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Balance Between Projecting Neuronal Populations of the Nucleus Accumbens Controls Social Behavior in Mice

Julie Le Merrer, Bérangère Detraux, Jorge Gandía, Aurélie De Groote, Mathieu Fonteneau, Alban de Kerchove d'Exaerde, and Jérôme A.J. Becker

ABSTRACT

BACKGROUND: Deficient social interactions are a hallmark of major neuropsychiatric disorders, and accumulating evidence points to altered social reward and motivation as key underlying mechanisms of these pathologies. In the present study, we further explored the role of the balance of activity between D₁ and D₂ receptor-expressing striatal projection neurons (D1R- and D2R-SPNs) in the control of social behavior, challenging the hypothesis that excessive D2R-SPN activity, rather than deficient D1R-SPN activity, compromises social behavior.

METHODS: We selectively ablated D1R- and D2R-SPNs using an inducible diphtheria toxin receptor-mediated cell targeting strategy and assessed social behavior as well as repetitive/perseverative behavior, motor function, and anxiety levels. We tested the effects of optogenetic stimulation of D2R-SPNs in the nucleus accumbens (NAc) and pharmacological compounds repressing D2R-SPN.

RESULTS: Targeted deletion of D1R-SPNs in the NAc blunted social behavior in mice, facilitated motor skill learning, and increased anxiety levels. These behaviors were normalized by pharmacological inhibition of D2R-SPN, which also repressed transcription in the efferent nucleus, the ventral pallidum. Ablation of D1R-SPNs in the dorsal striatum had no impact on social behavior but impaired motor skill learning and decreased anxiety levels. Deletion of D2R-SPNs in the NAc produced motor stereotypies but facilitated social behavior and impaired motor skill learning. We mimicked excessive D2R-SPN activity by optically stimulating D2R-SPNs in the NAc and observed a severe deficit in social interaction that was prevented by D2R-SPN pharmacological inhibition.

CONCLUSIONS: Repressing D2R-SPN activity may represent a promising therapeutic strategy to relieve social deficits in neuropsychiatric disorders.

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When conditions are favorable, interactions with conspecifics in social animals are pleasurable (1–3), which in turn fuels social motivation. Compromised social interactions are a hallmark of major neuropsychiatric disorders, such as autism spectrum disorder (ASD), schizophrenia, and depression. Although altered social reward and motivation appear as key underlying mechanisms in these pathologies (4–7), the neuronal and molecular underpinnings of social abilities remain scarcely known.

The identification of brain regions and specific networks underlying social processing in social animals has emerged and consolidated over the past 30 years (8–10). Interestingly, this social brain widely overlaps with the brain reward circuit (11–13). Within this circuit, the nucleus accumbens (NAc) works as a hub structure (14–16) and, as such, plays a unique role in reward processing and approach (15,17,18). The view of the NAc as a key substrate for modulating social behavior is supported by imaging studies revealing NAc activation in response to various social stimuli in humans (19,20), which is blunted in patients with ASD, depression, or schizophrenia

(21–23). Consistently, anomalies in the volume and/or connectivity of the NAc have been evidenced in patients with autism, schizophrenia, or depression (4,24–27) as well as in preclinical models of these pathologies (28–33). Dopamine (34), serotonin (35), opioid (36–38), and oxytocin (39,40) receptor activation in the NAc was shown to stimulate/drive social behavior in rodents. Thus, the NAc appears to be a key brain structure for the control of social abilities under physiological and pathological conditions. How the two main neuronal populations in the NAc, D₁ and D₂ receptor-expressing striatal projection neurons (D1R- and D2R-SPNs), modulate social behaviors, however, remains poorly understood.

D1R- and D2R-SPNs differ in their transcriptional and projection patterns. D1R-SPNs also express *Pdyn* (coding for prodynorphin) and *Tac1* (for substance P), whereas D2R expression is associated with *Penk* (for proenkephalin), *Adora2a* (for adenosine A_{2a} receptor), and *Gpr6* (for orphan receptor GPR6) (41–44) expression. D1R- and D2R-SPNs project to distinct brain areas. In the dorsal striatum (DS),

D1R-SPNs project to the substantia nigra pars reticulata (SNr) and internal globus pallidus (or entopeduncular nucleus in rodents), forming the direct pathway; D2R-SPNs project to the external globus pallidus that projects to the subthalamic nucleus, which in turn innervates the SNr and internal globus pallidus/entopeduncular nucleus, forming the indirect pathway (45). In the NAc, D1R-SPNs project to the ventral tegmental area (VTA) or SNr, while D2R-SPNs project to the ventral pallidum (VP); VP neurons then innervate the subthalamic nucleus, which in turn connects to the SNr (45). Clear-cut segregation, however, is not always verified, and a significant proportion of D1R-SPNs project to the VP (31,46,47).

D1R- and D2R-SPNs of the NAc play contrasting roles in modulating behavior, with the former proposed to drive pro-reward/pro-approach responses and the latter considered to inhibit these responses (45,48,49), although with some amendments (45,50–52). Indirect evidence argues for similar opposing roles of D1R-SPNs and D2R-SPNs in modulating social behavior. In typical mice, social interaction was associated with an activation of NAc D1R-SPNs (47), and reciprocally, optogenetic stimulation of these neurons increased social approach (13,53). In depressive-like mice, social avoidance increased with D2R-SPN excitability; repeated optogenetic stimulation of D1R-SPNs in the NAc restored their social interaction (31). Manipulating the expression of candidate genes in striatal SPNs has provided further evidence for their role in controlling social behavior. Deletion of *ErbB4* in both populations of NAc SPNs produced social impairment (54). Knocking down neuroligin-2 in D1R-SPNs led to a deficit in social interaction and dominance, while such manipulation in D2R-SPNs increased defensive response to social aggression (55). Suppressing endocannabinoid 2-AG (2-arachidonoylglycerol) signaling in all D1R-SPNs or locally in DS also blunted sociability (56). Finally, knocking down the *Shank3* gene in NAc D1R-SPNs impaired social preference (53). Together, these results have led to the view that D1R-SPNs, notably in the NAc, drive prosocial behavior, whereas activation of D2R-SPNs promotes social avoidance. In pathological context, stimulating D1-SPNs thus appears to be the best strategy to restore social behavior.

In the present study, we challenged the disruptive hypothesis that compromised social behavior in mice with low/deficient D1R-SPN function in the NAc would result from excessive remaining D2R-SPN activity, rather than from missing D1R-SPN outputs. To this aim, we used an inducible diphtheria toxin receptor (iDTR)-mediated cell targeting strategy (48,57) combined with pharmacological and optogenetic approaches. Social behavior was assessed in mice bearing D1R-SPN ablation in the NAc or DS or D2R-SPN ablation in the NAc. We revealed a severe social behavior deficit following ablation of D1R-SPNs in the NAc, but not in the DS, while ablation of D2R-SPNs in the NAc facilitated social interactions. Pharmacological repression of D2R-SPN activity relieved behavioral deficits in NAc D1R-SPN-ablated mice and repressed gene expression in the VP. Finally, we demonstrate that long, but not short, optogenetic stimulation of NAc D2R-SPNs induces a deficit in social interaction and increases Fos transcription in the VP; furthermore, pharmacological inhibition of D2R-SPNs prevented optogenetically induced social deficits in mice. These results converge to demonstrate that repressing

D2R-SPN activity represents a promising strategy for the treatment of ASD, schizophrenia, and depression.

METHODS AND MATERIALS

Animal Care

All experimental procedures were conducted in accordance with the European Communities Council Directive 2010/63/EU and the Institutional Animal Care Committee guidelines. They were approved by the Local Ethical Committees (Pôle Santé ULB). Animals were housed in groups (2–4 mice per cage) and maintained on a 12-hour light/dark cycle (light on at 8:00 AM) at a controlled temperature of 21 °C. Food and water were available ad libitum, except when otherwise stated.

Behavioral Experiments

Detailed protocols for behavioral tests are described in the [Supplement](#). Experiments were conducted and analyzed blind to genotype and experimental condition.

Statistics

Statistical analyses were performed using Statistica 9.0 software (StatSoft). For all comparisons, values of $p < .05$ were considered as significant. If normality was verified (Shapiro-Wilk test), statistical significance in behavioral and immunohistochemical experiments was assessed using a one- or two-way analysis of variance, followed by the Newman-Keuls post hoc test. If not, we used the nonparametric Mann-Whitney U test or Kruskal-Wallis analysis of variance (followed by two-tailed t test). When a parameter was measured repeatedly, an analysis of variance with a repeated measure was performed, followed by the Newman-Keuls post hoc test. Such analysis, however, may have exaggerated statistical significance (see [Tables S2, S4, S6, S8, and S10](#) for statistical results). Significance of quantitative real-time polymerase chain reaction (qRT-PCR) results was assessed after transformation using a two-tailed t test, as previously described (58,59); an adjusted p value was calculated using Benjamini-Hochberg correction for multiple testing. Outliers over 3 times the standard deviation were excluded from calculations as technical errors. Unsupervised clustering analysis was performed on transformed qRT-PCR data using complete linkage with correlation distance (Pearson correlation) for drug, treatment, and brain region (Cluster 3.0 [<https://www.encodeproject.org/software/cluster/>] and Java TreeView 3.0 [<https://jtreeview.sourceforge.net/>]). Results for qRT-PCR analysis and discarded or missing (undetected) values are detailed in [Tables S3, S5, S7, and S9](#).

Details for mouse lines, drugs, toxin ablation and optogenetic manipulation, methods of qRT-PCR analysis, immunohistochemistry, and cell counting are available in the [Supplement](#). The list of primers can be found in [Table S1](#).

RESULTS

Ablation of D1R-SPNs in the NAc Negatively Affects Social Behavior, Induces Motor Stereotypies, and Increases Conflict Anxiety

We first evaluated the consequences of D1R-SPN ablation in the NAc on social and stereotypic behavior, motor abilities,

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and anxiety (Figure 1A; see also Figure S2). In the direct social interaction test, lesioned mice displayed a severe deficit (Figure 1B); in the three-chamber social preference test (Figure 1C), these mice preferred to interact less with the living interactor than with the toy. Regarding stereotypic behavior, D1R-SPN-lesioned mice displayed more frequent spontaneous motor stereotypies (Figure 1D) and buried more marbles (Figure 1E) than sham control animals. In the Y maze (Figure 1F), however, they showed a similar number of perseverative same arm entries. Regarding motor function, nest building (Figure 1G) was impaired in ablated mice, but motor skill learning was facilitated (Figure S2E). Finally, lesioned mice showed reduced latency to feed in the novelty-suppressed feeding test (Figure S2F) and reduced exploration of the open arms in the elevated plus maze (EPM) (Figure S2G).

To assess the efficiency and specificity of D1R-SPN ablation in the NAc, we used qRT-PCR and measured in the NAc, medial DS (mDS), and SN pars compacta/VTA the expression of 11 marker genes of striatal neurons: D1R-SPNs (*Drd1a*, *Tac1*, *Pdyn*, *Chrm4*), D2R-SPNs (*Drd2*, *Adora2a*, *Penk*, *Gpr6*, *Grm4*), and cholinergic interneurons (CINs) (*Chat*, *Oprd1*), as well as the expression of *Fos*, as a marker of neuronal activity, 45 minutes after a direct social interaction (Figure 1H; Table S2). Ablation of NAc D1R-SPNs produced a significant decrease in NAc expression of *Tac1* and *Pdyn*, the two main marker genes of D1R-SPNs, confirming ablation (Figure 1I). In contrast, the expression of two marker genes of D2R-SPNs, *Drd2* and *Grm4*, and markers of CINs, *Oprd1* and *Chat*, were increased in the NAc. NAc D1R-SPN ablation produced a decrease in *Pdyn* expression (likely along the injection track) but an increase in *Drd1a* expression in

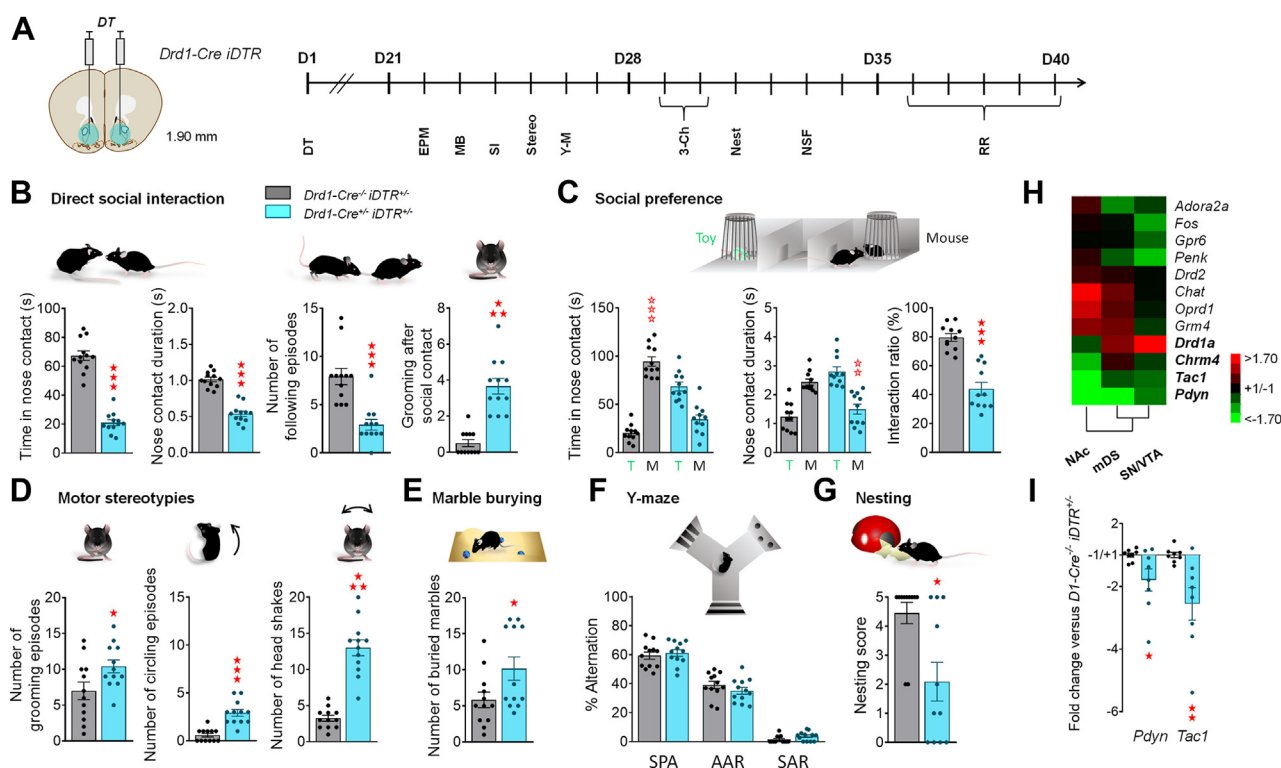


Figure 1. Behavioral and transcriptional consequences of ablating D1R-SPNs in the NAc. **(A)** Experimental design. Adult *Drd1-Cre^{+/+} iDTR^{+/+}* mice and *Drd1-Cre^{-/-} iDTR^{+/+}* control animals ($n = 11-14$ per group) were injected stereotaxically with DT in the NAc. Behavioral assays were performed successively between D21 and D40. **(B)** In the SI test, mice bearing D1R-SPN ablation (*Drd1-Cre^{+/+} iDTR^{+/+}*) in the NAc displayed shorter time spent in nose contact ($F_{1,22} = 141.4, p < .0001$), displayed reduced duration of these contacts ($F_{1,22} = 96.9, p < .0001$) and reduced number of following episodes ($U = 40.5, p < .0001$), and groomed more often immediately after a social contact ($U = 1.5, p < .0001$) compared with sham control animals (*Drd1-Cre^{+/+} iDTR^{+/+}*). **(C)** In the three-chamber test, mice with NAc D1R-SPN ablation preferred to stay longer in nose contact with the toy than with the mouse (stimulus \times ablation effect: $F_{1,20} = 40.9, p < .0001$) and made longer-lasting contact with the toy (stimulus \times ablation effect: $F_{1,20} = 18.21, p < .001$), resulting in a decrease in their interaction ratio compared with sham mice ($F_{1,20} = 45.8, p < .0001$). Compared with sham mice, lesioned mice **(D)** displayed more frequent grooming ($F_{1,22} = 5.1, p < .05$), circling ($U = 5.5, p < .0001$), and head shaking episodes ($F_{1,22} = 0.5, p < .0001$) and **(E)** buried more marbles in the marble burying test ($U = 37.0, p < .05$) without displaying perseverative same-arm entries in the Y maze. **(F)** Mice with NAc D1R-SPN ablation displayed lower nest-building scores than control animals ($U = 22.0, p < .05$). **(H)** Gene markers of D1R-SPNs (in bold) and not of D2R-SPNs were downregulated in the NAc of ablated mice, **(I)** notably the expression of the main markers *Pdyn* and *Tac1* (see Table S1). Behavioral and transcriptional results (fold change compared with sham control animals) are shown as scatter plots and mean \pm SEM. Solid stars indicate the ablation effect (one/two-way analysis of variance or Mann-Whitney U test), open stars indicate ablation \times stimulus interaction (two-way analysis of variance with repeated measures, mouse vs. object comparison). * $p < .05$, ** $p < .01$, *** $p < .001$. More behavioral parameters are shown in Figure S1. 3-Ch, three-chamber test; AAR, alternate-arm returns; D, day; D1R-SPN, D1 receptor-expressing striatal projection neuron; DT, diptheria toxin; EPM, elevated plus maze; M, mouse; MB, marble burying; mDS, medial dorsal striatum; NAc, nucleus accumbens; Nest, nesting; NSF, novelty-suppressed feeding; RR, rotarod; SAR, same-arm returns; SI, social interaction; SN, substantia nigra; SPA, spontaneous alternation; Stereo, stereotypies; T, toy; VTA, ventral tegmental area; Y-M, Y maze.

the mDS. Finally, in the SN/VTA, *Drd1a* expression was also upregulated, while *Penk* expression was downregulated, a possible compensation for NAc D1R-SPN ablation.

Therefore, selective ablation of D1R-SPNs in the NAc, evidenced by reduced expression of specific gene markers *Tac1* and *Pdyn* in this region, resulted in social interaction deficits and stereotypic behavior, facilitated motor skill learning, and increased anxiety levels in the EPM.

Ablation of D1R-SPNs in the DS Induces Stereotypies, Impairs Motor Learning, and Decreases Anxiety Without Impacting Social Behavior

We then assessed the effects of D1R-SPN ablation in the DS (Figure 2A; see also Figure S3). Selective lesions of D1R-SPNs had no detectable impact in the direct social interaction test (Figure 2B). Accordingly, DS D1R-SPN-ablated mice showed a similar preference to interact with a mouse in the three-chamber test as sham control animals (Figure 2C). In

contrast, these animals displayed more frequent motor stereotypies (Figure 2D) but unchanged marble burying (Figure 2E), exploration in the Y maze test (Figure 2F), and nest-building performance (Figure 2G). Nonetheless, this lesion produced a severe impairment in learning a motor skill on the accelerating rotarod (Figure S3E), as previously shown (57). Finally, mice bearing D1R-SPN ablation in the DS displayed shorter latencies to eat in the novelty-suppressed feeding test (Figure S3F) and explored the open arms more than sham control animals in the EPM test (Figure S3G), suggesting reduced levels of anxiety.

At the transcription level (Figure 2J-K; Table S3), deletion of DS D1R-SPNs produced a significant decrease in *Pdyn* and *Tac1* expression in the mDS. In the NAc, the expression of *Drd1a*, *Oprd1*, *Adora2a*, and *Pdyn* was upregulated; in the SN pars compacta/VTA, *Drd1a* and *Oprd1* expression was increased. These results indicate that D1R-SPN ablation in the DS did not spread to the NAc and that adaptations in gene expression developed in regions connected to DS.

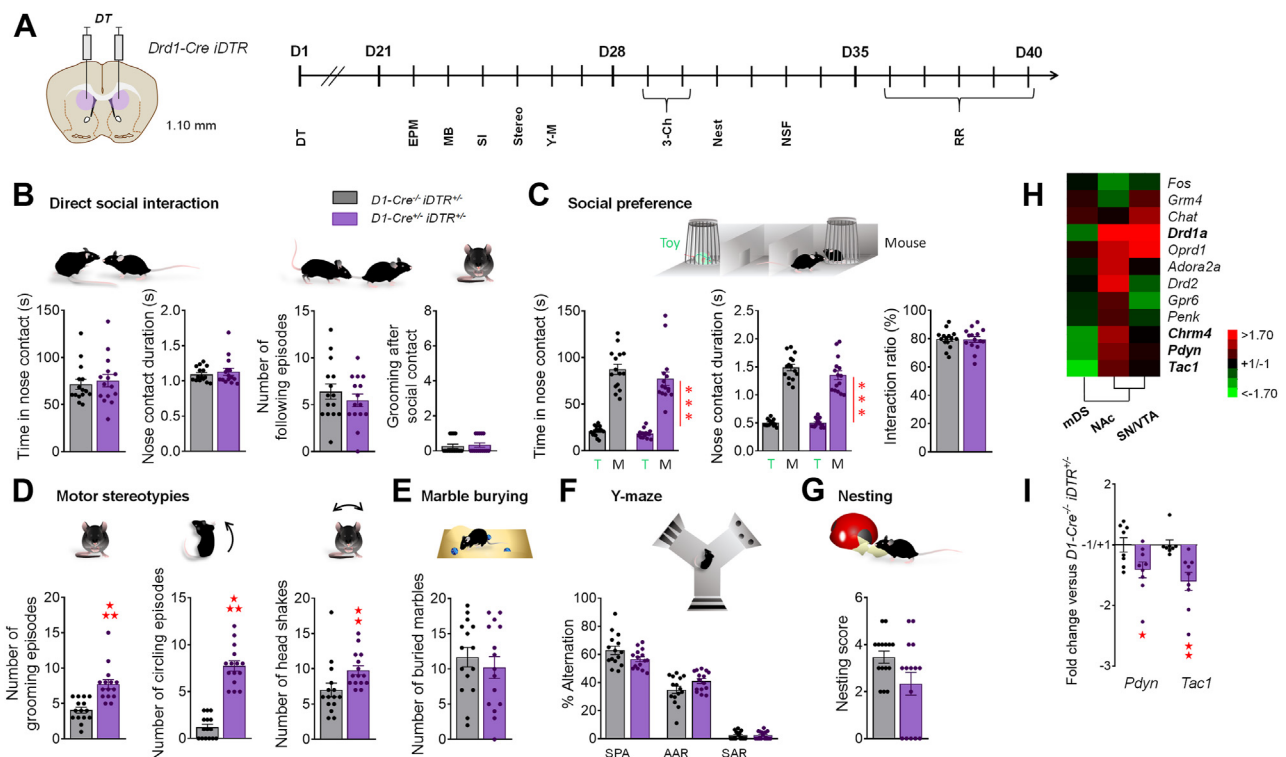


Figure 2. Behavioral and transcriptional consequences of ablating D1R-SPNs in the DS. **(A)** Experimental design. Adult *Drd1-Cre^{+/+} iDTR^{-/-}* mice and *D1-Cre^{-/-} iDTR^{-/-}* control animals ($n = 15$ per group) were injected stereotaxically with DT in the mDS. Behavioral assays were performed successively between D21 and D40. Mice with D1R-SPN ablation (*Drd1-Cre^{+/+} iDTR^{-/-}*) in the mDS showed similar **(B)** SI and **(C)** social preference to sham control animals (*D1-Cre^{+/+} iDTR^{-/-}*). **(D)** D1R-SPN ablation in the mDS increased spontaneous stereotypic grooming ($U = 16.0, p < .0001$), circling ($U = 0.0, p < .0001$), and head shaking ($U = 41.0, p < .001$), but ablated mice did not **(E)** bury more marbles in the marble burying test or **(F)** exhibit perseverative same-arm entries in the Y maze. **(G)** D1R-SPN ablation in the mDS failed to significantly affect nest building. **(H)** Gene markers of D1R-SPNs (in bold) were downregulated in the mDS of ablated mice, **(I)** notably the expression of the main markers *Pdyn* and *Tac1* (see Table S2). Behavioral and transcriptional results (fold change compared with sham control animals) are shown as scatter plots and mean \pm SEM. Solid stars indicate the ablation effect (one/two-way analysis of variance or Mann-Whitney U test) and asterisks indicate the stimulus effect (two-way analysis of variance with repeated measures). * $p < .05$, ** $p < .01$, *** $p < .001$. More behavioral parameters are shown in Figure S2. 3-Ch, three-chamber test; AAR, alternate-arm returns; D, day; D1R-SPN, D1 receptor-expressing striatal projection neuron; DS, dorsal striatum; DT, diptheria toxin; EPM, elevated plus maze; M, mouse; MB, marble burying; mDS, medial dorsal striatum; NAc, nucleus accumbens; Nest, nesting; NSF, novelty-suppressed feeding; RR, rotarod; SAR, same-arm returns; SI, social interaction; SN, substantia nigra; SPA, spontaneous alternation; Stereo, stereotypies; T, toy; VTA, ventral tegmental area; Y-M, Y maze.

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Together, these data indicate that the selective ablation of D1R-SPNs in the DS, confirmed by reduced expression of *Pdyn* and *Tac1* in this region, did not alter social behavior in mice, but rather increased spontaneous stereotypies, impaired motor skill learning, and decreased anxiety-like behavior.

Ablation of D2R-SPNs in the NAc Increases Social Approach and Impairs Motor Learning

Finally, we assessed the behavioral phenotype of mice bearing D2R-SPN ablation in the NAc (Figure 3A; see also Figure S4). Remarkably, D2R-SPN lesions increased direct social

interaction (Figure 3B) and preference for interacting with a mouse over a toy in the three-chamber test (Figure 3C). Ablated mice showed an increased number of spontaneous circling episodes (Figure 3D) but buried fewer marbles (Figure 3E). When exploring the Y maze, D2R-SPN-lesioned mice made more frequent alternations between two arms but fewer same-arm entries than control animals (Figure 3F); they showed normal performance in building a nest (Figure 3G) but impaired motor learning on the accelerating rotarod (Figure S4E). Finally, ablation of D2R-SPNs in the NAc had no detectable effect in the novelty-suppressed feeding (Figure S4F) and EPM (Figure S4G) tests.

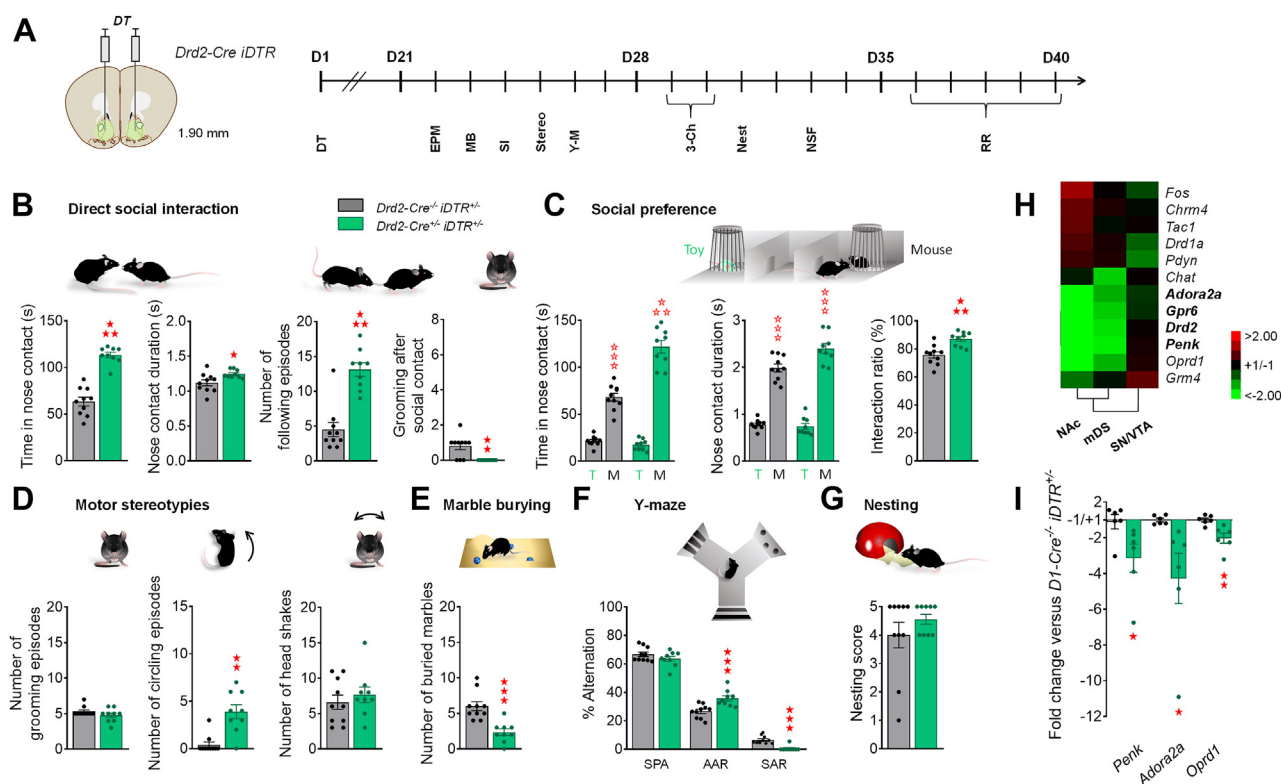
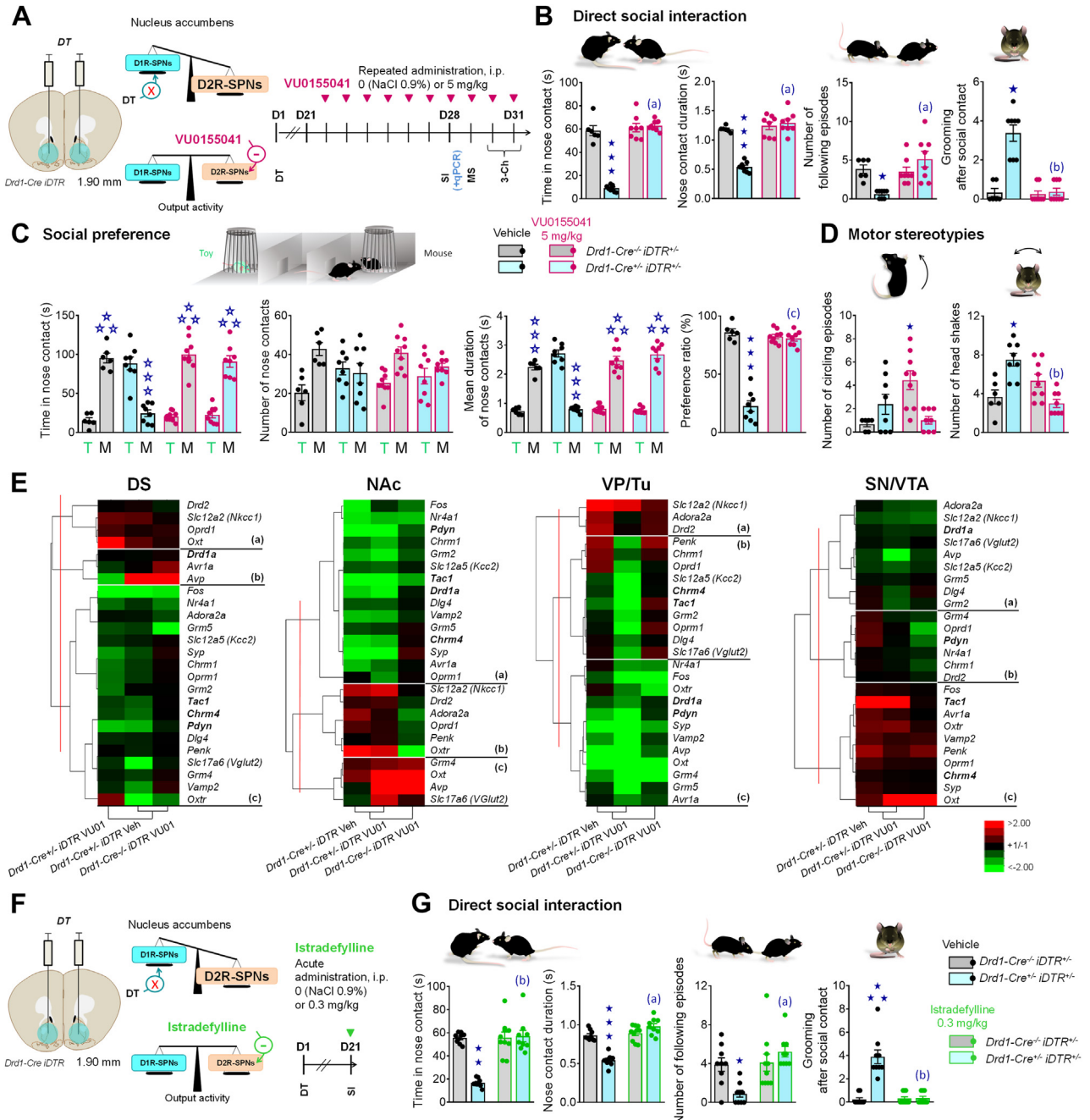


Figure 3. Behavioral and transcriptional consequences of D2R-SPN ablation in the NAc. **(A)** Experimental design. Adult *D2-Cre^{+/+} iDTR^{+/-}* mice and *D2-Cre^{-/-} iDTR^{+/-}* control animals ($n = 10$ per group) were injected stereotaxically with DT in the NAc. Behavioral assays were performed successively between D21 and D40. **(B)** In the SI test, mice bearing D2R-SPN ablation (*Drd2-Cre^{+/+} iDTR^{+/-}*) in the NAc spent more time in nose contact ($F_{1,17} = 74.8, p < .0001$) and made longer nose contacts ($F_{1,17} = 6.5, p < .05$) with the unfamiliar conspecific; they displayed more frequent following episodes ($U = 13.5, p < .001$) and groomed less after social contact ($U = 13.5, p < .01$) than sham control animals (*Drd2-Cre^{+/+} iDTR^{+/+}*). **(C)** In the three-chamber test, mice with NAc D2R-SPN ablation showed exacerbated social preference, as shown by a longer time spent in nose contact (stimulus \times ablation effect: $F_{1,17} = 39.3, p < .0001$) and a longer mean duration (stimulus \times ablation effect: $F_{1,17} = 9.7, p < .01$) of these contacts with the mouse over the toy than sham control animals, resulting in an increased interaction ratio ($F_{1,17} = 16.7, p < .001$). D2R-SPN ablation in the NAc resulted in **(D)** an increased number of spontaneous circling episodes ($U = 20.7, p < .001$) and **(E)** a decreased number of buried marbles in the marble burying test ($U = 3.5, p < .001$). Mice with NAc D2R-SPN ablation **(F)** made more AARs ($U = 7.0, p < .001$) and fewer SARs ($U = 5.0, p < .001$) in the Y maze and **(G)** displayed nesting scores similar to those of sham control animals. **(H)** Gene markers of D2R-SPNs (in bold) were downregulated in the NAc of ablated mice, as well as in the mDS, likely along the track of the injection cannula. **(I)** The two main gene markers of D2R-SPNs, *Penk* and *Adora2a*, were downregulated in the NAc of D2R-SPN-ablated mice, as was the CIN marker *Oprd1* (see Table S3). Behavioral and transcriptional results (fold change compared with sham control animals) are shown as scatter plots and mean \pm SEM. Solid stars indicate the ablation effect (one-way analysis of variance or Mann-Whitney *U* test) and open stars indicate the ablation \times stimulus interaction (two-way analysis of variance, mouse vs. object comparison), (a) indicates the ablation \times stimulus interaction (comparison with sham mouse condition, $p < .001$). * $p < .05$, ** $p < .01$, *** $p < .001$. More behavioral parameters are shown in Figure S3. 3-Ch, three-chamber test; AAR, alternate-arm returns; CIN, cholinergic interneuron; D, day; D2R-SPN, D₂ receptor-expressing striatal projection neuron; DS, dorsal striatum; DT, diptheria toxin; EPM, elevated plus maze; M, mouse; MB, marble burying; mDS, medial dorsal striatum; NAc, nucleus accumbens; Nest, nesting; NSF, novelty-suppressed feeding; RR, rotarod; SAR, same-arm returns; SI, social interaction; SN, substantia nigra; SPA, spontaneous alternation; Stereo, stereotypies; T, toy; VTA, ventral tegmental area; Y-M, Y maze.

At the transcription level (Figure 3H, I; Table S4), D2R-SPN ablation in the NAc decreased the expression of the D2R-SPN marker genes *Penk*, *Adora2a*, *Drd2*, and *Gpr6* and the CIN marker gene *Oprd1*; *Fos* expression was increased. In the mDS, the expression of *Penk*, *Adora2a*, *Gpr6*, and *Drd2* was downregulated, likely along the cannula track, as well as the

expression of CIN markers *Chat* and *Oprd1*. In the VTA, the expression of *Grm4* was upregulated.

Thus, D2R-SPN lesions in the NAc, likely associated with CIN lesions, as evidenced by reduced *Penk*, *Adora2a*, and *Oprd1* expression in the NAc, facilitated social interaction and preference, increased circling behavior but decreased marble



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burying and perseveration in the Y maze, and hindered motor skill learning, with no effect on anxiety-like behavior.

Pharmacological Inhibition of D2R-SPN Activity Relieves Behavioral Deficits and Represses VP Gene Expression in Mice With D1R-SPN Ablation in the NAc

We then tested whether administering pharmacological compounds that dampen D2R-SPN activity may alleviate the behavioral impairments of mice bearing NAc D1R-SPN ablation. We chronically treated lesioned mice with the positive allosteric modulator (PAM) of the mGlu4 receptor VU0155041 (5 mg/kg, intraperitoneal) (Figure 4A), a repressor of D2R-SPN activity (60). We then assessed social and stereotyped behaviors. Chronic VU0155041 administration normalized the social behavior of NAc D1R-SPN-ablated mice (Figure 4B) in the direct social interaction and three-chamber tests (Figure 4C), as well as normalized stereotyped circling and head shaking (Figure 4D; Figure S5). Chronic mGlu4 PAM treatment was thus able to relieve the deleterious behavioral effects of D1R-SPN ablation in the NAc.

In search for molecular substrates modified by chronic VU0155041 treatment, we assessed the expression of 24 genes in the NAc, mDS, VP/olfactory tubercle (VP/Tu), and SN pars compacta/VTA in the mGlu4 PAM versus vehicle-treated mice 45 minutes after a direct social interaction. These genes included marker genes of D1R-SPNs (*Drd1a*, *Tac1*, *Pdyn*, *Chrm4*), D2R-SPNs (*Drd2*, *Adora2*, *Penk*, *Gpr6*, *Grm4*), D1R/D2R-SPNs (*Chrm1*, *Grm2*, *Grm5*, *Nr4a1*, *Oprm1*), or CINs (*Chat*, *Oprd1*); genes of the oxytocin/vasopressin system (*Oxt*, *Oxtr*, *Avp*, *Avr1a*), involved in social behavior (61,62); genes coding for chloride and glutamate transporters (*Slc12a2*, *Slc12a5*, *Slc17a6*); *Fos* as a marker of neuronal transcription; and finally *Dlg4*, *Syp*, and *Vamp2* as marker genes of synaptic function. As shown by cluster analysis (Figure 4E; Table S5), chronic VU0155041 triggered a marked downregulation of

gene expression in the VP/Tu (clusters b, c), in addition to that produced by NAc D1R-SPN ablation (cluster c). To confirm our hypothesis of excessive D2R-SPN activity as the main contributor to altered behavior in mice bearing NAc D1R-SPN ablation, we evaluated the behavioral effects of another pharmacological repressor of D2R-SPN activity, the adenosine A2a receptor antagonist istradefylline (Figure 4F) (63). Single istradefylline injection (0.3 mg/kg) restored direct social interaction in mice bearing NAc D1R-SPN ablation (Figure 4G; Figure S5).

Thus, pharmacological treatments that repress the activity of D2R-SPNs can relieve the deleterious behavioral effects of NAc D1R-SPN lesions. Beneficial behavioral effects of the mGlu4 PAM VU0155041 in NAc D1R-SPN-ablated mice were associated with transcriptional inhibition in the VP/Tu.

Prolonged, and Not Short, Optical Stimulation of the D2R-SPNs in the NAc Hampers Social Interaction in Mice

To further challenge our hypothesis of a critical contribution of excessive D2R-SPN output to behavioral deficits in NAc D1R-SPN-ablated mice, we tested whether optical stimulation of D2R-SPNs may affect social behavior in mice. *Adora2a-Cre* mice were injected in the NAc with AAV5-EF1-DIO-h-ChR2-YFP containing a Cre-dependent channelrhodopsin (ChR2) or eYFP (Figure 5A). Based on a previous study showing differential outcomes on reward/aversion (64), we compared the effects of short (1 second) versus prolonged (60 seconds) optical D2R-SPN stimulation on direct social interaction in NAc adeno-associated virus-injected mice. We then assessed the effects of acute VU0155041 administration (10 mg/kg, 30 minutes before testing) on further long D2R-SPN optical stimulation.

Brief optical D2R-SPN stimulation facilitated direct social interaction (Figure 5B; Figure S6), while prolonged stimulation inhibited social contacts in *Adora2a-Cre-ChR2* mice; prior VU0155041 treatment prevented the long stimulation-

Figure 4. Behavioral and transcriptional consequences of D2R-SPN pharmacological inhibition in mice bearing D1R-SPN ablation in the NAc. **(A)** Experimental design. Mice bearing D1R-SPN ablation in the NAc and control animals were treated chronically with the mGlu4 PAM VU0155041 to rebalance the D1R/D2R-SPN output activity balance. They received VU0155041 (5 mg/kg, i.p.) or vehicle (NaCl, 0.9%) daily from D21 after surgery to D31. Behavioral testing was performed from D28 to D31 (cohort 1 [$n = 6-8$ per group]). In cohort 2 ($n = 6$ per group), mice were euthanized 45 minutes after direct SI (D28), and brain samples were collected for qRT-PCR analysis. **(B)** Chronic VU0155041 administration normalized SI parameters in NAc D1R-SPN-ablated mice, as illustrated by rescued time spent in (treatment \times ablation effect: $F_{1,26} = 84.8$, $p < .0001$) and mean duration of (treatment \times ablation effect: $F_{1,26} = 43.7$, $p < .0001$) nose contact, number of subsequent episodes ($H_{3,30} = 18.2$, $p < .001$), and grooming after social contact ($H_{3,30} = 19.9$, $p < .001$). **(C)** Similarly, in the three-chamber test, chronic facilitation of mGlu4 activity restored the preference for spending more time (stimulus \times treatment \times ablation: $F_{1,27} = 56.3$, $p < .0001$) and making longer nose contacts (stimulus \times treatment \times ablation: $F_{1,27} = 146.4$, $p < .05$) with the mouse over the toy in ablated mice, resulting in a normalized interaction ratio ($H_{3,31} = 4.3$, $p < .001$). **(D)** Finally, VU0155041 treatment suppressed spontaneous circling episodes ($H_{3,31} = 11.7$, $p < .01$) and head shakes ($H_{3,31} = 15.9$, $p < .01$) in mice bearing D1R-SPN ablation. **(E)** Gene expression was not restored upon chronic VU0155041 administration in the NAc of NAc D1R-SPN-ablated mice (cluster a) but was widely repressed in the VP/Tu of these animals (clusters b, c) compared with saline-treated sham control animals (see Table S4). **(F)** Experimental design. Mice bearing D1R-SPN ablation in the NAc and control animals were treated acutely with the A2a adenosine receptor antagonist istradefylline to rebalance D1R/D2R-SPN output activity balance. Istradefylline (0.3 mg/kg, i.p.) or vehicle (NaCl 0.9%) was administered acutely ($n = 9$ or 10 per group) 30 minutes before testing (D21 after surgery). **(G)** Acute istradefylline normalized the time spent in ($H_{3,37} = 20.0$, $p < .001$) and duration of (treatment \times ablation: $F_{1,33} = 48.9$, $p < .0001$) nose contact, as well as the number of following ($H_{3,37} = 17.9$, $p < .001$) and grooming ($H_{3,37} = 24.1$, $p < .0001$) episodes after social contact in NAc D1R-SPN-ablated mice. Behavioral results are shown as scatter plots and mean \pm SEM. Solid stars indicate the ablation \times treatment effect (two-way or Kruskal-Wallis analysis of variance, comparison with *D1-Cre^{-/-} iDTR^{+/-}* mice treated with saline), open stars indicate the ablation \times stimulus effect (3-way analysis of variance, mouse vs. object comparison). * $p < .05$, *** $p < .001$. Comparison with *Drd1-Cre^{+/-} iDTR^{+/-}* mice treated with saline: (a) $p < .001$, (b) $p < .01$, (c) $p < .05$. More behavioral parameters are shown in Figure S4. 3-Ch, three-chamber test; D, day; D1R-SPN, D₁ receptor-expressing striatal projection neuron; D2R-SPN, D₂ receptor-expressing striatal projection neuron; DT, diphtheria toxin; i.p., intraperitoneal; M, mouse; MS, motor stereotypies; NAc, nucleus accumbens; PAM, positive allosteric modulator; qRT-PCR, quantitative real-time polymerase chain reaction; SI, social interaction; SN, substantia nigra; T, toy; Tu, olfactory tubercle; Veh, vehicle; VP, ventral pallidum; VTA, ventral tegmental area.

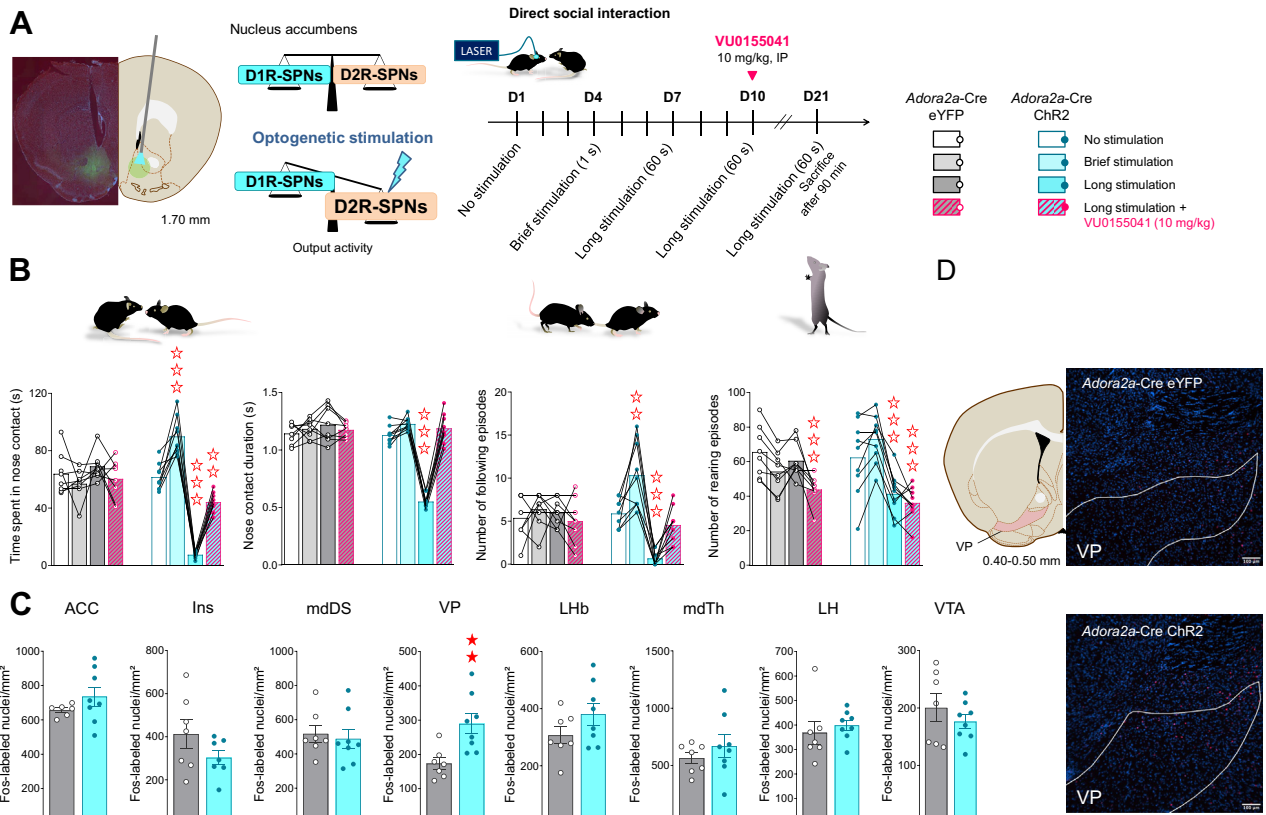


Figure 5. Effects of optical stimulation of D2R-SPN in the NAC on social interaction and Fos expression in *Adora2a-Cre* mice. **(A)** Experimental design. *Adora2a-Cre* mice ($n = 9$) were injected in the NAC with AAV5 EF1a-DIO-hChR2(H134R)-eYFP containing a Cre-dependent channelrhodopsin (ChR2) or eYFP, and direct optical stimulation was applied to mimic excessive D2R-SPN activity. Social interaction of *Adora2a-Cre-ChR2* mice and *Adora2a-Cre-YFP* control animals ($n = 8$) was first assessed in the absence of light (D1), then under short stimulation conditions (light on 1 second, D4) and under long stimulation conditions (light on 60 seconds, D7). On D10, all mice received VU0155041 (10 mg/kg) 30 minutes before being tested for social interaction under prolonged stimulation conditions. After a week drug-free, all mice were again submitted to a social interaction test under prolonged stimulation conditions (D21) and sacrificed 90 minutes later for Fos immunostaining experiments. **(B)** Brief optical D2R-SPN stimulation in the NAC facilitated direct social interaction, as shown by increased time spent in nose contact and the number of following episodes in *Adora2a-Cre-ChR2* mice. In contrast, prolonged stimulation markedly decreased social interaction, as evidenced by reduced time spent in and mean duration of nose contact, as well as a reduced number of subsequent episodes in *Adora2a-Cre-ChR2* mice. The number of rearing episodes was also reduced under these conditions. Finally, chronic VU0155041 treatment opposed the deleterious effects of prolonged stimulation on social interaction parameters, increasing the time spent in nose contact (viral construct \times condition effect: $F_{3,45} = 42.8, p < .0001$) and restoring nose contact duration (viral construct \times condition effect: $F_{3,45} = 80.4, p < .0001$) and the number of subsequent episodes (viral construct \times condition effect: $F_{3,45} = 11.0, p < .0001$) without normalizing the number of rearing episodes. **(C)** We detected an increase in Fos expression in the VP ($F_{1,13} = 10.7, p < .01$). **(D)** Representative microphotographs showing Fos-positive nuclei in the VP of *Adora2a-Cre-YFP* (upper panel) and *Adora2a-Cre-ChR2* (lower panel) after prolonged NAC D2R-SPN optical stimulation. Magnification = $\times 6.25$; scale bar = 100 μm . Behavioral and immunohistochemical results are shown as scatter plots and mean \pm SEM. Solid stars indicate optical stimulation \times stimulus interaction (two-way analysis of variance, compared with the no-light condition in *Adora2a-Cre-YFP* mice), open stars indicate optical stimulation \times stimulus interaction (Student's t test, compared with prolonged stimulation condition in *Adora2a-Cre-YFP* mice). ** $p < .01$, *** $p < .001$. More behavioral parameters are shown in Figure S5. ACC, anterior cingulate cortex; D, day; D1R-SPN, D₁ receptor-expressing striatal projection neuron; D2R-SPN, D₂ receptor-expressing striatal projection neuron; Ins, insular cortex; IP, intra-peritoneal; LH, lateral hypothalamus; LHb, lateral habenula; mDS, medial dorsal striatum; mdTh, medial dorsal thalamus; NAc, nucleus accumbens; VP, ventral pallidum; VTA, ventral tegmental area.

provoked deficit in social interaction. The beneficial effects of VU0155041 treatment were independent of the effects on vertical activity. We evaluated the neuronal consequences of long D2R-SPN stimulation by assessing Fos expression in several NAc afferences/efferences after a last social interaction test (Figure 5C) and detected an increase in Fos expression in the VP (Figure 5D).

Together, our data indicate that prolonged optical stimulation of NAC D2R-SPNs impedes social interaction, an effect

blocked by prior administration of an mGlu4 PAM and associated with increased neuronal activity in the VP.

DISCUSSION

Based on the classical dichotomy between D1R-SPNs and D2R-SPNs in driving reward versus aversion (45,48,49,52), activation of the D1R-SPNs has been proposed to drive social approach, while activation of the D2R-SPNs leads to social

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withdrawal. In the present study, we observed a severe deficit in social interaction and preference following selective ablation of D1R-SPNs in the NAc. When D1R-SPN ablation targeted the DS, no social deficit was detected. These findings are consistent with a prosocial role of NAc D1R-SPNs, in agreement with their activation during social encounter (47) and the effects of their optogenetic stimulation in typically behaving (13,53) or depressive-like (31) mice. In contrast, *Shank3* knockdown in NAc D1R-SPNs increased their excitability and concomitantly reduced social preference in mice (53), suggesting that excessive D1R-SPN activity may also lead to social avoidance, possibly by involving VP-projecting populations (47). However, increased D1R-SPN excitability in *Shank3* mutant mice can coexist with decreased output activity, as seen in mouse models of depression (31). Deleting the diacylglycerol lipase α gene (*Dagla*) from D1R-SPNs increased their spontaneous activity; when this deletion was restricted to the mDS, but not to the NAc, social preference was impaired (56), as observed following D1R stimulation (65), indicating that DS D1R-SPNs likely participate in controlling social behavior.

Mirroring the effects of D1R-SPN ablation, NAc D2R-SPN ablation facilitated social behavior. Accordingly, mice lacking the D2R, which inhibits the activity of D2R-SPNs, showed deficient social behavior (65). Spontaneous activity of D2R-SPNs was increased in the NAc of depressive-like mice, correlating negatively with social interaction (31). These elements argue for a repressing role of D2R-SPNs, notably of the NAc, on social behavior. Not only NAc D2R-SPNs were ablated in our study (66), however, as revealed by decreased CIN gene marker expression. Indeed, to drive iDTR expression, we used a *Drd2* promoter expressed in both striatal D2R-SPNs and CINs (67,68). CINs have also been implicated in the control of social behavior. Total striatal ablation of CINs exacerbated social behavior in mice (69). In contrast, DS-restricted CIN deletion reduced social interaction in male mice (70). Thus, increased social behavior in NAc DT-injected *Drd2-Cre^{+/-}iDTR^{+/-}* mice may have resulted, at least in part, from CIN ablation. The net effect of CIN ablation on striatal SPN output activity has not yet been fully elucidated; in the DS, CIN activation by thalamic inputs increases D2R-SPN responsiveness to corticostriatal inputs (71). If this was applied to NAc circuitry, NAc CIN ablation would reduce D2R-SPN activity, resembling D2R-SPN ablation; exacerbated social behavior following D2R-SPN and/or CIN ablation in the NAc may then result from reduced D2R-SPN output. However, the results from later pharmacological and optogenetic experiments, targeting selectively D2R-SPNs, suggest that the CIN contribution was minimal.

In addition to social behavior, SPN ablation increased motor stereotypies. Insufficient D1R-SPN output (72), excessive D2R-SPN activity, and conversely, insufficient D2R-SPN activity (73) were all reported to cause stereotypies. In contrast, complete ablation of striatal CINs did not produce such an outcome (69), suggesting a limited contribution of this neuronal population. The impact of SPN ablation on social behavior was well correlated with nest building, which is considered a social-related motor response (74), but not with motor skill learning. Therefore, social deficits in NAc D1R-SPN-lesioned mice were likely not a consequence of motor dysfunction, even though

NAc D1R-SPNs are involved in the control of motor skills (48,57,75). Finally, modulation of emotional response following SPN ablation was not predictive of modifications in social behavior, making it unlikely that modified levels of anxiety accounted for social behavior deficits or improvements. Globally, our data point to a dominant role of NAc D1R-SPNs in modulating anxiety-like behavior, in contrast to the traditional view (76–78). Of note, no retrograde ablation of neurons outside the striatum, projecting to SPNs and expressing DTR due to their expression of *Drd1a* or *Drd2* (such as dopamine neurons), was observed after D1R-SPN or D2R-SPN ablation, which could account for the observed behavioral changes [see (66)] (Table S2). Whether the effects of NAc D1R-SPN ablation indeed reflected deficient D1R-SPN output, however, is an intriguing question.

Previous studies have conceptualized a dualistic view of striatal outputs in which D1R-SPNs drive reward/social approach and D2R-SPNs trigger aversion/social avoidance. Here, we propose that the main driver of social deficit in NAc D1R-SPN-ablated mice is less related to the missing D1R-SPN outputs than to the resulting excessive weight of D2R-SPN outputs. In support of this hypothesis, our pharmacological experiments showed that facilitating mGlu4 activity or antagonizing A2a activity, two interventions that repress D2R-SPN activity, restored social behavior in NAc D1R-SPN-lesioned mice. These results are consistent with the beneficial effects of VU0155041 in the *Oprm1* mouse model of ASD (58,79); in *Elfn2* null mice, displaying ASD-like behavioral features (80); and in morphine-abstinent mice (60), in which NAc D2R-SPN activity is excessive (81). Similarly, A2a antagonism restored social behavior in mice exposed to social stress (82). Importantly, A2a receptors are specifically expressed in the D2R-SPN of the striatum (83), the only brain region where their distribution overlaps with that of mGlu4 receptors (84). These data point to an excess in D2R-SPN activity as the neuronal substrate of social deficit in NAc D1R-SPN-ablated mice. Beneficial effects of mGlu4 PAM treatment in these mice were associated with a repression of gene transcription in the VP/Tu region. Together, pharmacological and transcriptional data suggest a beneficial inhibitory action of mGlu4 PAM treatment on VP neurons in D1R-SPN-ablated mice.

The second piece of evidence supporting our hypothesis of excessive D2R-SPN activity as the main contributor to behavioral deficits in NAc D1R-SPN-ablated mice comes from optogenetic experiments. Upon short activation, shown to be rewarding (45,64,85), social investigation was facilitated in *Adora2a-Cre-ChR2* mice. Conversely, prolonged D2R-SPN stimulation in the NAc, which demonstrated aversive effects (64), produced a severe deficit in social interaction. Of note, the use of the *Adora2a* promoter allowed us to specifically target D2R-SPNs and not CINs. Long-lasting stimulation increased neuronal activity in the VP, consistent with prior electrophysiological evidence (64,86). Paradoxical increased activity in brain targets of GABAergic (gamma-aminobutyric acidergic) D2R-SPNs may involve enkephalin corelease, inducing delta opioid receptor-mediated presynaptic long-term depression (LTD) and decreasing GABA exocytosis (64,86). Increased VP activity may have dampened the activity of VTA dopamine neurons (64) and therefore contributed to supporting social aversion. Finally, mGlu4 facilitation prevented the deleterious

effects of prolonged NAc D2R-SPN stimulation on social behavior, possibly by reducing presynaptic GABA release from D2R-SPNs. Together, these results indicate that excessive activity of D2R-SPNs in the NAc produces a deficit in social behavior.

Converging preclinical data suggest that NAc D1R/D2R-SPN output balance could be tilted toward excessive D2R-SPN activity in depression, ASD, and schizophrenia. This has been evidenced in mouse models of depression, in which D1R-SPNs showed increased excitability and reduced output activity (31,87), morphological atrophy (88), and decreased excitatory inputs (31,87,89). Accordingly, elevated D1R-SPN activity promotes resilience to depressive-like outcomes (31,90). In contrast, D2R-SPNs showed increased excitatory inputs and spine density in these models (31,91), and their stimulation facilitated the development of depression-like behavior (31). In mouse models of ASD, even though experimental data consistently point to SPN dysfunction (65,92–96), D1R/D2R-SPN balance has rarely been assessed. Synaptic inhibition of D1R-SPNs but not D2R-SPNs is decreased in neuroigin-3 mutant mice (75), but D1R/D2R-SPN output activity has not been directly assessed. The excitability, dendritic complexity, and capacity to induce endocannabinoid-mediated LTD are reduced in D2R-SPNs from *Shank3* mutant mice (73). Remarkably, blunted presynaptic LTD in D2R-SPNs could facilitate their activity under sustained stimulation, as seen at D2R-SPN/VP synapses (86). Decreased endocannabinoid-mediated LTD was also observed in NAc SPNs of *Fmr1* knockout mice (94) and in DS SPNs of neuroigin-3 mutant mice (93), although the affected SPN population remains to be identified. Finally, striatal D2R availability is increased under dopamine depletion in schizophrenia (97,98). Blocking D2R activity with antipsychotics relieves positive symptoms (hallucinations, delusions) but fails to improve negative symptoms (anhedonia, social deficits), the severity of which is instead negatively correlated with NAc D2R availability in patients (97). An original therapeutic approach could thus be to combine D2R antagonism (antipsychotics) with molecules inhibiting D2R-SPN activity, ideally specifically in the NAc, to alleviate negative symptoms in schizophrenia. Preclinical assessment of D1R/D2R-SPN activity balance, not yet performed, may challenge this hypothesis. If excessive D2R-SPN activity represented a transnosographical feature between ASD, depression, and schizophrenia, this may account for shared deficits in social behavior and open novel therapeutic avenues.

There are limitations to our study. The primary focus of ablation experiments was NAc D1R-SPNs. We used D1R-SPN ablation in the DS and D2R-SPN ablation in the NAc as control experiments, but we did not explore the role of D2R-SPNs in the DS. We used qRT-PCR to assess the efficiency of SPN ablation. This technique has poor anatomical resolution but allowed us to quantitatively measure the expression of multiple genes in various brain regions, providing a wider picture of the consequences of ablation at the circuit level. Finally, in optogenetic studies, we did not assess the consequences of prolonged D1R-SPN stimulation of social behavior, which may result in social aversion (47,53). Further studies will be required to investigate the consequences of D2R-SPN ablation in DS and D1R-SPN stimulation in the NAc.

In conclusion, pharmacological compounds developed to repress D2R-SPN activity, such as mGlu4 PAMs or A2a antagonists, likely represent promising candidates to relieve the social deficit common to ASD, schizophrenia, and depression.

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JLM, AdKdE, and JAJB designed the experiments. BD performed stereotaxic surgeries for selective ablation experiments; BD, ADG, JLM, and JAJB performed behavioral testing. JG performed quantitative real-time polymerase chain reaction experiments. ADG performed stereotaxic surgeries, fiber implantations, optostimulation during behavior, and Fos immunohistochemistry for optogenetic experiments; MF quantified Fos expression. BD, JG, MF, ADG, JLM, AdKdE, and JAJB analyzed the data. JLM, AdKdE, and JAJB supervised the project and raised funding. JLM, AdKdE, and JAJB wrote the article. All authors discussed the findings and edited and contributed to the final version of the manuscript.

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REFERENCES

1. Fehr E, Camerer CF (2007): Social neuroeconomics: The neural circuitry of social preferences. *Trends Cogn Sci* 11:419–427.
2. Neuhaus E, Beauchaine TP, Bernier R (2010): Neurobiological correlates of social functioning in autism. *Clin Psychol Rev* 30:733–748.
3. Trezza V, Baarendse PJ, Vanderschuren LJ (2010): The pleasures of play: Pharmacological insights into social reward mechanisms. *Trends Pharmacol Sci* 31:463–469.
4. Enneking V, Krussel P, Zaremba D, Dohm K, Grotegerd D, Forster K, et al. (2019): Social anhedonia in major depressive disorder: A

Striatofugal Balance and Social Behavior

- symptom-specific neuroimaging approach. *Neuropsychopharmacology* 44:883–889.
5. Chevallier C, Kohls G, Troiani V, Brodtkin ES, Schultz RT (2012): The social motivation theory of autism. *Trends Cogn Sci* 16:231–239.
 6. Mow JL, Gandhi A, Fulford D (2020): Imaging the "social brain" in schizophrenia: A systematic review of neuroimaging studies of social reward and punishment. *Neurosci Biobehav Rev* 118:704–722.
 7. Fox ME, Lobo MK (2019): The molecular and cellular mechanisms of depression: A focus on reward circuitry. *Mol Psychiatry* 24:1798–1815.
 8. Chen P, Hong W (2018): Neural circuit mechanisms of social behavior. *Neuron* 98:16–30.
 9. Stanley DA, Adolphs R (2013): Toward a neural basis for social behavior. *Neuron* 80:816–826.
 10. Walsh JJ, Christoffel DJ, Malenka RC (2023): Neural circuits regulating prosocial behaviors. *Neuropsychopharmacology* 48:79–89.
 11. Gu R, Huang W, Camilleri J, Xu P, Wei P, Eickhoff SB, *et al.* (2019): Love is analogous to money in human brain: Coordinate-based and functional connectivity meta-analyses of social and monetary reward anticipation. *Neurosci Biobehav Rev* 100:108–128.
 12. Fernandez M, Mollinedo-Gajate I, Penagarikano O (2018): Neural circuits for social cognition: Implications for autism. *Neuroscience* 370:148–162.
 13. Gunaydin LA, Grosenick L, Finkelstein JC, Kauvar IV, Fenno LE, Adhikari A, *et al.* (2014): Natural neural projection dynamics underlying social behavior. *Cell* 157:1535–1551.
 14. Francis TC, Lobo MK (2017): Emerging role for nucleus accumbens medium spiny neuron subtypes in depression. *Biol Psychiatry* 81:645–653.
 15. Mannella F, Gurney K, Baldassarre G (2013): The nucleus accumbens as a nexus between values and goals in goal-directed behavior: A review and a new hypothesis. *Front Behav Neurosci* 7:135.
 16. Scofield MD, Heinsbroek JA, Gipson CD, Kupchik YM, Spencer S, Smith AC, *et al.* (2016): The nucleus accumbens: Mechanisms of addiction across drug classes reflect the importance of glutamate homeostasis. *Pharmacol Rev* 68:816–871.
 17. Floresco SB (2015): The nucleus accumbens: An interface between cognition, emotion, and action. *Annu Rev Psychol* 66:25–52.
 18. Humphries MD, Prescott TJ (2010): The ventral basal ganglia, a selection mechanism at the crossroads of space, strategy, and reward. *Prog Neurobiol* 90:385–417.
 19. Kohls G, Perino MT, Taylor JM, Madva EN, Cayless SJ, Troiani V, *et al.* (2013): The nucleus accumbens is involved in both the pursuit of social reward and the avoidance of social punishment. *Neuropsychologia* 51:2062–2069.
 20. Schmidt SNL, Fenske SC, Kirsch P, Mier D (2019): Nucleus accumbens activation is linked to salience in social decision making. *Eur Arch Psychiatry Clin Neurosci* 269:701–712.
 21. Pellissier LP, Gandia J, Laboute T, Becker JAJ, Le Merrer J (2018): μ Opioid receptor, social behaviour and autism spectrum disorder: Reward matters. *Br J Pharmacol* 175:2750–2769.
 22. Rademacher L, Schulte-Ruther M, Hanewald B, Lammertz S (2017): Reward: From basic reinforcers to anticipation of social cues. *Curr Top Behav Neurosci* 30:207–221.
 23. Lee J, Jimenez AM, Reavis EA, Horan WP, Wynn JK, Green MF (2019): Reduced neural sensitivity to social vs nonsocial reward in schizophrenia. *Schizophr Bull* 45:620–628.
 24. Heller AS, Johnstone T, Shackman AJ, Light SN, Peterson MJ, Kolden GG, *et al.* (2009): Reduced capacity to sustain positive emotion in major depression reflects diminished maintenance of fronto-striatal brain activation. *Proc Natl Acad Sci U S A* 106:22445–22450.
 25. Xu P, Klaasen NG, Opmeer EM, Pijnenborg GHM, van Tol MJ, Liemburg EJ, *et al.* (2019): Intrinsic mesocorticolimbic connectivity is negatively associated with social motivation in people with schizophrenia. *Schizophr Res* 208:353–359.
 26. Supekar K, Kochalka J, Schaefer M, Wakeman H, Qin S, Padmanabhan A, *et al.* (2018): Deficits in mesolimbic reward pathway underlie social interaction impairments in children with autism. *Brain* 141:2795–2805.
 27. Koshiyama D, Fukunaga M, Okada N, Yamashita F, Yamamori H, Yasuda Y, *et al.* (2018): Role of subcortical structures on cognitive and social function in schizophrenia. *Sci Rep* 8:1183.
 28. Fuccillo MV (2016): Striatal circuits as a common node for autism pathophysiology. *Front Neurosci* 10:27.
 29. Platt RJ, Zhou Y, Slaymaker IM, Shetty AS, Weisbach NR, Kim JA, *et al.* (2017): Chd8 mutation leads to autistic-like behaviors and impaired striatal circuits. *Cell Rep* 19:335–350.
 30. Asif-Malik A, Dautan D, Young AMJ, Gerdjikov TV (2017): Altered cortico-striatal crosstalk underlies object recognition memory deficits in the sub-chronic phencyclidine model of schizophrenia. *Brain Struct Funct* 222:3179–3190.
 31. Francis TC, Chandra R, Friend DM, Finkel E, Dayrit G, Miranda J, *et al.* (2015): Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biol Psychiatry* 77:212–222.
 32. Lipina TV, Fletcher PJ, Lee FH, Wong AH, Roder JC (2013): Disrupted-in-schizophrenia-1 Gln31Leu polymorphism results in social anhedonia associated with monoaminergic imbalance and reduction of CREB and beta-arrestin-1,2 in the nucleus accumbens in a mouse model of depression. *Neuropsychopharmacology* 38:423–436.
 33. Choe KY, Bethlehem RAI, Safran M, Dong H, Salman E, Li Y, *et al.* (2022): Oxytocin normalizes altered circuit connectivity for social rescue of the *Cntnap2* knockout mouse. *Neuron* 110:795–808 e796.
 34. Manduca A, Servadio M, Damsteegt R, Campolongo P, Vanderschuren LJ, Trezza V (2016): Dopaminergic neurotransmission in the nucleus accumbens modulates social play behavior in rats. *Neuropsychopharmacology* 41:2215–2223.
 35. Dolen G, Darvishzadeh A, Huang KW, Malenka RC (2013): Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature* 501:179–184.
 36. Trezza V, Damsteegt R, Achterberg EJ, Vanderschuren LJ (2011): Nucleus accumbens mu-opioid receptors mediate social reward. *J Neurosci* 31:6362–6370.
 37. Manduca A, Lassalle O, Sepers M, Campolongo P, Cuomo V, Marsicano G, *et al.* (2016): Interacting cannabinoid and opioid receptors in the nucleus accumbens core control adolescent social play. *Front Behav Neurosci* 10:211.
 38. Smith CJW, Wilkins KB, Li S, Tulimieri MT, Veenema AH (2018): Nucleus accumbens mu opioid receptors regulate context-specific social preferences in the juvenile rat. *Psychoneuroendocrinology* 89:59–68.
 39. Keebaugh AC, Barrett CE, Laprairie JL, Jenkins JJ, Young LJ (2015): RNAi knockdown of oxytocin receptor in the nucleus accumbens inhibits social attachment and parental care in monogamous female prairie voles. *Soc Neurosci* 10:561–570.
 40. Yu CJ, Zhang SW, Tai FD (2016): Effects of nucleus accumbens oxytocin and its antagonist on social approach behavior. *Behav Pharmacol* 27:672–680.
 41. Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, *et al.* (1990): D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250:1429–1432.
 42. Ho H, Both M, Siniard A, Sharma S, Notwell JH, Wallace M, *et al.* (2018): A guide to single-cell transcriptomics in adult rodent brain: The medium spiny neuron transcriptome revisited. *Front Cell Neurosci* 12:159.
 43. Lobo MK, Karsten SL, Gray M, Geschwind DH, Yang XW (2006): FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat Neurosci* 9:443–452.
 44. Heiman M, Schaefer A, Gong S, Peterson JD, Day M, Ramsey KE, *et al.* (2008): A translational profiling approach for the molecular characterization of CNS cell types. *Cell* 135:738–748.
 45. Soares-Cunha C, Coimbra B, Sousa N, Rodrigues AJ (2016): Reappraising striatal D1- and D2-neurons in reward and aversion. *Neurosci Biobehav Rev* 68:370–386.
 46. Kupchik YM, Brown RM, Heinsbroek JA, Lobo MK, Schwartz DJ, Kalivas PW (2015): Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nat Neurosci* 18:1230–1232.

47. Liu Z, Le Q, Lv Y, Chen X, Cui J, Zhou Y, *et al.* (2022): A distinct D1-MSN subpopulation down-regulates dopamine to promote negative emotional state. *Cell Res* 32:139–156.
48. Durieux PF, Bearzatto B, Guiducci S, Buch T, Waisman A, Zoli M, *et al.* (2009): D2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat Neurosci* 12:393–395.
49. Lobo MK, Covington HE 3rd, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, *et al.* (2010): Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* 330:385–390.
50. Soares-Cunha C, Coimbra B, Domingues AV, Vasconcelos N, Sousa N, Rodrigues AJ (2018): Nucleus accumbens microcircuit underlying D2R-MSN-driven increase in motivation. *eNeuro* 5:ENEURO.0386-18.2018.
51. Natsubori A, Tsutsui-Kimura I, Nishida H, Boucheikioua Y, Sekiya H, Uchigashima M, *et al.* (2017): Ventrolateral striatal medium spiny neurons positively regulate food-incentive, goal-directed behavior independently of D1 and D2 selectivity. *J Neurosci* 37:2723–2733.
52. Cole SL, Robinson MJF, Berridge KC (2018): Optogenetic self-stimulation in the nucleus accumbens: D1 reward versus D2 ambivalence. *PLoS One* 13:e0207694.
53. Tzanoulinou S, Musardo S, Contestabile A, Bariselli S, Casarotto G, Magrinelli E, *et al.* (2022): Inhibition of Trpv4 rescues circuit and social deficits unmasked by acute inflammatory response in a Shank3 mouse model of Autism. *Mol Psychiatry* 27:2080–2094.
54. Geng HY, Zhang J, Yang JM, Li Y, Wang N, Ye M, *et al.* (2017): Erbb4 deletion from medium spiny neurons of the nucleus accumbens core induces schizophrenia-like behaviors via elevated GABAA receptor alpha1 subunit expression. *J Neurosci* 37:7450–7464.
55. Heshmati M, Aleyasin H, Menard C, Christoffel DJ, Flanigan ME, Pfau ML, *et al.* (2018): Cell-type-specific role for nucleus accumbens neurotrophin-2 in depression and stress susceptibility. *Proc Natl Acad Sci U S A* 115:1111–1116.
56. Shonesy BC, Parrish WP, Haddad HK, Stephenson JR, Baldi R, Bluett RJ, *et al.* (2018): Role of striatal direct pathway 2-arachidonoylglycerol signaling in sociability and repetitive behavior. *Biol Psychiatry* 84:304–315.
57. Durieux PF, Schiffmann SN, de Kerchove d'Exaerde A (2012): Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. *EMBO J* 31:640–653.
58. Derieux C, Leaute A, Brugoux A, Jaccaz D, Terrier C, Pin JP, *et al.* (2022): Chronic sodium bromide treatment relieves autistic-like behavioral deficits in three mouse models of autism. *Neuropsychopharmacology* 47:1680–1692.
59. Becker JAJ, Pellissier LP, Corde Y, Laboute T, Leaute A, Gandia J, *et al.* (2021): Facilitating mGluR4 activity reverses the long-term deleterious consequences of chronic morphine exposure in male mice. *Neuropsychopharmacology* 46:1373–1385.
60. Amalric M, Lopez S, Goudet C, Fisone G, Battaglia G, Nicoletti F, *et al.* (2013): Group III and subtype 4 metabotropic glutamate receptor agonists: Discovery and pathophysiological applications in Parkinson's disease. *Neuropharmacology* 66:53–64.
61. Insel TR (2010): The challenge of translation in social neuroscience: A review of oxytocin, vasopressin, and affiliative behavior. *Neuron* 65:768–779.
62. Stoop R (2012): Neuromodulation by oxytocin and vasopressin. *Neuron* 76:142–159.
63. Ochi M, Shiozaki S, Kase H (2004): Adenosine A(2A) receptor-mediated modulation of GABA and glutamate release in the output regions of the basal ganglia in a rodent model of Parkinson's disease. *Neuroscience* 127:223–231.
64. Soares-Cunha C, de Vasconcelos NAP, Coimbra B, Domingues AV, Silva JM, Loureiro-Campos E, *et al.* (2020): Nucleus accumbens medium spiny neurons subtypes signal both reward and aversion. *Mol Psychiatry* 25:3241–3255.
65. Lee Y, Kim H, Kim JE, Park JY, Choi J, Lee JE, *et al.* (2018): Excessive D1 dopamine receptor activation in the dorsal striatum promotes autistic-like behaviors. *Mol Neurobiol* 55:5658–5671.
66. Detraux B, Vilella A, De Groote A, Schiffmann SN, Zoli M, de Kerchove d'Exaerde A (2021): Dorsal and ventral striatal neuronal subpopulations differentially disrupt male mouse copulatory behavior. *Eur Neuropsychopharmacol* 49:23–37.
67. Alcantara AA, Chen V, Herring BE, Mendenhall JM, Berlanga ML (2003): Localization of dopamine D2 receptors on cholinergic interneurons of the dorsal striatum and nucleus accumbens of the rat. *Brain Res* 986:22–29.
68. Tozzi A, de Iure A, Di Filippo M, Tantucci M, Costa C, Borsini F, *et al.* (2011): The distinct role of medium spiny neurons and cholinergic interneurons in the D(2)/A(2)A receptor interaction in the striatum: Implications for Parkinson's disease. *J Neurosci* 31:1850–1862.
69. Martos YV, Braz BY, Beccaria JP, Murer MG, Belforte JE (2017): Compulsive social behavior emerges after selective ablation of striatal cholinergic interneurons. *J Neurosci* 37:2849–2858.
70. Rapanelli M, Frick LR, Xu M, Groman SM, Jindachomthong K, Tamamaki N, *et al.* (2017): Targeted interneuron depletion in the dorsal striatum produces autism-like behavioral abnormalities in male but not female mice. *Biol Psychiatry* 82:194–203.
71. Ding JB, Guzman JN, Peterson JD, Goldberg JA, Surmeier DJ (2010): Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* 67:294–307.
72. Engeln M, Song Y, Chandra R, La A, Fox ME, Evans B, *et al.* (2021): Individual differences in stereotypy and neuron subtype transcriptome with TrkB deletion. *Mol Psychiatry* 26:1846–1859.
73. Wang W, Li C, Chen Q, van der Goes MS, Hawrot J, Yao AY, *et al.* (2017): Striatopallidal dysfunction underlies repetitive behavior in Shank3-deficient model of autism. *J Clin Invest* 127:1978–1990.
74. Gimenez-Llort L, Torres-Lista V (2021): Social nesting, animal welfare, and disease monitoring. *Animals (Basel)* 11:1079.
75. Rothwell PE, Fuccillo MV, Maxeiner S, Hayton SJ, Gokce O, Lim BK, *et al.* (2014): Autism-associated neurotrophin-3 mutations commonly impair striatal circuits to boost repetitive behaviors. *Cell* 158:198–212.
76. Blomeley C, Garau C, Burdakov D (2018): Accumbal D2 cells orchestrate innate risk-avoidance according to orexin signals. *Nat Neurosci* 21:29–32.
77. Yang H, de Jong JW, Tak Y, Peck J, Bateup HS, Lammel S (2018): Nucleus accumbens subnuclei regulate motivated behavior via direct inhibition and disinhibition of VTA dopamine subpopulations. *Neuron* 97:434–449 e434.
78. Koo JW, Lobo MK, Chaudhury D, Labonte B, Friedman A, Heller E, *et al.* (2014): Loss of BDNF signaling in D1R-expressing NAc neurons enhances morphine reward by reducing GABA inhibition. *Neuropsychopharmacology* 39:2646–2653.
79. Becker JA, Clesse D, Spiegelhalter C, Schwab Y, Le Merrer J, Kieffer BL (2014): Autistic-like syndrome in mu opioid receptor null mice is relieved by facilitated mGluR4 activity. *Neuropsychopharmacology* 39:2049–2060.
80. Dunn HA, Zucca S, Dao M, Orlandi C, Martemyanov KA (2019): ELFN2 is a postsynaptic cell adhesion molecule with essential roles in controlling group III mGluRs in the brain and neuropsychiatric behavior. *Mol Psychiatry* 24:1902–1919.
81. Muntean BS, Dao MT, Martemyanov KA (2019): Allostatic changes in the cAMP system drive opioid-induced adaptation in striatal dopamine signaling. *Cell Rep* 29:946–960 e942.
82. Ibrahim MK, Kamal M, Tikamdas R, Nouh RA, Tian J, Sayed M (2020): Effects of chronic caffeine administration on behavioral and molecular adaptations to sensory contact model induced stress in adolescent male mice. *Behav Genet* 50:374–383.
83. Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999): Distribution, biochemistry and function of striatal adenosine A2A receptors. *Prog Neurobiol* 59:355–396.
84. Corti C, Aldegheri L, Somogyi P, Ferraguti F (2002): Distribution and synaptic localisation of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. *Neuroscience* 110:403–420.
85. Yao Y, Gao G, Liu K, Shi X, Cheng M, Xiong Y, *et al.* (2021): Projections from D2 neurons in different subregions of nucleus accumbens shell to ventral pallidum play distinct roles in reward and aversion. *Neurosci Bull* 37:623–640.

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86. Creed M, Ntamati NR, Chandra R, Lobo MK, Luscher C (2016): Convergence of reinforcing and anhedonic cocaine effects in the ventral pallidum. *Neuron* 92:214–226.
87. Francis TC, Gaynor A, Chandra R, Fox ME, Lobo MK (2019): The selective RhoA inhibitor rhosin promotes stress resiliency through enhancing D1-medium spiny neuron plasticity and reducing hyperexcitability. *Biol Psychiatry* 85:1001–1010.
88. Fox ME, Chandra R, Menken MS, Larkin EJ, Nam H, Engeln M, *et al.* (2020): Dendritic remodeling of D1 neurons by RhoA/Rho-kinase mediates depression-like behavior. *Mol Psychiatry* 25:1022–1034.
89. Lim BK, Huang KW, Grueter BA, Rothwell PE, Malenka RC (2012): Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens. *Nature* 487:183–189.
90. Muir J, Lorsch ZS, Ramakrishnan C, Deisseroth K, Nestler EJ, Calipari ES, *et al.* (2018): In vivo fiber photometry reveals signature of future stress susceptibility in nucleus accumbens. *Neuropsychopharmacology* 43:255–263.
91. Fox ME, Figueiredo A, Menken MS, Lobo MK (2020): Dendritic spine density is increased on nucleus accumbens D2 neurons after chronic social defeat. *Sci Rep* 10:12393.
92. Schiavi S, Iezzi D, Manduca A, Leone S, Melancia F, Carbone C, *et al.* (2019): Reward-related behavioral, neurochemical and electrophysiological changes in a rat model of autism based on prenatal exposure to valproic acid. *Front Cell Neurosci* 13:479.
93. Martella G, Meringolo M, Trobiani L, De Jaco A, Pisani A, Bonsi P (2018): The neurobiological bases of autism spectrum disorders: The R451C-neuregulin 3 mutation hampers the expression of long-term synaptic depression in the dorsal striatum. *Eur J Neurosci* 47:701–708.
94. Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, *et al.* (2012): Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. *Nat Commun* 3:1080.
95. Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, *et al.* (2011): Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* 472:437–442.
96. Portmann T, Yang M, Mao R, Panagiotakos G, Ellegood J, Dolen G, *et al.* (2014): Behavioral abnormalities and circuit defects in the basal ganglia of a mouse model of 16p11.2 deletion syndrome. *Cell Rep* 7:1077–1092.
97. Kegeles LS, Abi-Dargham A, Frankle WG, Gil R, Cooper TB, Slifstein M, *et al.* (2010): Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch Gen Psychiatry* 67:231–239.
98. Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, *et al.* (2000): Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci U S A* 97:8104–8109.