl, $n = 18$; 1 mg kg^{-1} , $n = 18$; 5 mg kg^{-1} , $n = 14$; 10 mg kg^{-1} , $n = 15$; 20 mg kg^{-1} , $n = 13$) (right plot; Mann-Whitney tests, $D1^{wt}/D2$ versus $D1^{cKO}/D2$ for each dose: $^{***}P < 0.001$; Mann-Whitney tests, dose versus NaCl: $^{\#}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). **e**, Effect of repeated 10 mg kg^{-1} cocaine injections between $D1^{cKO}/D2$ ($n = 16$) and $D1^{wt}/D2$ mice ($n = 17$) on motor activity during 30 min of open field (one-way repeated-measures ANOVA over days in $D1^{wt}/D2$, $^{***}P < 0.001$, and $D1^{cKO}/D2$ mice, not significant; two-way repeated-measures ANOVA, genotype $\times$ days interaction $F(6,186) = 4.67$, $^{***}P < 0.0001$, followed by Sidák's post hoc tests versus day 1: $^*P < 0.05$, $^{**}P < 0.01$) (schematic illustrations were created with BioRender.com). **f**, Conditioned place preference for cocaine (10 mg kg^{-1}) in $D1^{wt}/D2$ mice ($n = 12$) and $D1^{cKO}/D2$ mice ($n = 9$) (two-way ANOVA followed by two-sided Sidák's tests between genotypes: not significant) (schematic illustrations were created with BioRender.com). Data are presented as mean values $\pm$ s.e.m. Detailed statistics are shown in Supplementary Table 1. Source data are provided as a source data file.

(Extended Data Fig. 9b); however, no difference was observed for the highest doses of cocaine or amphetamine tested (Fig. 6d and Extended Data Fig. 9b). Moreover, the temporal integration of the cocaine response differed between $D1^{cKO}/D2$ mice and controls.

Second, compared to control mice, $D1^{cKO}/D2$ mice were characterized by a different pattern of motor adaptive response to the cocaine sensitization paradigm (Fig. 6e). $D1^{wt}/D2$ mice displayed significant motor sensitization to daily administration of cocaine (Fig. 6e and Extended Data Fig. 9c). In contrast, in $D1^{cKO}/D2$ mice, the increase in locomotion induced by cocaine exposure remained similar between days (Fig. 6e and Extended Data Fig. 9c). In addition to reflecting a specific functional role of D1/D2-SPNs, this difference could be due to $D1^{cKO}/D2$ mice not sensitizing to cocaine or due to them already being in a 'presensitized' state. Indeed, following cocaine administration, locomotion was higher in $D1^{cKO}/D2$ mice than in $D1^{wt}/D2$ mice during the first 2 days. During the subsequent days of chronic cocaine treatment, the average locomotor activity was similar between $D1^{cKO}/D2$ and $D1^{wt}/D2$ mice (Fig. 6e). The analysis could also be confounded by the atypical delayed and long-lasting effects following cocaine administration in $D1^{cKO}/D2$ mice (Extended Data Fig. 9c). These findings demonstrate

that D1/D2-SPNs have essential roles in an integrated motor response to DA neurotransmission in collaboration with D1-SPNs and D2-SPNs. Notably, the rewarding effects of cocaine were not affected, as cocaine place preference was maintained in $D1^{cKO}/D2$ mice (Fig. 6f), indicating that the phenotype we observed seems to engage motor circuits rather than reward-related pathways. Altogether, these observations point to an essential role of D1/D2-SPNs in the integration of DA signals and the functioning of the striatum and suggest that the striatum cannot operate properly with only two efferent pathways.

Discussion

Our findings demonstrate that the striatum computes and relays information through three efferent pathways. We highlighted a third population of SPNs in the striatum that is distinguished by the coexpression of D1Rs and D2Rs and that projects specifically to the GPe. These neurons may partially account for the described genetic heterogeneity of SPNs^{22–27}. Accordingly, further functional subdivisions between SPN subtypes may arise by taking into consideration the existence of a genetic distinction between SPNs located in the matrix or striosomes and those located in the medial or lateral subdivisions

of the dorsal striatum^{22,23}. These additional subtypes would likely display unique properties, such as their input–output connectivity or their integration of various neurotransmitter signals that should be linked with specific functions during defined behaviors. Thanks to the comprehensive characterization of genetic markers expressed by SPNs^{22–27}, these issues could be addressed using a combination of multiple genetic markers, as we did in this study, with two-factor intersectional tools. This approach could also be enhanced using three-factor intersectional methods⁴⁴.

Our optogenetic studies revealed that D1/D2-SPNs provide key control over the global functioning of the dorsal striatum to control appropriate transitions between motor states. We observed that D1/D2-SPNs, when coactivated with D1- or D2-SPNs, act as conductors of striatal locomotion and shape the functional identity of D1- and D2-SPNs as described thus far, that is, prokinetic and antikinetic, respectively (Supplementary Note). Our study also revealed that the effects of D1- and D2-SPN excitation on motor states are not consistent with bidirectional control of locomotion; rather, D1-SPN activation induces a locked state in the promotion of small movements instead of locomotion, and D2-SPN activation induces a strong permissiveness to locomotor initiations.

Some of our observations do not entirely align with previously published data. Most of these differences may be due to the photoperiod during which motor behavior is investigated (Supplementary Note). Indeed, mice are nocturnal animals, and their motor behavior is variable depending on the time of the day^{45,46}. In addition, striatal physiology and response to pharmacological agents are affected by the circadian cycle^{47–49}. In our study, we decided to examine motor behaviors during the dark photoperiod, when mice are most active and more prone to engage in locomotion. This higher spontaneous locomotor drive may preclude the observation of higher-magnitude effects of direct pathway stimulation (Supplementary Fig. 1). Unfortunately, it is difficult to compare our results directly to previous data as the photoperiod during which experiments took place is not consistently mentioned (Supplementary Note). On top of the circadian effects, the comparison of different optogenetic stimulation trials in the same mouse suggests that the immediate outcome—in terms of alternation between motor states—can be greatly influenced by the ongoing motor state when stimulations arise. This is evocative of the observations that, for mice freely behaving in an open field, not all direct pathway or indirect pathway stimulations resulted in locomotion or immobility initiations, respectively, and the efficiency of the stimulation could be inferred by the level of activation of SNr neurons¹⁵. These results indicate that the ongoing motor state of the animal is an important determinant of whether the activation of one or the other striatal pathway will be efficient. These effects should be carefully investigated in future work and deciphered according to the activity level of downstream basal ganglia nuclei.

We also found that optogenetic activation of D2- and D1/D2-SPNs increased the animals' speed during locomotion episodes, whereas stimulation of D1-SPNs produced the opposite effect during open-field exploration. In addition to the above-mentioned experimental conditions, we replicated these effects on speed during locomotion episodes in a place preference/aversion paradigm by analyzing behavioral changes occurring in response to optogenetic stimulations delivered in a restricted area of the open field. Such a behavioral response could also be associated with an aversive effect of D2-SPN stimulation and a rewarding effect of D1-SPN stimulation^{41,42}. However, this hypothesis needs to be confronted with recent evidence highlighting dissociable effects between locomotor control and aversion that could be mediated through unique circuits, namely intrastriatal collaterals or projections to the GPe⁴³.

Optogenetics is a powerful tool to dissect neuronal circuits, but it also poses great challenges. Stimulation protocols eliciting a neuronal response should ideally be as close as possible to the physiological one,

which is remarkably difficult, as these are not always characterized. In vivo recordings demonstrate that SPNs are relatively quiet (<5 Hz) and change their firing rate in response to discrete stimuli or actions in less than a second or during a couple of seconds^{50–52}. As a consequence, one may argue that prolonged stimulation can lead to artificial effects not observed in natural conditions (Supplementary Note). It is important to note that optogenetics should be primarily considered a gain-of-function approach attempting to identify the respective contributions of different defined neuronal subpopulations; in addition, it does not allow the naturalistic recruitment of the targeted set of neurons. However, in the context of DA loss or cocaine use, long-lasting excitation or inhibition of some SPNs can occur^{21,53}. Therefore, we believe that additional experiments assessing the activity pattern of each of the three SPN populations using, for example, in vivo electrophysiology coupled with optotagging or in vivo calcium imaging during different behavioral tasks—including self-paced exploration or reinforcement learning—will be essential to understand better the functions of D1/D2-SPNs and how the three subpopulations co-orchestrate behavioral control. These experiments would complement the current understanding of how neurons in the direct and indirect pathways are jointly recruited during specific behaviors^{18–20,54,55}. In addition, evaluating how extracellular DA modulates the dynamics and size of movement-related D1/D2-SPNs would complement the observation that D1- and D2-SPN ensembles are not symmetrically modulated by variations of DA⁵⁶.

We have shown that the disruption of this pathway, which comprises a small subset of SPNs, has major functional consequences for spontaneous behavior and the integration of DA signals. The motor effects we observed in D1^{ckO}/D2 mice are reminiscent of the effects of the optogenetic stimulation of D1/D2-SPNs, especially when considering immobility and small movements that are both reciprocally modulated by gain of function or loss of function of D1/D2-SPNs. The main difference between the two experimental models is found for locomotion, which is unaffected by gain of function but increased by loss of function. This effect could be the consequence of the reorganization of the striatal network that contains only two output pathways in D1^{ckO}/D2 mice. Our optogenetic experiments revealed that coactivation of D1/D2-SPNs and D2-SPNs blocked a prokinetic effect induced by stimulation of D2-SPNs only. In D1^{ckO}/D2 mice, in the absence of D1/D2-SPNs, this blockade of a prokinetic effect of D2-SPNs when active would be prevented, thus potentially leading to an increase in locomotion. Moreover, the striking difference in psychostimulant effects in D1^{ckO}/D2 mice clearly demonstrates that, under normal conditions, DA activation of D1Rs in hybrid D1/D2-SPNs has opposite effects to D2R-induced facilitation of indirect D2-SPN activity in inhibiting locomotion. In D1^{ckO}/D2 mice, this motor inhibition could no longer be effective, but a more prominent locomotor behavior is observed, and the effects of cocaine are enhanced (or no longer buffered). This is a direct consequence of the unique electrophysiological properties of D1/D2-SPNs. Indeed, although D1/D2-SPNs displayed DA-induced depolarization following D1R activation, their firing rate remained unchanged. Therefore, we postulate that when extracellular DA increases, and upon glutamate activation by cortico-striatal and thalamo-striatal principal neurons, D1/D2-SPNs remain active, in contrast to D2-SPNs whose excitability is reduced by DA⁵⁷. D1/D2-SPNs would thus promote the activation of the indirect pathway, resulting in motor inhibition. Consequently, we hypothesize that, by maintaining an adequate inhibitory tone on the GPe, D1/D2-SPNs provide a necessary balance between the direct and indirect pathways to prevent DA-mediated convergent inhibition of the basal ganglia output nuclei, leading to overactivation of the thalamo-cortical systems. Moreover, the effects mediated by D1/D2-SPN activity could also be mediated by collaterals⁵⁸ to other SPNs or by specific targeting of GPe neuronal subpopulations that differentially modulate motor behaviors⁵⁹.

The information carried by individual SPN subpopulations may also be different for specific efferent targets^{43,58}. Therefore, we propose

that D1/D2-SPNs provide a key balance necessary for proper striatal functioning. This balance between D1/D2-, D1- and D2-SPNs is likely formed during embryonic stages and matures during early postnatal development, following a stage in which more SPNs coexpress both D1Rs and D2Rs^{60–62}. Only a fraction of SPNs in the indirect pathway will conserve the expression of both D1Rs and D2Rs, whereas most express only D2Rs, and all SPNs in the direct pathway express only D1Rs. It remains to be determined how these less abundant D1/D2-SPNs interact with and integrate into the other two SPN populations in different areas of the striatum during more elaborate striatal-dependent behaviors⁶³. Improper establishment of this balance or its subsequent dysregulation could lie at the core of certain neurophysiological disorders involving dysregulation of striatal function and, perhaps more preferentially, under conditions of hyperdopaminergic states. Thus, abnormal coordination of the three striatal output pathways, in particular the one formed by D1/D2-SPNs, could be an unsuspected susceptibility risk for psychiatric disorders and could be involved in motor control in neurological disorders such as Parkinson's disease.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41593-024-01694-4>.

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Methods

Generation of transgenic mice

Drd1a-FlpO (D1^{FlpO}) mice. The D1^{FlpO} mouse strain was engineered at the Mouse Clinical Institute (Institut Clinique de la Souris, MCI/ICS, Illkirch, France). Briefly, the FlpO-T2A-GFP cassette and a floxed NeoR/KanR selection cassette were inserted at the ATG site of the D1 DA receptor gene by homologous recombination into the RP24-266B bacterial artificial chromosome (Extended Data Fig. 3c). The modified bacterial artificial chromosome after the deletion of the selection cassette was microinjected into 300 C57BL/6N oocytes, and several founders were obtained. F1 offspring were evaluated for the expression pattern of FlpO (Institut Clinique de la Souris).

Drd1^{loxP/loxP} mice. The floxed *Drd1a* (D1^{loxP/loxP}) mice were produced as follows. The KOMP embryonic stem (ES) cell line Drd1tm1a(KOMP)Wts, clone EPD0507_1_B12, derived on the JM8A3.N1 cell line, was obtained from the KOMP Repository at the University of California, Davis (UC Davis). The ES cells were generated under National Institutes of Health (NIH) grant U01HG004080 by the CSD (CHORI–Sanger–Davis) consortium. Mice were generated from the ES cells by the UC Davis Mouse Biology Program (www.mousebiology.org) using blastocyst microinjection. The resulting chimeras were mated to C57BL/6NTac mice, and heterozygous *tm1a* (KO first) animals were generated through germline transmission. These ES cells are now distributed through the Mutant Mouse Resource and Research Center (MMRRC) at UC Davis, an NIH-funded strain repository (RRID: MMRRC_054799-UCD). For more information on obtaining KOMP products, email mmrrc@ucdavis.edu or go to www.mmrrc.org.

Mouse breeding

The *Adora2a*-Cre (A2a^{Cre}) driver line, which expresses the Cre recombinase selectively in D2-SPNs, thereby excluding cholinergic interneurons³⁵, was crossed with D1^{FlpO} mice (D1^{FlpO}/A2a^{Cre}) to perform intersectional approaches to target the various SPN subsets and was also used for control conditions. Single transgenic A2a^{Cre} and D1^{FlpO} mice were used to assess the functions of the classically characterized D2 and D1 'total' SPN populations, respectively. All mice were of a C57BL/6J genetic background.

Mice lacking the *Drd1a* gene in A2a-expressing neurons (D1^{CKO}/D2) were obtained by crossing heterozygous D1^{loxP/loxP} mice with A2a^{Cre} mice to obtain heterozygous offspring, which thereby produced WT (7 of 16), KO (3 of 16) and heterozygote (6 of 16) mice at the expected ratio. After being weaned and sexed, mice were housed in groups of four to five animals per cage.

For the collection of in vivo optogenetic data, ex vivo electrophysiology and FISH experiments, 8- to 10-week-old male and female mice were used. Experiments related to D1^{CKO}/D2 KO also used both males and females. Two to four mice were housed together in polycarbonate cages at constant temperature (21 ± 1 °C) and humidity (40–50%) under a 12-h light/dark cycle (lights off at 8 a.m.). Mice were provided with food and water ad libitum.

For experiments performed in Belgium, animals were treated according to protocols and guidelines approved by the Institutional Animal Care Committee, and experimental procedures were approved by the local ethics committees (ULB Pôle Santé, protocol 695N). For experiments performed in France, experimental procedures were approved by the Regional Ethics Committee No. 3 of the Ile-de-France region on Animal Experiments and followed the guidelines of the European Communities Council Directive (86/809/EEC) and the Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale (agreement A 94-028-21). For experiments performed in Canada, animal housing, breeding and care were operated in accordance with the Canadian Council on Animal Care guidelines, and all methods were approved by the Animal Care Committee from the Douglas Institute Research Center (protocol no. 5570). All methods were performed in accordance with the relevant guidelines and regulations.

Double-probe FISH

Sample collection. Mice were killed by cervical dislocation. The brains were removed, rapidly frozen in cold isopentane (between –30 and –35 °C) and serially sectioned in ten series on a cryostat at 16-μm thickness. Brain sections were processed by in situ hybridization to allow a systematic quantitative analysis throughout the whole dorsal and ventral striatum. Double-probe FISH was performed as previously reported⁶⁴.

DNA template for riboprobe synthesis. DNA templates with T3 and T7 promoter sequences on both sides of each cDNA target were produced by PCR using promoter-attached primers.

Probes. Double-probe FISH was performed using antisense riboprobes to detect the following mRNAs: D1, [NM_010076.3](#) sequence 1756–2707 and D2, [NM_010077.3](#) sequence 268–1187. Digoxigenin (DIG)- and fluorescein-labeled RNA probes were synthesized by a transcriptional reaction with incorporation of DIG- or fluorescein-labeled nucleotides (Sigma-Aldrich; reference nos. 11277073910 and 11685619910). Information on primer sequences for riboprobe synthesis is listed in the table below. The specificity of probes was verified using the National Center for Biotechnology Information BLAST (Basic Local Alignment Search Tool) application.

PCR primer name	PCR primer sequence	NM	Bases
mDrd1-S-T3	AATTAACCCTCACT AAAGGGATGGAGAA GCTGTCCCCGGCCT	NM_010076.3	1756–2707
mDrd1-AS-T7	TAATACGACTCACTAT AGGGCCTCGGGGA AAGCCTCTGCC		
mDrd2-S-T3	AATTAACCCTCACTAA AGGGAGGAAGCCTCG AGCAGCCGGC	NM_010077.3	268–1187
mDrd2-AS-T7	TAATACGACTCACTAT AGGGCTCCTGAGCTC GGCGGGCAG		

Procedure. Cryosections were air-dried, fixed in 4% paraformaldehyde (PFA) and acetylated in 0.25% acetic anhydride/100 mM triethanolamine (pH 8), followed by washes in PBS. Sections were hybridized for 18 h at 65 °C in 100 μl of formamide buffer containing 1 μg ml^{–1} DIG-labeled riboprobe and 1 μg ml^{–1} fluorescein-labeled riboprobe. Sections were washed at 65 °C with SSC buffer of decreasing strength and blocked with 20% FBS and 1% blocking solution. For revelation steps, DIG epitopes were detected with horseradish peroxidase (HRP) anti-DIG fab fragments at 1:2,500 (Sigma-Aldrich; reference no. 11207733910) and revealed using Cy3-tyramide at 1:100. Fluorescein epitopes were detected with HRP anti-fluorescein fab fragments at 1:5,000 (Sigma-Aldrich; reference no. 11426346910) and revealed using Cy2-tyramide at 1:250. Cy2-tyramide and Cy3-tyramide were synthesized as previously described⁶⁵. Nuclear staining was performed with 4',6-diamidino-2-phenylindole (DAPI). All slides were scanned at 20× resolution using NanoZoomer 2.0-HT (Hamamatsu). Laser intensity and time of acquisition were set separately for each riboprobe.

Automated quantitative analysis. Ten sections per brain in a total of three animals were selected for quantification. The dorsal and ventral parts of the striatum were differentiated for the quantification. A CellProfiler pipeline⁶⁶ was developed to analyze imaging data. Signal segmentation was performed through the recognition of objects based on the size, shape, intensity and texture of the signal. All sections were treated with the same segmentation. Cell segmentation was performed using DAPI staining to delineate the cell surface area.

We used the 'Identify Primary Object' module in CellProfiler to identify DAPI-positive cells, with the following parameters: typical diameter of object (15–40 pixels), adaptive threshold strategy, two-class Otsu thresholding method, and clumped object distinction based on the shape and minimal distance between local maxima of 5 pixels. Next, we established a pixel median threshold value in each fluorescent canal: red (D1 mRNA) and green (D2 mRNA). D1- and D2-positive cells were considered with a median pixel intensity of >0.25 and >0.10 , respectively. We then performed an automated quantitative analysis in which neurons were separated into cells expressing D1 mRNA only (DAPI + red), D2 mRNA only (DAPI + green) and both D1 and D2 mRNA (DAPI + red + green) in the dorsal and ventral striatum.

RNAscope FISH

Frozen brain samples were serially cut using a cryostat (10 μm in thickness) on superfrost-charged slides and kept at -80°C until further processing. In situ hybridization was performed using RNAscope probes and reagents (Advanced Cell Diagnostics) following the manufacturer's instructions. Briefly, sections were first fixed in chilled 10% neutral buffered formalin for 15 min at 4°C , dehydrated in increasing gradients of ethanol bath (50%, 70%, 95%, 100%) and left to air-dry for 5 min. Endogenous peroxidase activity was quenched with hydrogen peroxide reagent for 10 min, followed by protease digestion for 10 min at room temperature. The following sets of probes were then hybridized for 2 h at 40°C in a humidity-controlled oven (HybEZ II, ACDbio): MsDrD1 (cat. no. 461901) and MsDrD2 (cat. no. 406501-C2) to quantify D1/D2 coexpression. Successive addition of amplifiers was performed using the proprietary AMP reagents, and the signal was visualized through probe-specific HRP-based detection by tyramide signal amplification with Opal dyes (Opal 520 and Opal 570, Perkin Elmer) diluted 1:300. Slides were then coverslipped with Fluoromount-G mounting medium with DAPI for nuclear staining (Invitrogen Fisher, cat. no. 501128966) and kept at 4°C until imaging.

Virus-mediated targeting of channelrhodopsin and eYFP expression

We used an intersectional optogenetic viral strategy to trace, record from and control each SPN population, involving the stereotaxic injection into D1^{FlpO}/A2a^{Cre} mice of INTRSECT viruses³³ transfecting cells with eYFP-tagged ChR2(H134R), or eYFP alone for ex vivo recordings and control mice, in a manner conditional on the presence of Cre and/or FlpO in three configurations: Coff/Fon, Con/Foff and Con/Fon, targeting D1-, D2- and D1/D2-SPNs, respectively (AAV(DJ)-nEF-ConFon/Coff-Fon/ConFoff-hChR2(H134R)-eYFP, AAV(DJ)-hSyn-ConFon/CoffFon/ConFoff-eYFP; some aliquots were kindly provided by C. Ramakrishnan and K. Deisseroth of Stanford University, and most viral vectors were packaged and provided by the University of North Carolina (UNC) Vector Core facility). The virus injected was randomized between D1^{FlpO}/A2a^{Cre} mice. To compare the ChR2-induced effects in response to photostimulations in these three exclusive SPN populations to those classically described in mixed direct D1-expressing SPNs (combining D1- and D1/D2-SPNs) and mixed indirect D2-expressing SPNs (combining D2- and D1/D2-SPNs), we also expressed ChR2 in D1^{FlpO} and A2a^{Cre} mice using the FRT-ChR2 and lox-ChR2 constructs, respectively (AAV(DJ)-EF1a-fDIO-hChR2(H134R)-eYFP, AAV(5)-EF1a-DIO-hChR2(H134R)-eYFP; UNC Vector Core).

At 8–10 weeks old, mice used for tracing, quantification of virus-transfected cell densities, assessment of transfection specificity (Extended Data Fig. 1f) and in vivo experiments, or at approximately 6 weeks old, mice used for ex vivo experiments were anesthetized with isoflurane (4% induction, 1% maintenance: $0.5\text{ l min}^{-1}\text{ O}_2$). The recombinant adeno-associated virus (AAV) listed above was bilaterally injected (at $2 \times 1\text{ }\mu\text{l}$) under stereotaxic control into the dorsal striatum (anteroposterior +0.8 mm, mediolateral 1.5 mm and dorsoventral -2.7 mm relative to the bregma) through a 30-gauge cannula at a rate

of 100 nl min^{-1} . The injection needle was lowered through a hole drilled lateral to the midline (anteroposterior +0.8 mm, mediolateral 1.5 mm). Mice were allowed to recover for 10 days before being habituated to patchcord connections, and experiments started at least 3 weeks after viral injections.

To quantify the densities of cells transfected with the eYFP virus, we automated the quantification of positive cells (cells pointed with a red dot, Extended Data Fig. 1g,h), nuclei and region-of-interest areas by using in-house Python scripts. To minimize false positives, we eliminated detected cells with lower central fluorescence compared to their periphery (cells pointed with a blue dot). For this, the cell's central region was defined as a circle with a radius equal to one-fourth of the cell's diameter, and the average fluorescence in this region was compared to the average fluorescence in the rest of the cell.

Ex vivo electrophysiology experiment recording and analysis

Experiment preparation. In preparation for electrophysiology, brain slices containing the dorsal striatum were obtained from mice injected as described above. The animals were decapitated under deep anesthesia, and their brains were quickly removed. Coronal slices (250 μm in thickness) were cut with a vibrating microtome (VT1000S, Leica) in ice-cold (4°C) slicing artificial cerebrospinal fluid (ACSF) containing 140 mM choline chloride, 2.5 mM KCl, 1.25 mM NaH_2PO_4 , 7 mM MgCl_2 , 0.5 mM CaCl_2 , 25 mM NaHCO_3 and 14 mM D-glucose, pH 7.3, and constantly oxygenated (95% O_2 /5% CO_2). Slices were transferred to an incubation chamber filled with ACSF containing 124 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4 , 2 mM CaCl_2 , 1 mM MgCl_2 , 26 mM NaHCO_3 and 10 mM D-glucose. Slices were incubated for 1 h at 34°C and then maintained at room temperature.

Whole-cell recordings. Slices were placed on the stage of an Axio Examiner A1 microscope (Carl Zeiss) equipped with an infrared CCD camera (ORCA-05G, Hamamatsu) and visualized under infrared illumination. Slices were maintained immersed and continuously superfused at $2\text{--}5\text{ ml min}^{-1}$ with oxygenated ACSF. Neurons in the dorsal striatum were selected based on eYFP expression using 470-nm LED light (OptoLED, Carin Research) visualized through a FITC/Cy2 filter set (Carin Research). All electrophysiological experiments were performed with an EPC-10 patch-clamp amplifier (HEKA) attached to a computer running PatchMaster software (HEKA). Signals were low-pass filtered at 2.9 kHz and digitized at 20 kHz. Whole-cell patch-clamp recordings were performed with patch micropipettes (6–8 M Ω) pulled from borosilicate glass capillary tubes (1.5-mm outer diameter, 0.225-mm wall thickness; Hilgenberg) on a vertical puller (PIP5, HEKA) filled with 8 μl of an internal solution containing the following: 105 mM potassium gluconate, 30 mM KCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.3 mM ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 10 mM Na_2 -phosphocreatine, 4 mM Mg_2 -ATP, 0.3 mM Na_2 -GTP, pH 7.4, 290–300 mOsm, and 3 mg ml^{-1} biocytin. For ex vivo optogenetic stimulation, pulses of blue light (470 nm) were delivered using LEDs (OptoLED, Carin Research) controlled by PatchMaster (HEKA) through the EPC-10 patch-clamp amplifier (HEKA).

Recording analysis. Data analysis was performed blind to the identity of the neuron using Neuromatic (version 2.001) running under Igor Pro 6 (WaveMetrics). The intrinsic membrane properties of neurons were assessed by applying current steps (1 s) from -500 pA to firing saturation in 10-pA increments. To describe the electrophysiological properties of the recorded SPNs comprehensively, we measured 30 parameters reflecting the SPN properties^{36–38,67} on traces corresponding to the voltage responses induced by hyperpolarizing and depolarizing current pulses. These parameters follow the Petilla nomenclature⁶⁸. (1) Resting membrane potential (RMP) was measured immediately after electrical access to the intracellular compartment of the cell. (2) Input

membrane resistance (R_m), (3) membrane time constant (τ_m) and (4) membrane capacitance (C_m) were determined in response to hyperpolarizing current pulses resulting in voltage drops of 10–15 mV relative to rest. R_m was measured following Ohm's law, and τ_m was computed as the time constant of a single exponential fit of the voltage response from onset to maximum hyperpolarization. C_m was obtained using the formula $C_m = \tau_m/R_m$. Some neurons have been described to undergo partial repolarization following a hyperpolarization peak in response to negative currents. To quantify this, we measured whole-cell conductance when the sag conductance was inactive (G_{hyp}) or active (G_{sag}). G_{sag} was measured as the slope of the linear portion of a current–voltage (I – V) plot in which V was determined at the end of 1-s hyperpolarizing current pulses (–500 to 0 pA). G_{hyp} was measured as the slope of the linear portion of the I – V plot in which V was determined as the maximal negative potential during the 1-s hyperpolarizing pulses. (5) The sag index (Sag) was calculated as a relative decrease in membrane following $(G_{sag} - G_{hyp})/G_{sag}$. (6) The step intensity to the first spike (I_{th}) was evaluated as the minimal intensity of the 1-s current pulse that elicited an action potential. As SPNs display a delayed firing supported by I_A currents, (7) the first spike latency (lat) was computed as the time needed to elicit a spike after the onset of a current pulse corresponding to the step intensity to the first spike. (8) The action potential threshold (V_{th}) was measured as the potential of the first spike onset when the rate of membrane potential change exceeded 30 mV ms^{-1} . To describe the firing behavior of the recorded SPNs around the threshold, we measured the instantaneous firing frequency in response to the minimal current injection eliciting more than three action potentials and fitted it to a linear curve with (9) adaptation (A_{th}) corresponding to the slope of the linear fit and (10) minimal steady-state frequency (F_{min}) corresponding to its y-intercept ($F(t) = A_{th} \times t + F_{min}$). Then, to evaluate the change in firing activity in relation to increased depolarizing current steps, we calculated (11) the slope of the current–frequency relationship (I – f slope) in its initial linear portion. (12) The maximum step intensity before the depolarization block (I_{sat}) was defined as the maximal 1-s current pulse eliciting robust firing activity without inducing firing saturation. At high firing frequencies, neurons display spike frequency adaptation, which is characterized by an early exponential decrease in spike frequency followed by a linear decrease. To quantify this adaptation, we fitted the instantaneous firing frequency during the train of spike induced by the maximal depolarizing current injection not inducing saturation to a decaying single exponential and to a linear function using the formula $F(t) = A_{sat} \times \exp(-t/\tau_{sat}) + m_{sat} \times t + F_{max}$ (ref. 69), with the fitting parameters defined as the (13) amplitude of early adaptation (A_{sat}), (14) time constant of early adaptation (τ_{sat}), (15) slope of late adaptation (m_{sat}) and (16) maximal steady-state frequency (F_{max}). To evaluate firing adaptation at an intermediate level of activation, we measured (17) spike frequency adaptation (SFA) as the ratio between the instantaneous firing frequency between the first two spikes over the instantaneous firing frequency over the last three spikes on traces corresponding to the depolarizing current injection eliciting a sustained action potential train of about 20 spikes. Spike waveforms have been described to vary between neuronal cell types. To quantify these variations, we measured six parameters on the first and second spikes of traces corresponding to the depolarizing current injection eliciting a sustained action potential train of about 20 spikes. (18, 19) The amplitudes of the first ($A1$) and second ($A2$) action potentials were defined as the difference in membrane potential between their onsets and peaks. (20, 21) The spike durations of the first ($D1$) and second ($D2$) spikes were defined as the spike widths between spike onset and spike offset. (22, 23) The spike half-widths of the first ($W1$) and second ($W2$) spikes were defined as the spike width at half amplitude. (24, 25) The afterhyperpolarization potential (AHP) maxima (AHP1 and AHP2) were calculated using the membrane potential at the onset of the action potential as a reference. Comparatively, (26, 27) AHP latencies (tAHP1 and tAHP2) were calculated as the time interval between

the AHP maximum and the onset of the action potential. During a spike train, spikes can display variations in their amplitude and duration. To quantify this phenomenon, we computed the (28) amplitude reduction (A.Red), (29) duration increase (D.Inc) and (30) half-width duration increase (W.Inc) as $(A1 - A2)/A1$, $(D2 - D1)/D1$ and $(W2 - W1)/W1$, respectively.

Pharmacology. The following drugs were used: DA HCl (40 μM , Sigma-Aldrich), sulpiride (10 μM , Sigma-Aldrich) and SCH23390 (10 μM , Sigma-Aldrich). Stock solutions of these compounds were stored as frozen aliquots at -20°C and dissolved in ACSF to their working concentrations immediately before application. Pharmacological experiments were carried out in the current-clamp mode. Neurons were depolarized to approximately -60 mV by injecting a holding current. Changes in membrane voltage were measured at this holding current. To elicit firing activity and quantify membrane resistance, we stimulated neurons using a 1-s depolarizing pulse set to elicit 10–15 action potentials. After being held for 1 s at the holding current, neurons were stimulated with a -50 -pA hyperpolarizing pulse from the holding current lasting 500 ms. This stimulation protocol was repeated every 10 s throughout the recording. Moreover, a current ramp (1 s from -200 to 600 pA) was applied during baseline and at the peak of pharmacological effect to extract the action potential threshold and current to the first action potential as well as to interpolate current–frequency curves. Recordings were discarded if access resistance changed by more than 20% throughout the experiment. Results are not corrected for liquid junction potential.

Three-dimensional reconstruction of biocytin-filled SPNs

Brain slices containing SPNs filled with biocytin were incubated overnight at 4°C in PFA solution (4% PFA in 0.01 mM PBS). The slices were then washed in PBS and permeabilized in PBS with 0.1% Triton X-100 (Sigma-Aldrich). The slices were incubated with streptavidin-coupled Alexa Fluor 488 (1:200, Jackson ImmunoResearch) for 2 h at room temperature under constant agitation. After being washed in PBS, the slices were mounted with FluorSave medium (Fisher Scientific) between the slide and the coverslip (VWR).

Serial optical sections in the x , y and z planes (Z -stacks) were acquired on a laser-scanning confocal system (LSM 510, Zeiss) mounted on an Axiovert 200M inverted microscope (Zeiss) and equipped with an LD C-Apochromat $40\times/1.2 \text{ W}$ objective (Zeiss). The excitation beam of an argon laser (488 nm) was used, and fluorescence was measured between 500 and 550 nm for the detection of the Alexa 488 fluorophore. Once acquired, ZEN software (Zeiss) was used to stitch the images. Manual reconstruction of the dendritic arborization of the neurons was done with the help of the computer filament tracing function in the three-dimensional image analysis software Imaris (Bitplane). Total dendritic arborization reconstruction allowed the study of dendrite branching and length, as well as Sholl analysis. The soma volume was automatically measured by creating a surface that delineated the soma region.

Optic fiber implantation for in vivo experiments

At 8–10 weeks old, within the same surgery following virus injection, mice subjected to in vivo experiments were bilaterally implanted with homemade optic fiber implants. A cleaved piece of optic fiber $\sim 12 \text{ mm}$ in length (400 μm in diameter, numerical aperture 0.39; Thorlabs) was inserted and glued into a metal ferrule (1.25 mm in diameter; Thorlabs). Optic fiber implants that were inserted were verified before implantation to transmit at least 80% light illumination. During the surgery, before the viral injection, the skull was completely cleared of all connective tissue and thoroughly dried using alcohol. To insert the optic fiber implant, we drilled a hole on the skull at the following coordinates: anteroposterior $+0.9 \text{ mm}$ and mediolateral 2.17 mm relative to the bregma. After gently cutting through the dura, we lowered

the implant through the hole at an angle of 10° with the optic fiber end location targeted 200 μm above the injection site in the dorsal striatum. Following optic fiber placement, dental cement (Metabond) was applied to fix the implant permanently to the skull.

Rotational behavior

Animals were placed in circular chambers (17 cm in diameter) to examine rotational behavior under unilateral stimulations. A camera (Basler ace acA1600-60gc) was placed above the chambers to record videos at 30 frames per second, and rotations were scored manually. Rotations were defined as each 360° rotation that contained no turn of more than 90° in the opposite direction. We analyzed them over three periods—the ‘Pre’ period, which corresponded to 60 s preceding laser illumination; the ‘ON’ period, which corresponded to constant 60-s unilateral laser illumination (473 nm, Laserglow); and the ‘OFF’ period of 60 s at the end of laser illumination. Both right and left hemisphere laser illuminations were performed in a randomized manner in two distinct trials separated by a 1- to 2-h interval period during which mice returned to their home cage. Data collection was performed blind to the identity of the SPN population targeted.

Open-field measures

The open field was a white square enclosure of 40 × 40 × 40 cm where animals could move freely and be video-monitored from above (Basler ace acA1600-60gc; sampling rate, 30 frames per second). All behavioral experiments were performed during the dark photoperiod between 9 and 11 a.m. Detection and calculation of the animal's center of mass coordinates were performed using EthoVision XT 14.0 software (Noldus). Data collection was performed blind to the identity of the SPN population targeted. The animal's speed was calculated from changes in the center of mass coordinates between two successive frames and smoothed (moving average, 0.5-s width) to reduce noise and tracking artifacts. The animal's behavior in the open field was then classified into three motor states: locomotion when velocity reaches 5 cm s^{-1} and remains above 2 cm s^{-1} for at least 0.5 s, immobility when velocity is below 0.5 cm s^{-1} for at least 0.5 s, and small movement when velocity lays between 0.5 and 2 cm s^{-1} . Any unassigned bout (lasting shorter than 0.5 s) is affected for its first half to the previous state and for its second half to the next state. Averaged temporal evolutions of mouse velocity and acceleration were examined around locomotion onset, and measures of averaged velocity and top velocity (ninth decile) were analyzed only during locomotion episodes.

Animals were recorded for a 10-min reference baseline period before any laser illumination (‘Pre’ period). Mice exhibiting a biased low or high baseline activity, measured as distance traveled below 1,700 cm or above 4,500 cm over the 10-min baseline, were excluded from analysis. Then, the laser was illuminated in a series of ten trials. Each trial lasted 30 s with constant illumination (ON period), followed by a 90-s period during which the laser was turned off (OFF period). The total time spent in each motor state, the frequency and the mean episode duration of each state were calculated over the 10-min baseline (Pre) and over the average of the ten trials for the ON and OFF periods. Other stimulation patterns were also tested: (1) continuous 5 Hz for 30 s (mid-frequency tonic activation; 10 trials with 90-s intervals), (2) 20 Hz for 500 ms (phasic activation, 20 trials with 30-s intervals) and (3) continuous 20 Hz for 30 s (high-frequency tonic activation, 4 trials with 90-s intervals).

For optogenetic real-time place preference, the open-field arena was divided into two equal zones (20 × 40 cm). After a 10-min reference baseline period, a 20-min optogenetic stimulation period started with the online-tracked mouse body center triggering a closed-loop optogenetic stimulation restricted in one of the two zones. The stimulation period was followed by a 10-min poststimulation period. Place preference was evaluated as the proportion of time spent in the stimulated zone during baseline (Pre) and during the stimulation period (ON). Mice

that displayed a biased preference for the stimulated area during baseline above 65% or below 35% were excluded from subsequent analyses.

To better characterize how each SPN population activation affects motor state dynamics, we computed transition probabilities between states using a Markov chain model from time series of motor states resampled at 5 Hz. Briefly, for each period (Pre, ON, OFF) defined above and for each mouse, we calculated the transition probability from state X to Y ($P(Y|X)$) as the number of time bins in state X immediately followed by state Y over the total number of time bins in state X .

To catch supplementary information on the dynamics of motor states during laser illumination, we also computed probabilities to observe motor states for all stimulation trials. The 95% CIs for motor state probability were calculated using bootstrap from time series of motor states resampled at 10 Hz. For each experimental group, we repeatedly resampled data aligned to laser illumination (from 30 s before laser onset to 30 s after laser offset) by randomly generating the same number of total trials (random sampling with replacement). For each of the 10,000 random iterations, we calculated the mean probability to observe each motor state across trials. The lower and upper CIs were obtained for each time point from the distribution of resampled mean values. The 95% CIs for changes in probabilities were obtained the same way by calculating the difference in mean probabilities during early (from 0 to 5 s after laser onset), intermediate (from 10 to 15 s after laser onset) and late (from 20 to 25 s after laser onset) periods of laser illumination minus the mean probabilities before laser illumination (over 10 s before laser onset). Chance levels for changes in probabilities were calculated the same way by selecting a random time point for trial onset. The 95% CIs for actual trials lying outside the 95% CI for chance were considered significantly different. Significance levels for the computation of CIs were corrected for multiple comparisons using Šidák correction.

For open-field experiments performed on $D1^{cKO}/D2$ mice and their WT littermates, mice were placed in the open field and video-tracked for 60 min.

All these analyses were performed using MATLAB (MathWorks).

Histological confirmation of optic fiber placement and construct expression

Following the completion of all behavioral optogenetic experiments, mice were deeply anesthetized with one injection of avertin (2,2,2-tribromoethanol 1.25%, 2-methyl-2-butanol 0.78%; 20 $\mu\text{l g}^{-1}$, intraperitoneal, Sigma-Aldrich). Mice were then perfused transcardially with 1× PBS, pH 7.4, followed by 4% PFA in PBS. The brains were then extracted and postfixed overnight in PFA at 4 °C and subsequently cryoprotected in 30% sucrose dissolved in PBS for an additional 24 h at 4 °C. Each brain was then frozen and sectioned at 40 μm using a cryostat. To confirm construct expression and optic fiber placement, we first washed 1:3 sections over the entire striatum in PBS 1×–Triton (0.3%) (PBST), incubated in blocking solution (10% donkey serum in PBST) for 1 h at room temperature and subsequently incubated for 1 h with GFP boosters (ATTO488, Chromotek) diluted 1:400 in 1% donkey serum. Sections were then counterstained for 10 min with the chromosomal dye Hoechst 33258 (prepared in 1× PBS at 1:10,000) and then mounted on glass slides and permanently coverslipped with Fluoromount. Fluorescent images were collected using a fluorescent microscope (V16, Zeiss). Only mice with histologically confirmed bilateral correct placement of the optic fiber and with proper construct expression in the dorsal striatum were used in the present study.

Psychostimulant dose–response and behavioral sensitization

Locomotor response to acute psychostimulant treatment was assessed by intraperitoneal administration of a single dose of amphetamine HCl (0, 1 and 3 mg kg^{-1} in 0.9% saline) or cocaine HCl (0, 1, 5, 10 and 20 mg kg^{-1} in 0.9% saline) to $D1^{cKO}/D2$ mice and their WT littermates. Mice were randomly assigned to drug doses. The test environment

consisted of transparent activity boxes (VersaMax automated activity monitor, AccuScan Instruments) measuring 42 cm × 42 cm × 30 cm. Mice were first habituated to the environment by being placed in the activity boxes for 20 min daily for three consecutive days. Acute injections were made on day 4, in which mice were individually placed in activity boxes for 15 min and, after injection, were immediately put back into the boxes for an additional 120 min. Locomotor activity was recorded in 5-min intervals.

To induce behavioral sensitization, D1^{CKO}/D2 mice and their WT littermates received daily administration of cocaine (10 mg kg⁻¹) for six consecutive days. On day 14, a challenge dose of cocaine (10 mg kg⁻¹) was administered to both groups. Injections were made 15 min after placing the mice in the monitor boxes, and locomotion recording continued for 120 min after cocaine administration.

Conditioned place preference

The Pavlovian conditioning protocol, using distinctive floor textures as conditioned stimuli, was adapted from Cunningham et al.⁷⁰. The apparatus consisted of a plexiglass box divided into two compartments (5 cm wide, 15 cm long, 15 cm high) with interchangeable floors made of either stainless steel or plastic. Each apparatus was equipped with photocell beams allowing the automatic recording of the animal's position and horizontal activity. The experiment involved three phases during which each animal was weighed and injected immediately before being placed in the apparatus. (1) During the habituation session, each animal received saline and had free access to both compartments with distinctive floors for 30 min. (2) The conditioning phase (eight sessions) lasted four consecutive days, with two 30-min trials a day. Mice were restricted to a single compartment, with the less preferred floor paired four times with cocaine (10 mg kg⁻¹, 10 ml kg⁻¹, intraperitoneal) and the other floor texture paired four times with saline. (3) Place preference was assessed 24 h after the last conditioning trial. All animals received a saline injection before being exposed to both compartments with distinctive floors for 30 min. The cocaine-conditioned preference was determined by comparing the time spent in the cocaine-associated box after versus before chronic injection. Data collection and analysis were performed blind to the animals' genotype.

Statistical analysis

No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous similar publications^{15,35,39,43,67}. Statistical analysis was performed using GraphPad Prism 7.0e and MATLAB. All data are presented as mean ± s.e.m. unless explicitly stated otherwise. Statistical significance was set at 0.05 for all procedures. Normality and homoscedasticity assumptions were verified before the use of any parametric tests (Shapiro–Wilk normality test and equality of variance *F* test). In case of violation of any of these assumptions, standard transforms were applied to meet the above criteria. Statistics were performed with repeated-measures two- or three-way ANOVAs to test the significance between experimental groups (genotype) and stimulation conditions (in the case of optogenetics) or groups (floxed and WT littermates), drugs and time. Details of the statistical tests are available in the statistical table in Supplementary Table 1. Cell count and in vivo analyses were performed by researchers blinded to genotype and experimental conditions.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data supporting the findings of this study are available within the article and its supplementary materials and are available from the corresponding authors upon request. Source data are provided with this paper.

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Author contributions

Conceptualization: B.G., P.B. and A.d.K.d'E. Design and investigation for optogenetic experiments: P.B. with support from P.M.O. Methodology and investigation for motor analysis: P.B., C.V. with support from A.C. Methodology and investigation for electrophysiological experiments: C.V. Investigation for three-dimensional reconstruction: A.D.G. Investigation for behavioral experiments on D1^{CKO}/D2: G.F., Q.R. with support from K.X. and E.I. Analysis: P.B. and C.V. Investigation for FISH experiments: S.D. with support from A.C. for automated analysis. Investigation for viral-based

cell quantification and tracing: P.B. in collaboration with A.C. and R.L.L. (automated analysis), with support from E.P.F. (viral injections). Supervision for the animal colonies: E.V. Resources for the D¹^{Cre} strain: E.T. Formal analysis: P.B. and C.V. Validation and supervision: P.B., B.G. and A.d.K.d'E. Writing: P.B., C.V., A.d.K.d'E. and B.G.

Competing interests

The authors declare no competing interests.

Additional information

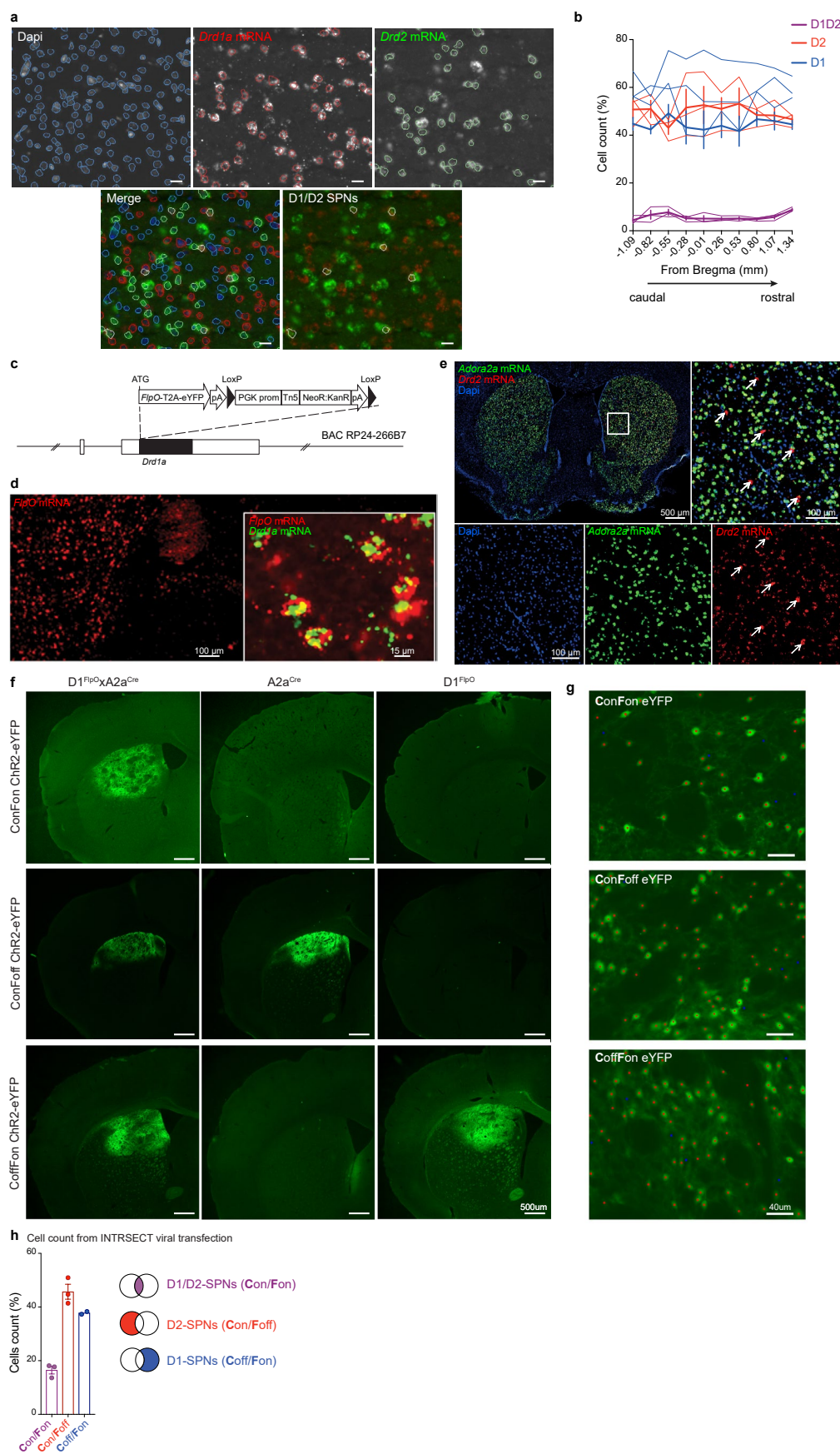
Extended data is available for this paper at <https://doi.org/10.1038/s41593-024-01694-4>.

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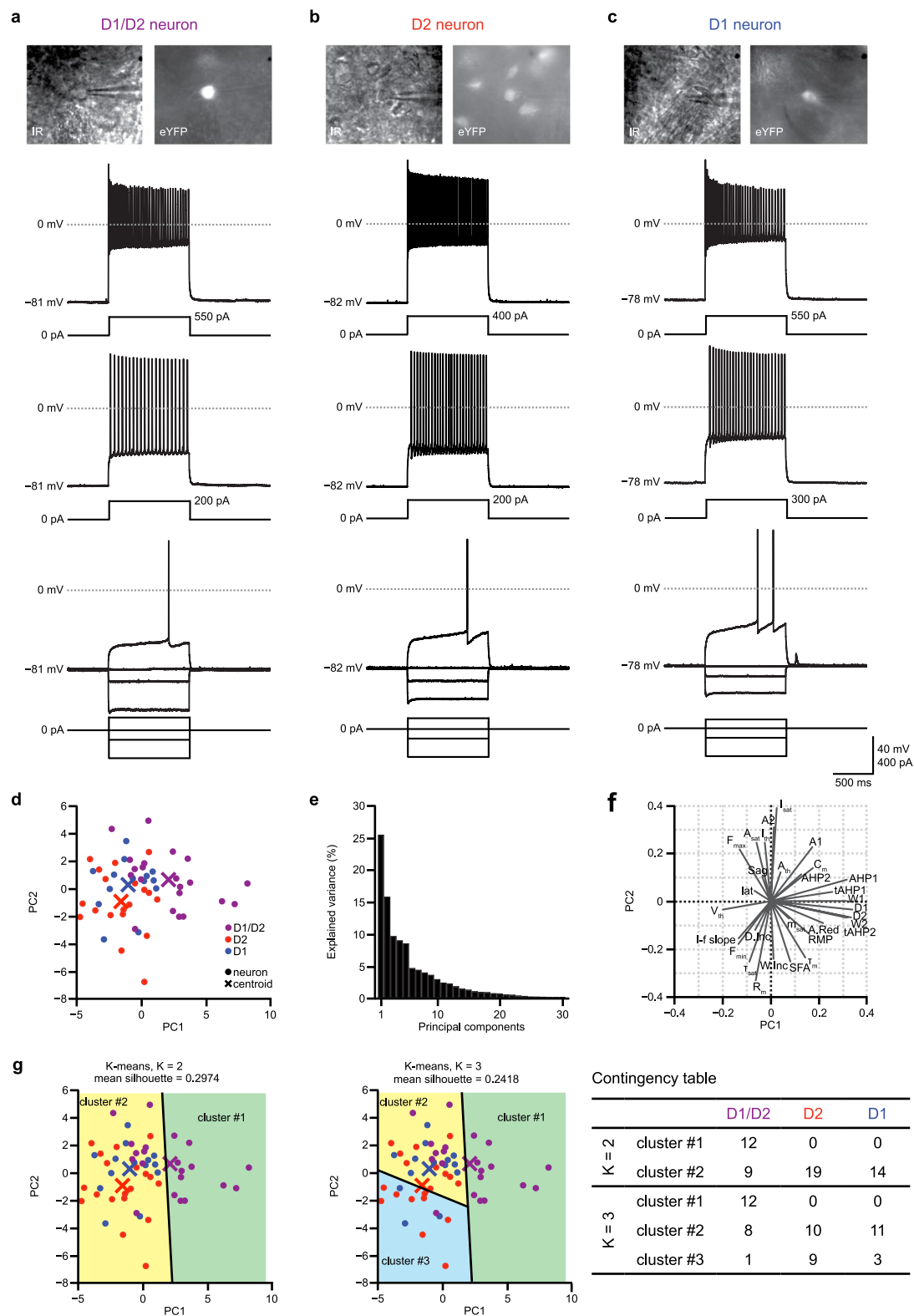


Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Anatomical characterization of D1/D2-SPNs and generation and validation of a novel D1^{FlpO}/A2a^{Cre} transgenic line.

a, Representative coronal section in the dorsal striatum illustrating fluorescent in situ hybridization of *Drd1a* and *Drd2* mRNAs and Dapi staining. Using a CellProfiler pipeline, segmentation was first performed on dapi staining to outline all cells (blue contours). Then D1- and D2-positive cells are identified using an intensity threshold in red and green channels, respectively (red and green contours). Scale: 20 μ m. **b**, Quantification of D1-, D2-, and D1/D2-positive cells throughout the rostro-caudal extent of the dorsal striatum ($n = 3$ mice). **c**, Diagram illustrating the generation of D1^{FlpO} mice. **d**, Photomicrograph of a coronal brain section at the level of the dorsal striatum after in situ hybridization of FlpO (red) and *Drd1a* (green) mRNAs illustrating expression of FlpO-recombinase in *Drd1a*-expressing SPNs. The experiment was repeated on 3 mice. Scales: left: 100 μ m; right zoom: 15 μ m. **e**, Coronal brain section after in situ hybridization of *Drd2* (green) and *Adora2a* (red) mRNAs counterstained with Dapi (blue) illustrating specific expression of *Adora2* in D2-SPNs³⁴. Some cells expressing *Drd2* and not *Adora2* (arrows) correspond to cholinergic interneurons. The experiment was repeated on 3 mice. Scales: top left whole section: 500 μ m; zoom below: 100 μ m. **f**, INTRSECT ChR2 virus specificity. Representative coronal sections showing

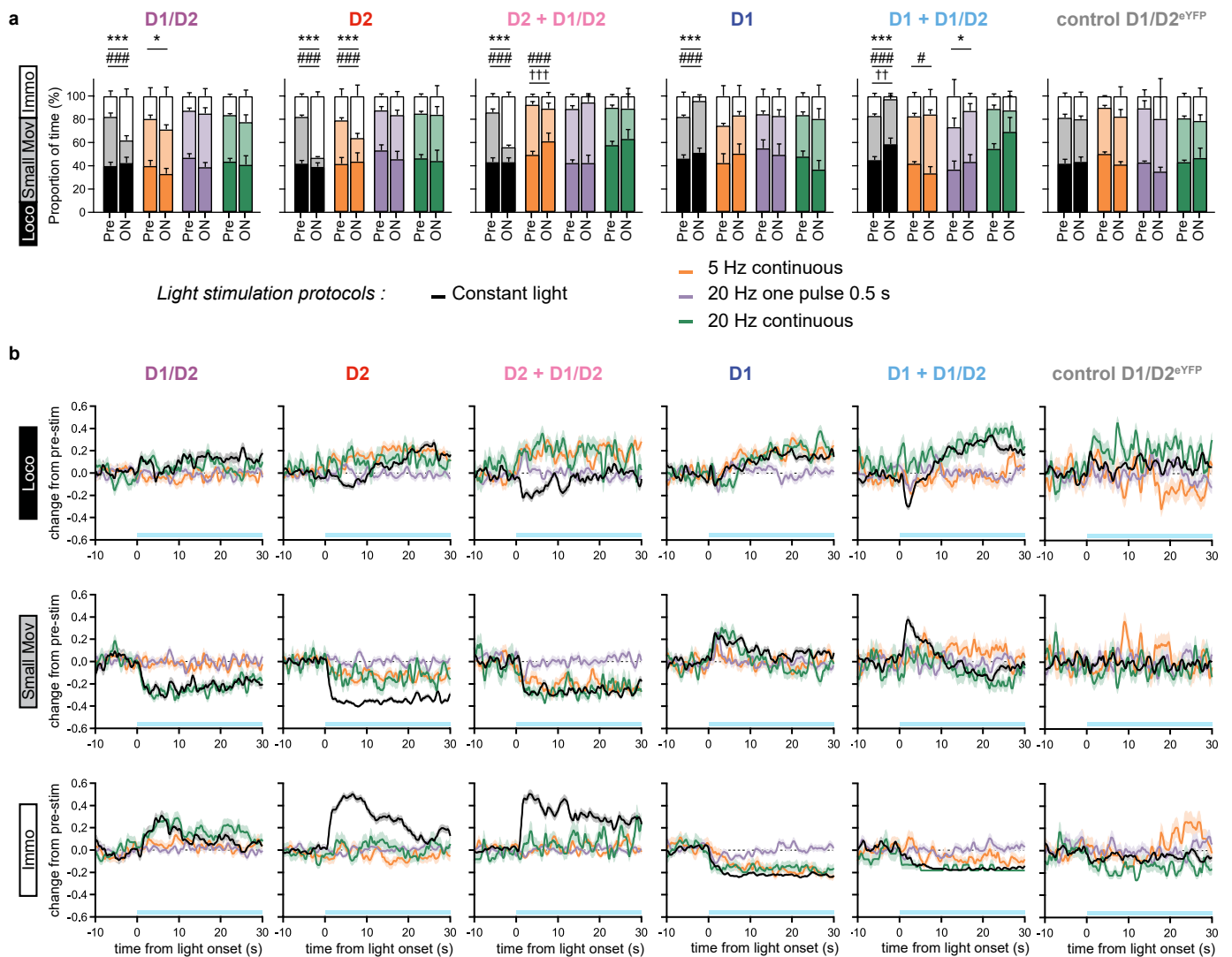
Con/Fon- (first line), Con/Foff (second line), Coff/Fon-ChR2eYFP (third line) expression in double D1^{FlpO} x A2a^{Cre} mice (left column), in single A2a^{Cre} line (middle column) and in single D1^{FlpO} line (right column). We controlled the absence of leaky expression by verifying the absence of recombination of the Con/Fon construct in all single lines, as well as the absence of recombination of the Con/Foff construct in D1^{FlpO} or the Coff/Fon construct in A2a^{Cre}. Scale: 500 μ m. **g, h**, Quantification of the density of INTRSECT virus transfected cells using automated detection. **g**, Representative sections in the dorsal striatum illustrating Con/Fon-, Con/Foff, Coff/Fon-eYFP virus expression, from top to bottom respectively. Red dots: positive cells. Blue dots: false positive cells with lower fluorescence. Scale: 40 μ m. **h**, Density of cells transfected with Con/Fon-eYFP (purple), Con/Foff-eYFP (red) and Coff/Fon-eYFP (blue) virus. We found 228.3 ± 26.9 cells/mm² for D1/D2-SPNs, 675.1 ± 58.2 cells/mm² for D2-SPNs, and 561.2 ± 37.1 cells/mm² for D1-SPNs which correspond to proportions of $16.6 \pm 2.4\%$ D1/D2-SPNs, $45.2 \pm 5.2\%$ D2-SPNs, and $38.2 \pm 0.7\%$ D1-SPNs relative to total SPNs (Dapi normalized, $n = 3$ mice for Con/Fon-eYFP and Con/Foff-eYFP virus, $n = 2$ mice for Coff/Fon-eYFP virus). Detailed statistics are displayed in Supplementary Table 1. Source data are provided as a Source Data file.



Extended Data Fig. 2 | See next page for caption.

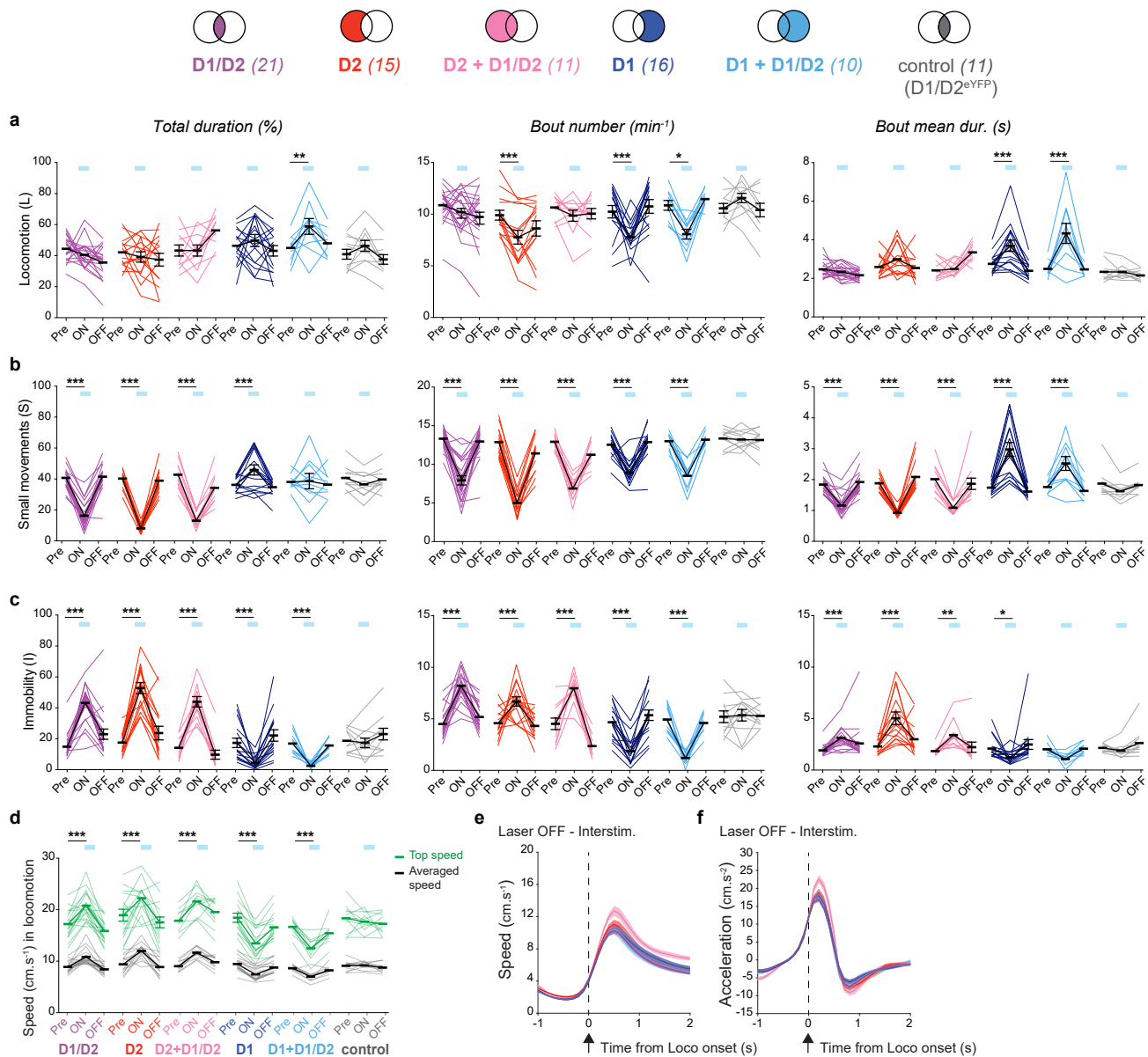
Extended Data Fig. 2 | Electrophysiological characterization of the three SPNs subpopulations. a–c. Representative example of voltage response to current steps in one D1/D2-SPN (**a**), one D1-SPN (**b**) and one D2-SPN (**c**), with their corresponding photomicrograph under infrared illumination (IR, top left) and under fluorescent illumination (eYFP, top right). **d.** Two-dimensional projection of the set of neurons recorded following PCA into the first- and second-order components. Individual cells are represented in violet (D1/D2), blue (D1) and red (D2) circles; crosses represent centroids of D1/D2-SPNs (violet), D1-SPNs (blue) and D2-SPNs (red). **e.** Percent of variance explained as a function of the number of principal components. **f.** Two-dimensional projection of orthonormal principal component coefficients for each parameter. (Abbreviations: *RMP*: resting membrane potential; *R_m*: input membrane resistance; *τ_m*: membrane time constant; *C_m*: membrane capacitance; *Sag*: Sag index; *I_{thr}*: step intensity to first spike; *lat*: first spike latency; *V_{thr}*: action potential; *A_{thr}*: adaptation around threshold; *F_{min}*: minimal steady state frequency around threshold; *I–f* slope: early slope of the current–frequency relationship; *I_{sat}*: maximum intensity before depolarization block;

A_{sat}: amplitude of early adaptation at high firing; *τ_{sat}*: time constant of early adaptation at high firing; *m_{sat}*: slope of late adaptation at high firing; *F_{max}*: maximal steady state frequency at high firing; *SFA*: spike frequency adaptation at high firing; *A1*: amplitude of the first action potential; *A2*: amplitude of the second action potential; *D1*: duration of the first action potential; *D2*: duration of the second action potential; *W1*: spike half-widths of the first action potential; *W2*: spike half-widths of the second action potential; *AHP1*: AHP maximum of the first action potential; *AHP2*: AHP potential maximum of the second action potential; *tAHP1*: AHP latency after of the first action potential; *tAHP2*: AHP latency of the second action potential; *A.Red*: amplitude reduction; *D.Inc*: duration increase; *W.Inc*: half-width duration increase). **g.** Clusters obtained after k-means partitioning (left, *k* = 2; middle, *k* = 3) based on all the electrophysiological parameters recorded, and contingency table (right) comparing clusters assignments (lines) and cell identity defined by INTRSECT eYFP labelling. Source data are provided as a Source Data file.



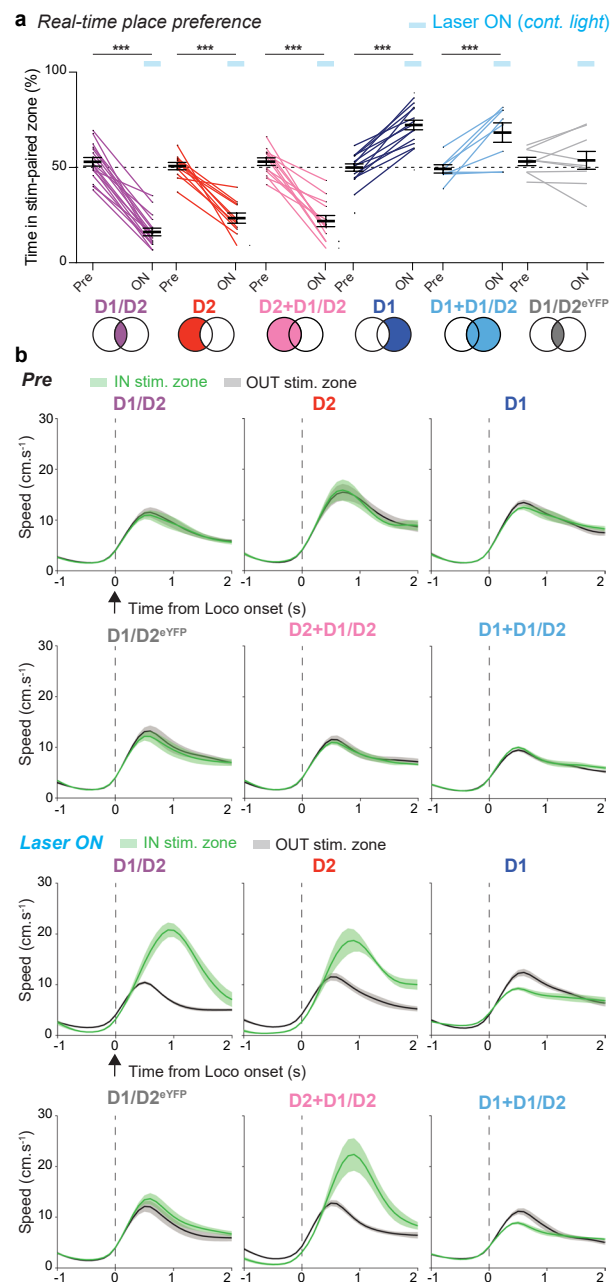
Extended Data Fig. 3 | Comparison between optogenetic stimulation protocols to elicit behavioral changes. **a**, Effect of four different optogenetic stimulation protocols (constant light ON during 30 s, black; 5 Hz stimulation during 30 s, orange; one pulse at 20 Hz lasting 0.5 s, violet; 20 Hz stimulation during 30 s, green; D1/D2, $n = 21, 8, 8, 13$, respectively; D2, $n = 15, 8, 8, 8$, respectively; D2 + D1/D2, $n = 11, 6, 6, 5$, respectively; D1, $n = 16, 6, 6, 11$, respectively; D1 + D1/D2, $n = 10, 5, 5, 5$, respectively; D1/D2^{eYFP}, $n = 11, 5, 5, 6$, respectively) on the proportion of time spent in locomotion, small movements, and immobility (three-ways ANOVA followed by two-sided Tukey's tests pre vs. light ON: for locomotion, $^{**}p < 0.01$,

$^{***}p < 0.001$; for small movements, $^{\#}p < 0.05$, $^{###}p < 0.001$; for immobility, $^{*}p < 0.05$, $^{***}p < 0.001$). Data are presented as mean values $\pm$ SEM. **b**, Temporal evolution of changes in locomotion (top), small movements (middle), and immobility (bottom) occurrences induced by the four different optogenetic stimulation protocols (constant light ON during 30 s, black; 5 Hz stimulation during 30 s, orange; one pulse at 20 Hz lasting 0.5 s, violet; 20 Hz stimulation during 30 s, green) (solid line, mean; shading, bootstrap 95% CI). Detailed statistics are displayed in Supplementary Table 1. Source data are provided as a Source Data file.



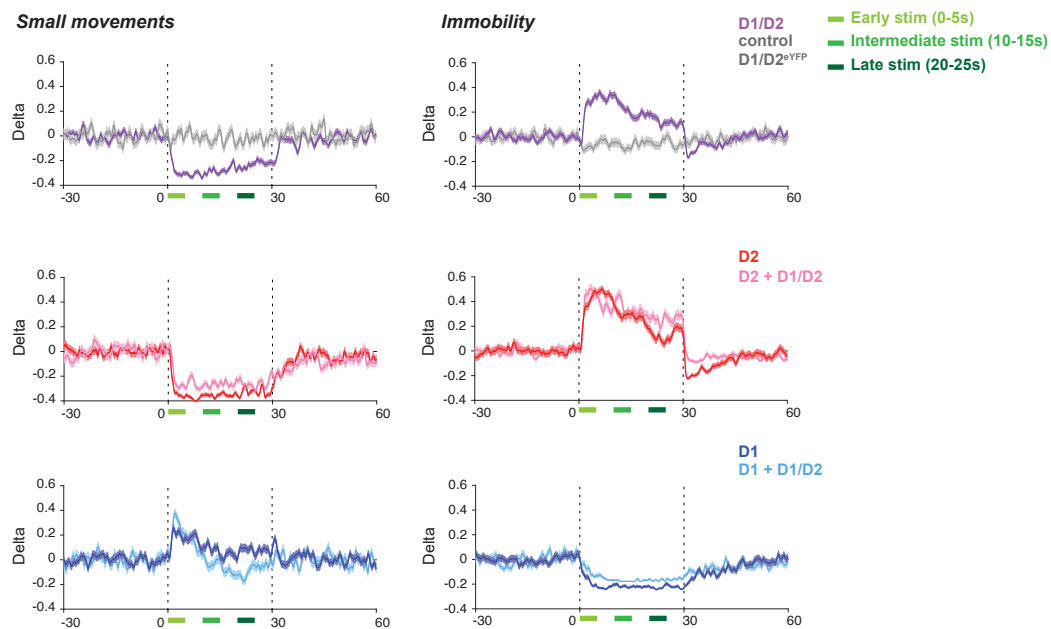
Extended Data Fig. 4 | Architecture of motor states and locomotion speed during optogenetic stimulation. **a-c**, Effect of optogenetic stimulation motor architecture during locomotion (**a**), small movement (**b**), and immobility (**c**) quantified by the proportion of time spent in each state (left panels), bouts frequency (middle panels), and the average episode duration (right panels) in D1/D2, control D1/D2^{eYFP}, D1, D1 + D1/D2, D2, and D2 + D1/D2 mice (two-ways RM ANOVA followed by two-sided Tukey's tests pre vs. light ON: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). **d**, Effect of laser illumination on average speed (black) and top

speed (9th decile, green) during locomotion (two-ways RM ANOVA followed by two-sided Tukey's tests pre vs. light ON: *** $p < 0.001$). **e, f**, Averaged temporal evolution of mice speed (**e**) and acceleration (**f**) around locomotion onset during laser 'OFF' periods between stimulation trials. For all graphs: D1/D2, $n = 21$; D2, $n = 15$; D2 + D1/D2, $n = 11$; D1, $n = 16$; D1 + D1/D2, $n = 10$ and control D1/D2^{eYFP}, $n = 11$. Data are presented as mean values $\pm$ SEM. Detailed statistics are displayed in Supplementary Table 1. Source data are provided as a Source Data file.



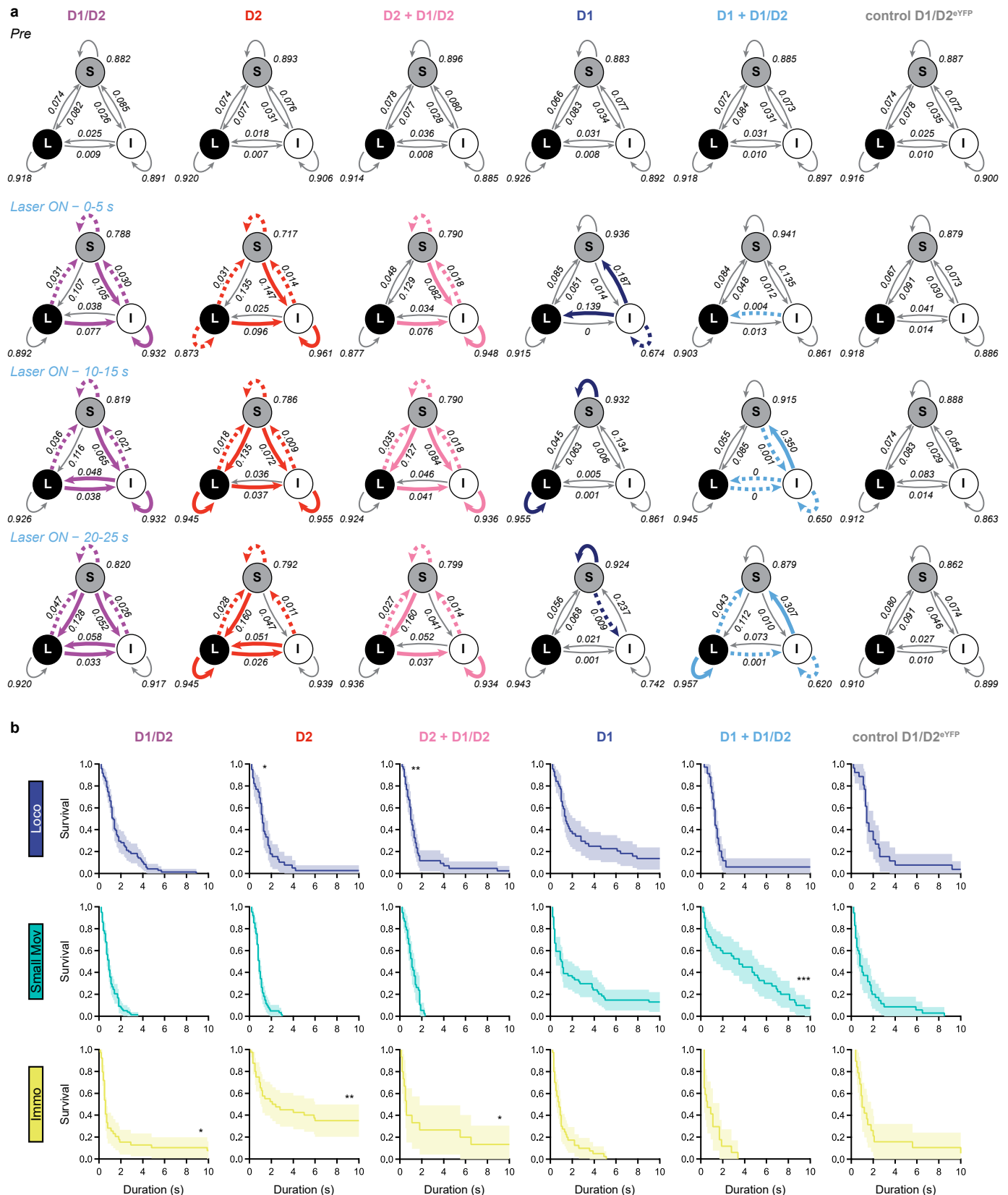
Extended Data Fig. 5 | Effect of optogenetic stimulation on real-time place preference. **a**, Proportion of time spent in the laser-paired area before (Pre) and during (ON) the optical activation (continuous blue light) of D1/D2-SPNs ($n = 17$), D2-SPNs ($n = 11$), D2 + D1/D2-SPNs ($n = 13$), D1-SPNs ($n = 13$), D1 + D1/D2-SPNs ($n = 8$) or in control (D1/D2^{eYFP}; $n = 9$) mice in the laser-paired zone (two-ways RM ANOVA followed by two-sided Tukey's tests Pre vs. LaserON: ns $p > 0.05$, *** $p < 0.001$). **b**, Average temporal evolution of mice speed aligned to locomotion episode onsets when the animal is exploring the laser-paired zone (green curves) or the unpaired zone (black curves) during the baseline period (Pre, top

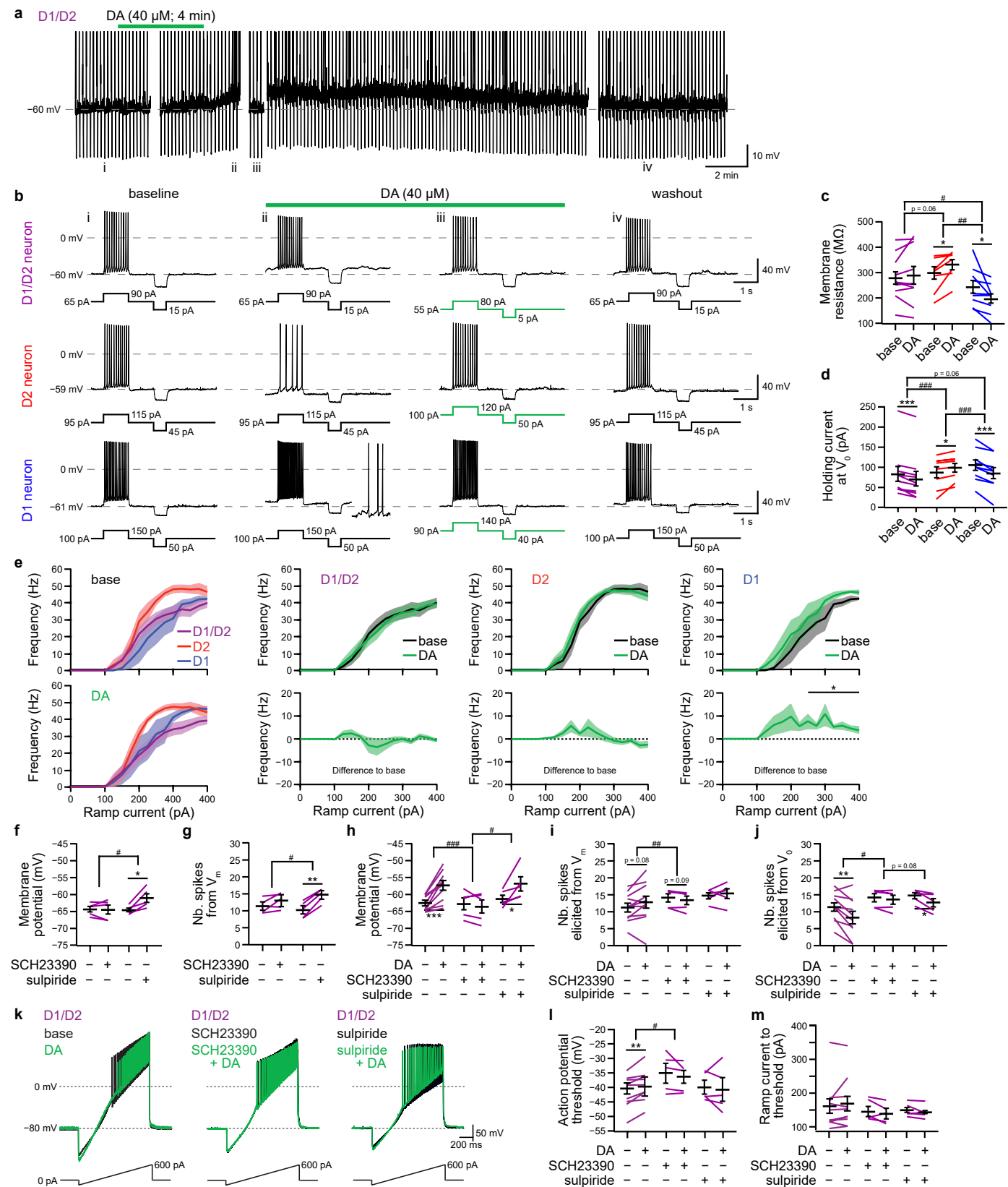
panels) and during the optogenetic stimulation in the laser paired zone (Laser ON, bottom panels). Note during the Laser ON condition, the large increase in locomotion speed elicited by the stimulation of D1/D2, D2, and D2 + D1/D2-SPNs suggesting the aversive effect of the stimulation, and the decrease in speed elicited by the stimulation of D1, and D1 + D1/D2-SPNs suggesting the appetitive effect of the stimulation. Data are presented as mean values $\pm$ SEM. Detailed statistics are displayed in Supplementary Table 1. Source data are provided as a Source Data file.



Extended Data Fig. 6 | Dynamic changes in small movements and immobility occurrence during optogenetic stimulation. Temporal evolution of changes in small movements (left panels), and immobility (right panels) occurrences induced by optogenetic stimulation (constant light ON during 30 s; D1/D2, $n = 21$;

D1/D2^{eYFP}, $n = 11$; D2, $n = 15$; D2 + D1/D2, $n = 11$; D1, $n = 16$; D1 + D1/D2) (solid line, mean; shading, bootstrap 95% CI). X-axes correspond to time (s) and optogenetic stimulation is applied for 30s from time 0. Source data are provided as a Source Data file.

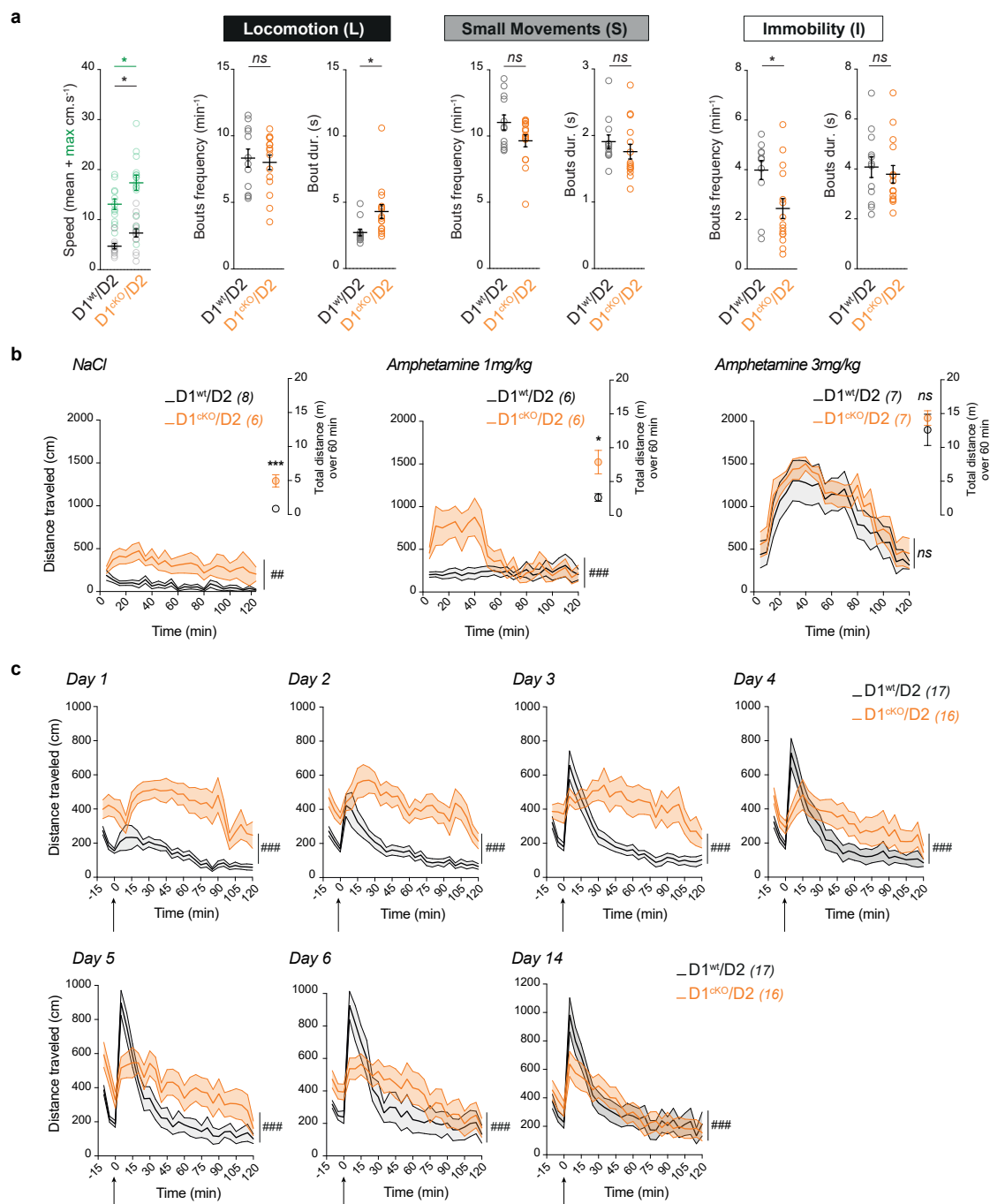




Extended Data Fig. 8 | Effects of DA application on SPNs subpopulations.

a, Representative example of the effect of bath applied DA (40 μ M, 4 min) on membrane potential and evoked firing activity in one D1/D2-SPN. **b**, Voltage traces recorded in one representative D1/D2-SPN (top), one D1-SPN (middle), and one D2-SPN (bottom) in response to depolarizing and hyperpolarizing current steps during baseline (first column), at peak of DA effect from the same holding current than in the beginning of the recording (second column), at peak of DA from holding current to restore voltage at the start of the recording (third column), and after DA washout (last column). For the D1/D2-SPN example i–iv represent times indicated in **a**. **c**, **d**, Quantification of changes in membrane resistance (around -60 mV) induced by DA application (**c**), and change in holding necessary to maintain cells at the same potential than at the beginning of the recording before and after DA application (**d**) in D1/D2 ($n = 10$ cells from 6 mice, violet), D1- ($n = 9$ cells from 4 mice, blue), and D2-SPNs ($n = 7$ cells from 4 mice, red) and (two-sided paired t-tests between baseline and after DA application: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; two-sided t-tests comparing the magnitude of DA effect between SPN subpopulations: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$). **e**, Changes in average current–frequency relationship evaluated from ramp current injection during baseline and at the peak of DA effect in D1/D2- ($n = 11$ cells from 6 mice), D1- ($n = 6$ cells from 4 mice), and D2-SPNs ($n = 8$ cells from 4 mice) and (two-sided paired t-tests between baseline and after DA application: $^*p < 0.05$). **f**, **g**, Effect of D1R antagonist (SCH23390, 10 μ M) and D2R antagonist (sulpiride, 10 μ M) application on membrane potential (**f**) and number of spikes

elicited by a depolarizing current step (**g**) in D1/D2-SPNs (SCH23390, $n = 5$ cells from 3 mice; sulpiride, $n = 5$ cells from 3 mice) (two-sided paired t-tests between baseline and after antagonist application: $^*p < 0.05$, $^{**}p < 0.01$; two-sided t-tests comparing the magnitude of effect between treatments: $^{\#}p < 0.05$). **h–j**, Effect of DA application on D1/D2-SPNs without any pre-treatment, in the presence of SCH23390, or in the presence of sulpiride on the membrane potential (**h**), the number of elicited spikes by a depolarizing pulse from current membrane potential (**i**), and the number of spikes elicited from initial membrane potential (**j**) (no treatment, $n = 11$ cells from 6 mice; SCH23390, $n = 5$ cells from 3 mice; sulpiride, $n = 5$ cells from 3 mice) (two-sided paired t-tests between baseline and after DA application: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; two-sided t-tests comparing the magnitude of DA effect between treatments: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$). **k**, Voltage response and APs of representative D1/D2-SPNs to ramp current injection before (black) and after (green) DA application without pre-treatment (left), in the presence of SCH23390 (middle), or in the presence of sulpiride (right). **l–m**, Effect of DA application on AP threshold (**l**) and minimal ramp current to elicit discharge (**m**) in D1/D2-SPNs without antagonists ($n = 10$ cells from 6 mice), or in the presence of SCH23390 ($n = 5$ cells from 3 mice), or sulpiride ($n = 5$ cells from 3 mice) (two-sided paired t-tests between baseline and after DA application: $^*p < 0.05$, $^{**}p < 0.01$; two-sided t-tests comparing the magnitude of DA effect between conditions: $^{\#}p < 0.05$). Data are presented as mean values $\pm$ SEM. Detailed statistics are displayed in Supplementary Table 1. Source data are provided as a Source Data file.



Extended Data Fig. 9 | Supplements on the phenotypic characterization of D1^{KO}/D2 mice. **a**, Comparison of spontaneous locomotor behavior between D1^{wt}/D2 ($n = 13$) and D1^{KO}/D2 ($n = 15$) mice in term of average and top speed during locomotion episodes (left panel) and motor states architecture (two-sided t-tests, D1^{wt}/D2 vs. D1^{KO}/D2 mice: ns $p > 0.05$, * $p < 0.05$). **b**, Temporal distribution of the locomotor effects of acute amphetamine or saline injections in D1^{wt}/D2 (NaCl, $n = 8$; 1 mg/kg, $n = 6$; 3 mg/kg, $n = 7$) and D1^{KO}/D2 mice (NaCl, $n = 6$; 1 mg/kg, $n = 6$; 3 mg/kg, $n = 7$) and (inserts) total distance traveled over the first 60 min after injection (three-ways ANOVA followed by post-hoc two-ways ANOVA for interaction genotype $\times$ time: NaCl, $F(23,276) = 1.9$; 1 mg/kg, $F(23,230) = 5.2$; 3 mg/kg,

$F(23,276) = 0.57$; ns $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$; and post-hoc two-sided Mann Whitney tests D1^{wt}/D2 vs. D1^{KO}/D2 mice on distance traveled over 60 min: ns $p > 0.05$, * $p < 0.05$, *** $p < 0.001$). **c**, Temporal evolution of traveled distance across repeated 10 mg/kg cocaine injection in D1^{wt}/D2 (black) and D1^{KO}/D2 mice (orange) (three-ways ANOVA followed by post-hoc two-ways ANOVA for interaction genotype $\times$ time: Day1, $F(26,806) = 1.9$; Day2, $F(26,806) = 2.29$; Day3, $F(26,806) = 6.55$; Day4, $F(26,806) = 4.96$; Day5, $F(26,806) = 6.08$; Day6, $F(26,806) = 4.27$; Day14, $F(26,806) = 2.72$; *** $p < 0.001$). Data are presented as mean values $\pm$ SEM. Detailed statistics are displayed in Supplementary Table 1. Source data are provided as a Source Data file.

Extended Data Table 1 | Complete list of electrophysiological properties recorded in D1/D2-, D1- and D2-SPNs

	D1/D2 neurons n = 21	D2 neurons n = 19	D1 neurons n = 14
Passive membrane properties			
(1) Resting potential (mV) ANOVA: NS ($F_{2,51} = 2.02$; $p = 0.14$)	-69.67 ± 0.77	-71.05 ± 1.13	-72.86 ± 1.49
(2) Input Resistance (M Ω) ANOVA: NS ($F_{2,51} = 0.83$; $p = 0.44$)	110.13 ± 6.93	129.94 ± 12.43	115.81 ± 16.75
(3) Membrane time constant (ms) ANOVA: NS ($F_{2,51} = 2.55$; $p = 0.09$)	6.48 ± 0.35	6.02 ± 0.39	5.16 ± 0.48
(4) Membrane capacitance (pF) ANOVA: NS ($F_{2,51} = 2.10$; $p = 0.13$)	61.14 ± 3.40	50.20 ± 3.58	49.70 ± 4.98
(5) Sag index (%) ANOVA: NS ($F_{2,51} = 0.24$; $p = 0.79$)	13.39 ± 1.02	13.83 ± 1.12	12.04 ± 1.63
Just above threshold properties			
(6) Step intensity to first spike (pA) ANOVA: $p = 0.014$ ($F_{2,51} = 4.65$)	123.33 ± 10.74 < D1	121.58 ± 11.68 < D1	176.43 ± 19.66 > D1/D2 . D2
(7) Latency to first spike (ms) ANOVA: NS ($F_{2,51} = 0.01$; $p = 0.99$)	528.12 ± 53.15	521.17 ± 49.89	516.11 ± 66.61
(8) Action potential threshold (mV) ANOVA: $p < 0.001$ ($F_{2,51} = 15.03$)	-41.39 ± 1.05 <<< D1 < D2	-37.15 ± 1.11 > D1/D2 << D1	-31.46 ± 1.70 >>> D1/D2 >> D2
(9) Adaptation (Hz/s) ANOVA: NS ($F_{2,51} = 0.58$; $p = 0.57$)	4.46 ± 1.08	3.67 ± 0.61	3.06 ± 0.87
(10) Minimal steady state frequency (Hz) ANOVA: NS ($F_{2,51} = 2.00$; $p = 0.15$)	6.96 ± 0.46	8.12 ± 0.49	8.25 ± 0.64
Firing properties			
(11) Early slope of current-frequency curve (Hz/pA) ANOVA: $p < 0.001$ ($F_{2,51} = 1.55$)	0.20 ± 0.01 << D2	0.25 ± 0.02 >>> D1 >> D1/D2	0.17 ± 0.01 <<< D2
(12) Maximum intensity before depolarization block (pA) ANOVA: $p < 0.001$ ($F_{2,51} = 9.00$)	666.67 ± 49.80 >> D2	470.00 ± 27.98 <<< D1 << D1/D2	717.86 ± 46.77 >>> D2
(13) Amplitude of early frequency adaptation (Hz) ANOVA: NS ($F_{2,51} = 0.83$; $p = 0.44$)	34.56 ± 3.15	28.64 ± 4.83	27.85 ± 4.33
(14) Time constant of early frequency adaptation (ms) ANOVA: $p = 0.006$ ($F_{2,51} = 5.60$)	42.23 ± 4.34 << D2	67.53 ± 5.20 >> D1/D2	54.97 ± 8.10
(15) Slope of late adaptation (Hz/s) ANOVA: NS ($F_{2,51} = 1.66$; $p = 0.20$)	-14.09 ± 1.14	-14.37 ± 1.60	-18.20 ± 2.47
(16) Maximal steady state frequency (Hz) ANOVA: NS ($F_{2,51} = 0.75$; $p = 0.48$)	40.95 ± 2.07	39.86 ± 2.53	43.81 ± 1.57
(17) Spike frequency adaptation (%) ANOVA: NS ($F_{2,51} = 1.34$; $p = 0.27$)	97.70 ± 5.06	104.64 ± 5.50	92.45 ± 3.68
Action potentials properties			
(18) First spike amplitude (mV) ANOVA: $p < 0.001$ ($F_{2,51} = 10.74$)	95.12 ± 1.29 >>> D1 >> D2	87.14 ± 1.68 << D1/D2	84.03 ± 2.52 <<< D1/D2
(19) Second spike amplitude (mV) ANOVA: $p = 0.049$ ($F_{2,51} = 3.08$)	85.87 ± 1.68 > D1	82.87 ± 1.82	79.46 ± 1.76 < D1/D2
(20) First spike duration (ms) ANOVA: $p < 0.001$ ($F_{2,51} = 12.75$)	3.01 ± 0.15 >>> D2 >> D1	2.22 ± 0.08 <<< D1/D2	2.46 ± 0.09 << D1/D2
(21) Second spike duration (ms) ANOVA: $p < 0.001$ ($F_{2,51} = 10.14$)	3.36 ± 0.17 >>> D2 > D1	2.57 ± 0.10 <<< D1/D2	2.81 ± 0.09 < D1/D2
(22) First spike half-width (ms) ANOVA: $p < 0.001$ ($F_{2,51} = 13.36$)	1.71 ± 0.09 >>> D2 >> D1	1.40 ± 0.06 <<< D1/D2	1.38 ± 0.05 << D1/D2
(23) Second spike half-width (ms) ANOVA: $p < 0.001$ ($F_{2,51} = 9.94$)	1.87 ± 0.10 >>> D2 > D1	1.40 ± 0.06 <<< D1/D2	1.55 ± 0.05 < D1/D2
(24) First spike AHP (mV) ANOVA: $p < 0.001$ ($F_{2,51} = 10.37$)	-11.03 ± 0.91 >>> D2 > D1	-16.77 ± 0.89 <<< D1/D2	-14.51 ± 1.04 < D1/D2
(25) Second spike AHP (mV) ANOVA: NS ($F_{2,51} = 0.931$; $p = 0.40$)	-14.35 ± 0.89	-16.01 ± 0.77	-15.28 ± 1.10
(26) First spike AHP latency (ms) ANOVA: $p < 0.001$ ($F_{2,51} = 8.38$)	5.48 ± 0.26 >>> D2 > D1	4.29 ± 0.15 <<< D1/D2	4.67 ± 0.20 < D1/D2
(27) Second spike AHP latency (ms) ANOVA: $p = 0.002$ ($F_{2,51} = 6.93$)	8.44 ± 0.69 >> D2 > D1	5.97 ± 0.43 << D1/D2	5.97 ± 0.35 < D1/D2
(28) Spike amplitude reduction (%) ANOVA: $p = 0.034$ ($F_{2,51} = 3.61$)	9.66 ± 1.47 > D2 . D1	4.86 ± 1.13 < D1/D2	4.92 ± 1.99 < D1/D2
(29) Spike duration increase (%) ANOVA: NS ($F_{2,51} = 1.69$; $p = 0.20$)	12.00 ± 1.27	15.47 ± 1.59	14.71 ± 1.58
(30) Spike half-width increase (%) ANOVA: NS ($F_{2,51} = 1.51$; $p = 0.23$)	8.92 ± 1.62	12.59 ± 2.00	13.06 ± 2.09

n: number of cells; NS: nonsignificant. Two-sided Tukey's post-hoc tests: <, <<, <<<: inferior with $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively; >, >>, >>>: superior with $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.