A circuit-based mechanism underlying familiarity signaling and the preference for novelty

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Novelty preference (NP) is an evolutionarily conserved, essential survival mechanism often dysregulated in neuropsychiatric disorders. NP is mediated by a motivational dopamine signal that increases in response to novel stimuli, thereby driving exploration. However, the mechanism by which once-novel stimuli transition to familiar stimuli is unknown. Here we describe a neuroanatomical substrate for familiarity signaling, the interpeduncular nucleus (IPN) of the midbrain, which is activated as novel stimuli become familiar with multiple exposures. In mice, optogenetic silencing of IPN neurons increases salience of and interaction with familiar stimuli without affecting novelty responses, whereas photoactivation of the same neurons reduces exploration of novel stimuli mimicking familiarity. Bidirectional control of NP by the IPN depends on familiarity signals and novelty signals arising from excitatory habenula and dopaminergic ventral tegmentum inputs, which activate and reduce IPN activity, respectively. These results demonstrate that familiarity signals through unique IPN circuitry that opposes novelty seeking to control NP.

The capacity to detect and react to novel as opposed to familiar stimuli provides an opportunity for adaptation in a rapidly changing environment1. Inappropriate responses toward novelty are associated with a number of neurodevelopmental and neuropsychiatric disorders, including schizophrenia2, autistic-related behaviors3, attention deficit hyperactivity disorders and addiction4–6. Although the expression of NP involves cognitive function and recognition memory7,8, responses to novel events depend on the activation of midbrain reward systems9, including the dopaminergic (DAergic) neuron-rich substantia nigra and ventral tegmental area (VTA), which exhibit greater activity when individuals face novel stimuli and lower activity once these stimuli become familiar. This response to novelty is likely correlated with DAergic neuron responses linked to higher novel-stimuli saliency as compared to familiar stimuli10. However, how a novel stimulus transitions to a familiar stimulus after repeated exposures and how the brain detects familiarity to cease responding is unknown. In addition, whether signals for novel and familiar stimuli act through distinct neural pathways or share common circuitry has not been explored.

RESULTS

To address the behavioral and neural responses to familiar and novel stimuli, we adapted a model of social interaction using a three-chamber interaction context11. In this procedure (Fig. 1a), adult male C57BL/6 mice were tested for sociability toward familiar or novel individuals by meeting the same 4–7-week-old juvenile C57BL/6 male mouse for 3 consecutive days (‘Familiar Social’ or FS) or meeting a new (stranger) individual on the third day (‘Novel Social’ or NS). The FS group exhibited a high social investigation rate during the first encounter to a novel social stimulus (N1), which decreased on days 2 and 3 as the stimulus mouse became familiar (F1 and F2, respectively; Fig. 1b,c). Nonsocial investigation (of the empty cylinder) progressively increased from N1 to F1 and F2 (Fig. 1b,d), resulting overall in a reduced social preference ratio across sessions (Fig. 1e). Conversely, the NS group, presented with a novel social stimulus (N2) on day 3, exhibited rebounded social investigation (Fig. 1b,c) and reduced nonsocial investigation (Fig. 1b,d), altogether increasing the social preference ratio (Fig. 1e). Both FS and NS groups demonstrated similar total amounts of investigation (Fig. 1f).

To explore the neural circuits underlying the response to novelty and familiarity, we focused on the IPN, a brain region medial and ventral to the VTA but with opposing roles in reward versus aversion signaling12. Recently, IPN activity has been associated with social conflict resolution in zebrafish13. However, no studies have addressed the potential role of the IPN in response to social novelty and familiarity in mammals. To map IPN activity during familiar and novel events, we examined c-Fos immunoreactivity, a marker for neuronal activation14, 90 min after a social encounter. In control animals that did not experience any social interaction (nonsocial), few c-Fos+ nuclei were observed in the IPN (Fig. 1h). However, the FS group exhibited elevated numbers of c-Fos+ nuclei, particularly in the central and ventral portions of the IPN (Fig. 1g). In contrast, this neuronal activation was not observed in the NS group (Fig. 1g). Notably, each juvenile mouse in this task was placed in the same cylinder and compartment within each experiment to avoid effects of novel spatial information; thus, IPN activation solely reflected novel social interaction. These results suggested that the IPN was exclusively activated by an experience-dependent (familiar) social stimulus but not by social interaction per se. We next asked whether activation of the IPN persisted or increased with the degree of familiarity. To this end, another group of C57BL/6 mice progressively encountered the same

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Figure 1  Familiar and novel social encounters differentially activate the IPN. (a) Schematic of experimental approach used to measure interactions with FS (n = 20 mice) and NS stimuli (n = 16 mice). Subject mice (black) were exposed to the same juvenile mouse on day 1 (N1) and day 2 (F1). On day 3 mice were split into two groups and were exposed either to the same juvenile (F2) or a new juvenile (N2). (b) Representative spatial heat maps showing the location of the test mouse on each of the social interaction test. (c) Time spent investigating the social cylinder decreased across consecutive days (two-way repeated-measures (RM) ANOVA; day effect, F2,68 = 41.31, P < 0.0001). Exposure to a new juvenile increased social investigation (day × stimulus group interaction, F2,68 = 4.22, P = 0.0187; post hoc test, **P = 0.0035). (d) Time spent investigating the nonsocial cylinder decreased in the NS group (two-way RM ANOVA; day × stimulus group interaction, F2,68 = 3.95, P = 0.0239; post hoc test, *P = 0.0311). (c) Preference ratio for a social stimulus decreased across consecutive days (two-way RM ANOVA; day effect, F2,68 = 23.32, ***P < 0.0001) but rebounded in the NS group (day × stimulus group interaction, F2,68 = 7.35, P = 0.0013; post hoc test, ***P < 0.0001). (f) Total investigation time decreased across days (two-way RM ANOVA; day effect, F2,68 = 24.43, P < 0.0001) but similarly between groups (day × stimulus group interaction, F2,68 = 1.10, P = 0.3382). (g) Representative images of c-Fos immunoreactivity in coronal sections containing the IPN. Scale bar, 50 μm. (h) Normalized c-Fos immunopositive nuclei in the IPN of mice encountering a familiar (F2) social stimulus (FS) were significantly higher than in control animals (nonsocial encounters, Non-S) or mice encountering a novel (N2) social stimulus (NS; one-way ANOVA; F2,12 = 5.49, P = 0.0203; post hoc test, *P = 0.0385 and ***P = 0.0466, respectively; n = 5 mice per group). (i) Representative image of Gad1/2 (red) and c-Fos (green) immunofluorescence in the IPN of the FS group. Arrowheads show co-localized nuclei (inset). Nuclei were labeled with DAPI (blue). Scale bars, 50 μm and 20 μm (inset). Data are expressed as mean ± s.e.m.; n.s., nonsignificant.

To define the IPN neuronal population activated by familiar stimuli, we used immuno-colocalization analysis of c-Fos with glutamate acid decarboxylase (Gad1/2), a marker for GABAergic neurons15. Double-labeling (Fig. 1i) revealed that ~70% (75.19 ± 19.15%; n = 3 mice) of the c-Fos-immunopositive neurons in the FS group co-localized with Gad1/2 staining, suggesting that the majority of activated neurons in the IPN were inhibitory. We hypothesized that IPN GABAergic neuronal activation may act as a brake for novelty-induced exploratory behavior as novel stimuli become familiar. To test this hypothesis, we selectively targeted IPN GABAergic neurons by injecting a Cre-dependent adeno-associated virus (AAV) encoding halorhodopsin (Nphr3.0) fused to enhanced yellow fluorescent protein (eYFP)16 into the IPN of mice expressing Cre under the control of the promoter for the gene encoding Gad2, Gad2::Cre mice (Gad2::Cre::Nphr3.0). Numerous eYFP+ cell bodies in the IPN co-localized with Gad2 staining, verifying that the virus was restricted to GABAergic neurons (Fig. 2b). Efficient photostimulation-dependent silencing was confirmed in spontaneously active eYFP::Nphr3.0 current-clamped neurons from the injected animals (Fig. 2c). Notably, Gad2Cre::Nphr3.0 photostimulation parameters produced robust IPN inhibition without rebound neuronal firing. To assess the requirement of IPN GABAergic activity in familiarity responses, Gad2Cre::Nphr3.0 mice implanted with an optic fiber in the IPN were presented with the same social stimulus for 2 consecutive days without light delivery, to avoid any putative effect on memory acquisition. On the third day, mice were offered the choice between FS and NS stimuli whilst half of the group received light illumination (Fig. 2d). Control mice without light exhibited more investigation toward NS (Fig. 2c.g) compared to FS stimuli (Fig. 2e.f) and a positive NP ratio (Fig. 2h). In contrast, photoinhibited mice (593 nm, constant light, 20 s ON and
10 s OFF) showed significantly more FS investigation compared to controls (light-OFF mice; Fig. 2e,f), mimicking a novelty situation. NS investigation was not affected by Gad2Cre::NpHRIPN photoinhibition (Fig. 2c,g), confirming that the activity of IPN GABAergic neurons was specific for familiarity signaling. Consequently, the NP ratio was significantly reduced in photoactive Gad2Cre::NpHRIPN mice (Fig. 2h), without interfering with total time of exploratory behavior (Fig. 2i). Locomotor activity (Supplementary Fig. 2a) was not significantly affected by Gad2Cre::NpHRIPN photoinhibition \((P = 0.2808)\). Notably, Gad2Cre::NpHRIPN photostimulation increased exploration only toward FS stimuli but not NS stimuli when mice were exposed to familiar or novel mice once daily during consecutive days (i.e., when mice did not have a choice between exploring novel or familiar stimuli during the same session; Supplementary Fig. 2b–f). These data suggest that the activity of IPN GABAergic neurons might reversibly control the motivational salience of familiar versus novel exploration, rather than memory discrimination. To confirm this idea, we also used a modified version of the conditioned place preference (CPP) model, in which mice associated one of two compartments with either FS or NS presentations (Supplementary Fig. 3a). Control mice, without light, developed a CPP for the NS compartment \((P = 0.0561)\). Data are expressed as mean ± s.e.m.; n.s., nonsignificant.
IPN activation determined the reduced salience of familiarity itself.

To test more directly whether familiarity responses could be triggered by optogenetic activation of IPN GABAergic neurons, we expressed Cre-dependent ChR2-eYFP in the IPN of Gad2::Cre mice (Fig. 2a)\(^17\). Light delivery (20 Hz) elicited neuronal firing from ChR2\(^+\) Gad2 cells with high temporal precision (Fig. 2c), confirming the functional expression of ChR2. When mice were offered the choice between NS and FS stimuli (Fig. 2d), photoactivation of IPN GABAergic cell bodies (473 nm, 20 Hz, 12-ms pulse for 5 min) significantly decreased NS investigation (Fig. 2j,l) compared to control mice (light-OFF), mimicking a familiarity response. In contrast, FS investigation remained unaffected (Fig. 2j,k), thus significantly decreasing the NP ratio (Fig. 2m) but not total exploratory behavior (Fig. 2n). Similar results were obtained when comparing ChR2\(^+\) mice with nonopsin, eYFP\(^+\) animals receiving photostimulation.
(Supplementary Fig. 5a–d), excluding putative behavioral outcomes due to light-driven effects. In addition, when we examined the response toward inanimate objects (Supplementary Fig. 6), Gad2Cre::ChR2IPN stimulation significantly decreased NO investigation (P = 0.0348; Supplementary Fig. 6a,c), while leaving exploration of a FO intact (Supplementary Fig. 6a,b). Consequently, the NP ratio was significantly reduced (P = 0.0246; Supplementary Fig. 6d) without interfering with the total time of exploration (Supplementary Fig. 6e). Notably, neither photoinhibition (Supplementary Fig. 7a–d) nor photoactivation (Supplementary Fig. 7e–h) of IPN Gad2+ neurons altered anxiety-like behaviors. These data confirm that activation of IPN GABAergic neurons was both necessary and sufficient to reduce exploration toward familiar social and object stimuli and, therefore, the expression of NP.

To determine whether activation of the IPN and familiarity signaling is controlled by the major excitatory input innervating the IPN, which arises from medial habenula (mHb) cholinergic–glutamatergic neurons, we injected AAV2 encoding either NpHR3.0-eYFP or ChR2-eYFP into the mHb of mice expressing Cre under the control of the promoter for the gene encoding choline acetyltransferase (ChatCre mice), and directly stimulated mHb axon terminals in the IPN (Fig. 3a–c). Cholinergic neurotransmission plays an important role in novelty processing and familiarity-based responses, raising the possibility that cholinergic signaling in the IPN contributes to NP. We observed robust eYFP expression in mHb cell bodies that co-localized with ChAT immunostaining (Fig. 3b), as well as abundant eYFP axon bundles of the fasciculus retroflexus descending and innervating the dorsal and ventral central regions of the IPN (Fig. 3c), indicating that photoactivation of ChR2+ mHb ChAT+ terminals in the IPN resulted in light-dependent increases in action potentials (Fig. 3d).
In addition, photoinhibition of the same terminals expressing NpHR reduced IPN neuronal activity (Fig. 3d). We investigated the ChAT+ mHb→IPN functional connection on NP using previously established parameters of in vivo optogenetics for social (Fig. 2d) and inanimate (Supplementary Fig. 4a) stimuli interactions. Optical stimulation of mHb cholinergic–glutamatergic terminals in the IPN (473 nm, 20 Hz, 12-ms pulse for 5 min) decreased exploration of NS (Fig. 3c,e) and NO stimuli (Supplementary Fig. 8a,c) compared to control mice (light-OFF), mimicking familiar-stimuli–exploration responses, but did not interfere with FS (Fig. 3e,f) or FO stimulation (Supplementary Fig. 8a,b). The net Chat<sup>Cre::ChR2<sup>+</sup>mHb→IPN</sup> effect resulted in overall significantly decreased NP ratio for social (Fig. 3h) and inanimate stimuli (Supplementary Fig. 8d). These results were not attributable to nonspecific effects of light stimulation (Supplementary Fig. 9a–d).

Conversely, Chat<sup>Cre::NpHR<sup>+</sup>mHb→IPN</sup> photoinhibition (593 nm, constant light, 20 s ON, 10 s OFF) of mHb cholinergic–glutamatergic IPN terminals in Chat<sup>Cre::NpHR<sup>+</sup>mHb→IPN</sup> mice increased interest toward FS (Fig. 3j,k) and FO stimuli (Supplementary Fig. 8g,h), mimicking exploration of novel stimuli. Investigation of NS (Fig. 3j,l) or NO stimuli (Supplementary Fig. 8g,i) were not affected by photoinhibition of cholinergic–glutamatergic IPN terminals, altogether significantly decreasing the ratio of NP for social (Fig. 3m) or inanimate events (Supplementary Fig. 8j). Activation (in Chat<sup>Cre::ChR2<sup>+</sup>mHb→IPN</sup>) or inhibition (in Chat<sup>Cre::NpHR<sup>+</sup>mHb→IPN</sup>) mice of mHb→IPN inputs did not significantly impact total time of investigation (Fig. 3n and Supplementary Fig. 8e,k) or locomotor activity (Supplementary Fig. 8l). Taken together, these data indicate that the mHb→IPN circuit bidirectionally modulated the social evaluation of FS and NO stimuli.
exploration of novel versus familiar stimuli and was both necessary and sufficient for the expression of NP.

Given that (i) novelty responses are mediated by VTA DAergic activity\(^1\), (ii) the IPN is located ventromedial to the VTA and (iii) previous studies indicate the existence of a meso-interpeduncular circuit\(^2\), we asked whether VTA DAergic neurons could potentially innervate the IPN to control NP. To this aim, we selectively expressed Cre-dependent ChR2-eYFP in the VTA of mice expressing Cre under the control of the promoter for Slc6a3, the gene encoding the DA transporter (DAT::Cre mice) via AAV2-mediated gene delivery (Fig. 4a) and observed VTA→IPN axonal projections (Fig. 4b). Axons arising from VTA DAergic neurons were more abundant in lateral and central regions of the caudal IPN (Supplementary Fig. 10a). To determine the functional contribution of DA\(^{VTA} \rightarrow\)IPN, we performed optogenetic circuit-specific terminal photostimulation in combination with slice electrophysiology. Whole-cell voltage-clamp recordings confirmed light-dependent inward currents in VTA ChR2\(^+\) DAergic cell bodies (Supplementary Fig. 10b). Cell-attached recordings in caudal IPN neurons surrounded by eYFP\(^+\) VTA terminals revealed that DAT\(^{Cre}::ChR2^{VTA} \rightarrow\)IPN photostimulation elicited an increase in action potentials in 10 of 16 neurons, which was completely blocked by preapplication of the D1 receptor antagonist SCH39166 (Fig. 4c,d), suggesting that activation of DAergic terminals leads to D1 receptor-dependent modulation of caudal IPN neuronal activity. In addition, in vivo photoactivation of presynaptic VTA DAergic terminals in the IPN triggered an overall reduced number of IPN c-Fos\(^+\) neurons (Supplementary Fig. 10c,d), suggesting these terminals may broadly inhibit IPN via activation of a small population of dopaminergic neurons to drive a novelty signal.

To investigate the functional consequences of DA\(^{VTA} \rightarrow\)IPN neuronal firing in the responses to novel versus familiar stimuli, we photostimulated IPN DAergic terminals during the social NP test (Fig. 4e). Stimulation was delivered in 30-Hz bursts of light (8 pulses of 5 ms each) every 5 s throughout the 5-min assay, a phasic pattern shown to evoke high levels of DA release\(^2\), DAT\(^{Cre}::ChR2^{VTA} \rightarrow\)IPN phasic stimulation significantly increased Fs investigation (Fig. 4f,g), mimicking a novelty response. In contrast, NS investigation remained intact (Fig. 4h), altogether resulting in a significantly reduced NP ratio compared to control animals without light delivery (Fig. 4i) or to animals injected with nonopsin, eYFP-encoding virus and receiving photobleaching (Supplementary Fig. 10i–h). Total exploratory behavior (Fig. 4j) or locomotion (Fig. 4k) was not affected by manipulating DA\(^{VTA} \rightarrow\)IPN activity. When examining responses to inanimate objects (Supplementary Fig. 11a), DAT\(^{Cre}::ChR2^{VTA} \rightarrow\)IPN photoactivation did not significantly affect either FO (\(P = 0.3612\); Supplementary Fig. 11b,c) or NO investigation (\(P = 0.9999\); Supplementary Fig. 11d,e) or total exploratory behavior (\(P = 0.8008\); Supplementary Fig. 11f).

If, under social novelty circumstances, the VTA provides a DA signal in the IPN, this suggests that IPN neurons may express DA receptors and that these neurons should respond to novel social information. Our recordings from caudal IPN slices demonstrated that D1 is critical in DAT\(^{Cre}::ChR2^{VTA} \rightarrow\)IPN transmission, and previous reports indicate D1 responses are linked to NP\(^2\). Together implying IPN D1 activity may be important for the expression of NP. We examined transgenic Drd1atdTomato mice, which use the mouse Drd1 gene promoter to drive the expression of DsRed fluorescent protein and found a rostrocaudal gradient in the relatively sparse density of D1\(^+\) cell bodies in the IPN (Fig. 5a,b). Intra-IPN infusion of the D1 agonist SKF82958 elicited an increase in c-Fos expression in the majority of IPN D1\(^+\) neurons, confirming a functional IPN response to DAergic input (Supplementary Fig. 11g,h). To test how the IPN D1 neural subpopulation responds to novel and familiar events, Drd1atdTomato mice encountered either FS or NS stimuli as represented in Figure 1a. FS Drd1atdTomato mice showed elevated numbers of c-Fos\(^+\) nuclei in the IPN compared to the NS group (Fig. 5c,d). However, a high number of c-Fos\(^+\) nuclei co-localized with DsRed fluorescence only in NS Drd1atdTomato mice (Fig. 5c,e), indicating that activation of sparse IPN D1\(^+\) neurons occurred upon exposure to novel but not familiar social conditions. Finally, to examine whether IPN D1 activity contributes to the expression of social NP, we preinformed the D1-like receptor family antagonist SCH39166 before phasic DAT\(^{Cre}::ChR2^{VTA} \rightarrow\)IPN photostimulation (Fig. 5f,g), without significantly affecting NS investigation (Fig. 5h,i), and increased the NP ratio to a more positive value (Fig. 5k). Preinflation of the drug did not alter total time of exploration (Fig. 5l). These results impliciate the meso-interpeduncular circuit as a key modulator of NP through D1-receptor signaling in the IPN.

DISCUSSION

Taken together, our data indicate that IPN GABAergic neuron activity is controlled by coordinated habenular cholinergic–glutamatergic and VTA DAergic neuron input to form a critical circuit-based mechanism mediating the signaling of familiarity and the expression of NP. As opposed to dopaminergic midbrain areas that are activated by novel stimuli\(^9\), overall IPN neuronal activity progressively increases with multiple exposures to the same stimulus (FS and FO) as the stimulus becomes familiar but decreases when interactions occur with novel stimuli (NS and NO). Optogenetic activation of IPN GABAergic neurons can inhibit investigation of a novel stimulus, mimicking familiarity, whereas inhibition of these neurons induces increased investigation toward familiar stimuli without affecting exploration of a true novel stimulus, highlighting that the IPN is a critical node for familiarity signaling. Thus, when given a choice between novel and familiar stimuli, the IPN acts as a brake that is necessary and sufficient for reduced exploration of a familiar but not novel signal to control NP. Notably, the results from both single object or social encounters and the CPP assay demonstrate that the IPN controlled NP by assigning the motivational salience of novel versus familiar information, as opposed to the discrimination of novel versus familiar stimuli, which presumably is mediated by cortical\(^25\) and hippocampal\(^28\) areas. In particular, reducing activation of the IPN during the CPP assay increases the rewarding properties of a familiar social encounter to those of a novel encounter, whereas stimulation of IPN neurons reduces the rewarding value of novel social stimuli (data not shown), suggesting that the IPN controls the valence of motivation toward familiarity.

Stimulating or inhibiting excitatory mHb cholinergic–glutamatergic axon terminals in IPN bidirectionally controls NP similarly to directly activating or silencing IPN GABAergic neurons, indicating that the mHb acts upstream of the IPN to mediate familiarity-based responses. The mHb receives most afferent projections from the septum\(^27\), which may provide encoded familiarity-based information. Via its projections to the IPN, the mHb represents a link between the forebrain and midbrain regions involved in reward and emotional processing\(^28\). Therefore, the mHb→IPN circuit could contribute to the cholinergic network extensively implicated in the evaluation of novel versus familiar information\(^29\).

Several behaviors have been attributed to the mHb→IPN axis, including anxiety\(^30,31\), fear-related responses\(^31–33\), aversive memories\(^12\) and nicotine aversion\(^34,35\) or withdrawal\(^21,22,36\), most requiring
conditioned learning or drug exposure. We excluded the possibility that our results could be attributed to fear responses because our experimental procedures did not employ conditioned learning assays but instead used innate exploratory behavior. Furthermore, with manipulation of IPN activity, animals did not remain immobile but continued exploring, redirecting their levels of investigation exclusively toward familiar or novel stimuli.

Recent data indicate that there may be direct synaptic connectivity between the VTA and IPN22. Notably, DAergic responses are linked to stimulus saliency, as substantia nigra–VTA functional connectivity provides integrative information about novelty and reward10. Our study provides the first functional evidence that VTA DAergic neurons, specifically, innervate the IPN and modulate IPN neuronal activity to control novel versus familiar interactions through novelty-induced activation of D1 receptor-expressing IPN neurons. While familiarity increases IPN activation overall, a relatively restricted subpopulation of dopaminceptive IPN neurons is activated by novel stimuli, and selective activation of DAergic VTA → IPN input specifically increased the activity of neurons in the IPN, essentially mimicking novelty-like exploration, an effect blocked by IPN infusion of a D1 receptor antagonist. These data raise the interesting possibility that IPN controls familiarity signaling and NP through two distinct inputs: familiarity signaling is received from the mHb, which activates GABAergic IPN neurons to reduce the motivational salience of novel stimuli as they become familiar, thereby reducing exploration; and novelty signaling is received from VTA DAergic neurons, activating a discreet dopaminceptive IPN neuron population to presumably reduce activity of familiarity-signaling IPN neurons, enhancing salience and increasing exploration toward novel stimuli. Future studies will be needed to further characterize the neuronal identity and projection pattern of IPN dopaminceptive neurons and to determine whether their activation by novel stimuli directly inhibits the activity of familiarity-signaling IPN neurons or whether these neurons project to novelty-associated brain areas outside the IPN.

Notably, we found that stimulation of VTA → IPN DAergic terminals was sufficient to control the expression of NP for social but not inanimate stimuli. This is consistent with recent work indicating that optogenetic activation of VTA DAergic neurons enhances social interaction with novel stimuli without affecting novel object interaction, despite 

in vivo imaging data indicating that VTA DAergic neuron activity is modulated by both novel social and novel object interactions35,57. This result likely reflects the circuit complexity and raises the possibility that other VTA neuronal subtypes may mediate responses to nonsocial novel stimuli.

In summary, we have identified a previously unknown functional role for the IPN as a neuroanatomical substrate for familiarity signaling, together with convergent inputs from the mHb and VTA. To our knowledge, this is the first report indicating that information regarding familiar and novel stimuli is signaled by independent yet connected circuits that affect familiarity-signaling directly to control NP. Of note, we expect that dysregulation of familiarity signaling within IPN and associated circuits could play a role in neuropsychiatric disorders characterized by impaired novelty versus familiarity interactions, such as schizophrenia, autism and addiction. Thus, targeting components of this circuit may provide novel therapeutic strategies for treating several neuropsychiatric conditions.

METHODS
Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.
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ONLINE METHODS

Animals. All experiments were conducted in accordance with the guidelines for care and use of laboratory animals provided by the National Research Council, as well as with an approved animal protocol from the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School (UMMS). C57Bl/6J (#000664), Gad2Cre (#10802), Clut4Cre (#006410), DATCre (#006660) and Drd1adTomato (#001624) mice were obtained from The Jackson Laboratory, bred in the UMMS animal facility and used in behavioral, optogenetic and biochemical experiments as indicated. Cre lines were crossed with C57Bl/6J mice and only heterozygous animals carrying one copy of the Cre recombinase gene were used for experimental purposes. For social experiments, juvenile stimuli always consisted of male C57Bl/6J mice (4–7 weeks old) bred in the UMMS animal facility. All mice were housed together and kept on a standard 12-h light/dark cycle (lights ON at 7 a.m.) with ad libitum access to food and water. Three to four weeks before experimentation, subject mice were kept under a reverse 12-h light/dark cycle (lights ON at 7 p.m.) and individually housed for at least 5 d before any behavioral testing.

Viral constructs. The pAAV-Ef1a-DIO-ENopHR3.0-eYFP and pAAV-Ef1a-DIO-ChR2-eYFP plasmids (generously provided by K. Deisseroth) and the pAAV-Vectopax virus particles by the UMMS Viral Vector Core. AAV2 was used as the serotype for all virus-mediated gene deliveries, as AAV2 preferentially infects neurons and exhibits minimal retrograde infection. Viral titrations consisted of 5 × 1012–1013 genome copies per mL for AAV2-Ef1a-DIO-ENopHR3.0-eYFP, 2.5 × 1012 viral particles per mL for pAAV-Ef1a-DIO-ChR2-eYFP and 5 × 1012 viral particles per mL for pAAV-Ef1a-DIO-eYFP. All viral injections were performed 4–6 weeks before experiments to allow sufficient time for transgene expression.

Stereotoxic injections. cannula and optic fiber implantation. Surgery and injections were performed under aseptic conditions and stereotaxic guidance as previously described. Mice (age 6–8 weeks) were deeply anesthetized using a 100 mg kg–1 ketamine (Vedco) and 10 mg kg–1 xylazine (Lloyd) mixture. Following anesthesia treatment, a 0.4-mm drill was used for craniotomies at the target bregma coordinates. All mice were microinjected at a controlled rate of 30 nl min–1 using a gas-tight 33-gauge 10-µl syringe (1701RN; Hamilton) in a microsyringe pump (Stoelting Co). After infusion, we left the needle in place for another 10 min slowly withdrawing it. Injection parameters included, in mm from bregma (anteroposterior (AP), mediolateral (ML), dorsoventral (DV) and angle): IPN (−3.4, −0.5, −4.8, 0°), VTA (−3.4, 0.5, −4.5, 0°) and Mhb (−1.5, ±0.25, −2.8, 0°). Viral volumes were 300 nl for unilateral IPN and bilateral Mhb injections and 800 nl for bilateral VTA injections. For behavioral optogenetic experiments, 4 weeks after injection, an unilateral optic fiber implant (200-µm core diameter; 0.53 NA, Doric Lenses) was held in a magnetic aluminum receptacle (Doric Lenses) was placed above the IPN (−3.4, −0.5, −4.8, 0°) and secured into the skull using adhesives (C&B Metabond cement, Parkell Inc.) followed by dental cement (Cerebong, PlasticsOne). For pharmacological experiments, a stainless steel guide cannula (23-gauge with 4-mm pedestals, PlasticsOne) was inserted above the IPN (−3.4, 0, −4.8, 0°) and secured to the skull with Cerebong. All mice received intraperitoneal (i.p.) injections of 1 mg kg–1 ketoprofen analgesic (Zoetis) and placed on heating pads until recovery from anesthesia. Mice were allowed to recover in their home cages for 5 d before any behavioral testing. Injection sites and viral expression were confirmed for all animals by experimenters blinded to behavioral outcome as previously described. Animals showing no viral or off-target site viral expression or incorrect optic fiber placement were excluded from analysis. From a total of 206 mice, 34 mice were excluded from analysis due to incorrect viral expression or optic fiber placement.

Infusion of drug solutions. Mice were anesthetized with 2% isoflurane via a nose cone adapter at a flow rate of 800 ml L–1, as previously described. An internal infusion cannula (30 gauge) designed to reach IPN coordinates (−4.8 mm) was inserted into the guide cannula and vehicle (2% DMSO and 98% sterile saline) or D1 antagonist SCH39166 (70 ng µL–1 dissolved in 2% DMSO and 98% sterile saline) was infused at a rate of 0.3 µL min–1 for 1 min. After infusion, the injection cannula was left in place for an additional 2 min before removal. Subsequently, an optic fiber implant was inserted through the guide cannula and instant adhesive was used to stabilize the fiber to the cannula. SKF82958 (0.2 µg) was dissolved in sterile saline and also administered at a rate of 0.3 µL min–1. The drug solutions were infused 10 min before behavioral testing. Animals showing incorrect cannula placement were excluded from analysis by experimenters blinded to treatment. From a total of 30 mice, 8 mice were excluded from analysis due to incorrect cannula placement.

Behavioral assays. All behavioral experiments were conducted during the active dark phase (8 a.m. to 6 p.m.) of mice aged between 9–12 weeks old. Animals were acclimated to the testing room for 30 min before any experiment, and all testing was performed under dim red light conditions. For experiments involving C57Bl/6J and Drd1adTomato mice, animals were used only in one behavioral module. For optogenetic experiments, animals used for both social and object interactions underwent a minimum of at least a 4-d washout period between photostimulations. All social behavior experiments were performed in male mice that interacted with male juvenile stimulus mice. Data for object interaction using the Gad2Cre line included both males and females, as no sex differences were detected. All experiments were conducted in at least three cohorts of animals (i.e., in triplicate), which were randomly allocated into experimental groups and counterbalanced across cohorts.

Social interaction tests. For experiments involving C57Bl/6J and Drd1adTomato mice, social approach testing was performed using the standard three-chamber apparatus design as previously described. The Plexiglas apparatus consisted of two identical compartments (each 42 × 24 × 30 cm) connected via a neutral central zone (42 × 15 × 30 cm) that allowed the animal to freely move between compartments. Each of the outer compartments contained an inverted plastic cylinder (14 cm × 11 cm diameter) with holes in it (1.5 cm diameter), allowing for direct physical contact (i.e. visual, gustatory and olfactory interaction) between the stimulus and subject animals. Notably, in this protocol social approach is established by the testing animal, as juvenile mice are constrained inside the cylinder, thus removing the potential stress caused by any stimulus-directed physical contact. Subject mice were first habituated to the apparatus for a 5-min period. Following habituation, a juvenile C57Bl/6J mouse (4–7 weeks of age) was placed under one of the two inverted cylinders (counterbalanced). The subject mouse was then placed in the central zone and allowed to freely explore all three compartments for 5 min. This testing phase was repeated 24 h later, on day 2, using the same juvenile mouse placed in the same compartment. Social investigation on days 1 and 2 was used to determine baseline exploratory behavior and to counterbalance sociability among subject animals. On day 3, half of the subject mice were presented for 5 min with the same familiar juvenile mouse as used on days 1 and 2, whereas the other half were presented with a new juvenile stimulus mouse, located in the same compartment as on days 1 and 2. Interaction groups receiving a familiar or novel stimulus were balanced in an unbiased way to account for individual animals’ social interests. A control group (Nonsocial) was similarly presented with the apparatus and cylinders for three consecutive days, but never exposed to a social stimulus. For progressive social interactions, subject mice were presented for 5 min with the same familiar juvenile social stimulus and in the same cylinder for seven consecutive days. All sessions were video recorded from above (HDR-CX4440 camera, Sony) and mouse behavior was tracked automatically using EthoVision XT 11.5 (Noldus Apparatus). Heat maps were generated in EthoVision XT with pseudocolor representing the relative time spent by the mouse at each position, with the maximum and minimum calculated within each session. Times exploring the social and nonsocial cylinders were manually scored by an experimenter blind to experimental conditions. Exploration was defined as sniffing when a mouse directed its nose at the cylinder from a distance of less than 2 cm. Sitting or resting next to the cylinder was not considered exploration. The social preference ratio was calculated as: (total social investigation – total nonsocial investigation)/total investigation over the 5-min session. The apparatus and cylinders were cleaned with Micro-90 solution (International Products Corporation) to eliminate olfactory traces after each session.

For optogenetic experiments, subject mice were tested in an open-field apparatus (42 × 38 × 30 cm) containing two plastic cylinders on opposite corners of the maze. Optic fiber implants were connected to a patch cable using magnetic force (Doric Lenses), which in turn was connected to a commutator (rotary joint; LEDFRJ-B; FC for blue light and LEDFRJ-A; FC for yellow light; Doric Lenses) by means of an FC/SMC adaptor to allow unrestricted movement. On day 1, mice were subjected to a 5-min habituation session followed by a 5-min test session in which a juvenile mouse was placed inside one of the two cylinders (counterbalanced).
This test session was repeated 24 h later, with the same social stimulus located in the same cylinder. No light was delivered on days 1 and 2. Interaction groups receiving or not receiving photostimulation were balanced in an unbiased way to account for individual animals’ social interest. On day 3, a familiar mouse (encountered twice previously) was placed simultaneously with a novel juvenile stimulus in the opposite cylinder, and half of the subject mice received paired optical stimulation (ChR2: 473 nm, 20 Hz, 12-ms pulse for 5 min; NpHR: 593 nm constant light, 20 s ON, 10 s OFF for 5 min). The optogenetic parameters used for DAVTa-MPN were consistent with a previously published protocol for DArgeic firing (8 pulses of 5-ms pulse-width, at 30 Hz, delivered every 5 s for 5 min)17. A high-powered LED driver (DC2200, Thorlabs) was used to generate light pulses at intensity ~15–20 mW. A control group injected with Cre-dependent nonop- sin virus (eYFP) was added to test light-derived effects and received the same optogenetic stimulation pattern as used for ChR2-injected animals. For these choice experiments, the preference ratio was calculated as: (total novel stimulus investigation − total familiar stimulus investigation) / (total investigation) over the 5-min session. For single presentations of social stimulus, similar experimental conditions were used. In this protocol, the subject mice encountered the same juvenile social stimulus mouse in the same location (counterbalanced) for four consecutive days. On day 5, a novel juvenile stimulus mouse was placed in that position. Half of the animals received paired photostimulation (NpHR: 593 nm constant light, 20 s ON, 10 s OFF, ~15–20 mW, for 5 min) on days 3 and 5.

CPP test. The CPP apparatus (Med Associates) consisted of two outer conditioning chambers (each 13 × 15 × 12 cm) connected by a central neutral zone (13 × 10 × 12 cm). One chamber had black walls with a striped metal floor, and the other had white walls with a grid metal floor. For the novel social CPP, each chamber contained a plastic cylinder (9 cm × 7 cm diameter). The protocol consisted of three phases: pretest, acquisition, and test, all of them conducted under dim light and sound-attenuated conditions. During the pretest phase, mice were placed in the central zone and allowed to explore the entire apparatus for 20 min. Conditioning groups were balanced in an unbiased fashion to account for potential baseline chamber preference. Five to 6 h after the pretest session, subject mice were presented with a juvenile stimulus mouse placed inside a cylinder in a neutral arena for 10 min. The acquisition phase consisted of three successive days with two conditioning trials each day, separated 6 h apart. On days 2, 3 and 4 mice were confined to one chamber for 10 min while in the presence of either a familiar juvenile social stimulus (encountered in the neutral arena) or a novel juvenile stimulus (which changed every day) constrained inside a cylin- der. Familiar and novel social stimuli were counterdistributed for conditioning chamber and morning/afternoon trials. Half of the subject mice were paired with photostimulation when presented with the familiar social stimulus on days 3 and 4 (NpHR: 593 nm constant light, 20 s ON, 10 s OFF, ~15–20 mW, for 10 min). On the test day, subject mice were placed into the central zone area without social stimuli or light delivery and allowed to freely explore the apparatus for 20 min. Analysis of duration spent within each compartment was automatically recorded by MED-PC IV software (MED Associates Inc.). The subtracted CPP score was calculated as: (test-phase duration spent in the social novel-paired chamber) − (test-phase duration spent in the social familiar-paired chamber). For the real-time place preference, mice were positioned in the central zone area and allowed to explore the entire apparatus for 20 min. Half of the animals did not receive photostimulation and the absolute times spent in the left (L) and right (R) chambers were automatically measured. The other half of mice received photostimulation (NpHR: 593 nm constant light, ~15–20 mW) when they were present in either one of the two chambers (counterbalanced between animals) during the 20 min test duration.

Object interaction tests. The apparatus consisted of a T-shaped maze (three arms, each 9 × 30 × 20 cm, connected through a 9 × 9-cm central zone) made of white Plexiglas. For experiments using C57BL/6J mice, interest in familiar and novel events was examined as described for social interaction but in the T-maze and using inanimate objects instead of social stimuli. Briefly, after 5 min of habituation to the apparatus, half of the mice were presented with an inanimate object located at one end of the T-maze arms (counterbalanced) for 5 min/d for three consecutive days. On day 3, the other half of mice were presented with a new inanimate object, placed in the same location as the previous object. All objects were plastic and induced similar levels of exploration. For optogenetic experiments, following 5 min of habituation, mice were presented with two inanimate objects positioned at each end of the T-maze arms. This session was repeated 24 h later. No light was delivered on days 1 and 2. On day 3, a novel object replaced the previous objects and half of the mice received paired optical stimulation (parameters identical to those used for social interactions). For single presentations of inanimate objects, all subject mice encountered one inanimate object, which remained unchanged and in the same location for two consecu- tive days. No light was delivered on days 1 and 2. Interaction groups with either a novel or familiar object stimuli were balanced in an unbiased way to account for individual animals’ rates of investigation. On days 3 and 4, half the subject mice were presented with the same familiar object, whereas the other half encountered a novel inanimate object in the same position as before. Within the novel and familiar groups, half of the animals received paired photostimulation only on day 3 (parameters identical to those used for social interactions).

Open field. Mice were individually placed facing one of the walls of a Plexiglass open-field (42 × 38 × 30 cm). Mice were allowed to explore freely for 10 min and the time spent in the center of the chamber compared to the outer areas was automatically tracked using EthoVision XT 11.5 (Noldus Apparatus). Half of the animals received light stimulation for the entire 10-min session (with the same parameters as described above).

Marble burying. The test was performed in standard mouse cages filled with a 5–6-cm thick layer of bedding material. All mice were habituated to the test cages for two consecutive days (30–40 min per d) without light stimulation. On the third day, 15 sterilized 1.5-cm glass marbles were evenly spaced above the bedding in five rows of three, each 4 cm apart. Implanted mice were placed in the test cage and left for 20 min, and the number of marbles buried with bedding (to 2/3 their depth) was counted. Half of the animals received photoluminonisation during the 20-min session.

Elevated plus maze. Implanted mice were plugged into a patch cord by magnetic force before the beginning of the session and subsequently placed in an elevated plus maze. The apparatus consisted of four arms connected by a central axis (5 × 5 cm) and was elevated 45 cm above the floor. Two of the arms were enclosed with plastic black walls (5 × 30 × 15 cm) while the other two remained open (5 × 30 × 0.25 cm). At the start of each session, mice were placed at the intersection, facing into an arm with no walls and allowed 5 min of free exploration. Optical stimulation was delivered to half of the animals and during the entire 5-min session (with the same parameters as described above). The number of entries into the open and closed arms and the total time spent in the open and closed arms were measured by MED-PC IV software. The time spent in open arms was considered an index of anxiety-like behavior and the total number of entries as an index of locomotor activity. The apparatus was cleaned between animals with Micro-90 solution.

Immunohistochemistry. Immunohistochemistry and microscopy were performed as described previously30. In brief, mice were given a fatal dose of sodium pentobarbital (200 mg kg−1) and transcardially perfused with 0.1 M ice-cold phosphate buffer saline (PBS, pH 7.4) followed by 10 mL of cold 4% (W/V) paraformaldehyde (PFA) in 0.1 M PBS. Brains were postfixed for 4 h in 4% PFA and submerged in 30% sucrose for 48 h. Coronal sections (25 µm) were obtained using a freezing microtome (HM430; Thermo Fisher Scientific, MA, USA) to assess viral placement and immunohistochemistry. For immunohistochemical experiments, brain sections were permeabilized with 0.5% Triton X-100 in 0.1 M PBS for 10 min, blocked with 5% donkey serum (DS, Sigma) in 0.1 M PBS for 30 min and then incubated overnight (o.n.) with the corresponding primary antibodies in 0.1 M PBS 3% DS at 4 °C. Primary antibodies used: rabbit anti-c-Fos (1:700, Santa Cruz Biotechnology, SC-52); mouse anti-TH (1:500, Millipore, MAB318); rabbit anti-GAD1/2 (1:1000, Sigma, GS163), mouse anti-Gad2 (1:800, Sigma, G1166); and goat anti-ChAT (1:800, Millipore, AB144P). Slices were subsequently washed in 0.1 M PBS and incubated in secondary antibodies for 2 h (all at 1:800 and from Life Technologies: donkey anti-rabbit Alexa Fluor 488 (A21206); donkey anti-rabbit Alexa Fluor 594 (A21207); donkey anti-mouse Alexa Fluor 594 (A21203); goat anti-mouse Alexa Fluor 488 (A11017); and donkey anti- Alexa Fluor 594 (A11058)). After washing in 0.1 M PBS, nuclei were counterstained with DAPI, sections were mounted, air-dried and coverslipped with Mowiol (Sigma). All slices were imaged using a fluorescent microscope (Zeiss, Carl Zeiss MicroImmagine, Inc., NY, USA) connected to computer-associated image analyzer software (Axiovision Rel., 4.6.1). All antibodies used have been verified previously for the intended use by the manufacturer. For c-Fos studies, fluorescent images were acquired at 20× magnification, and analysis of c-Fos

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nuclei was performed using ImageJ software. c-Fos+ nuclei were counted in all IPN serial slices and averaged in each mouse. For co-localization analysis between c-Fos and Gad1/2, the percentage of co-localized nuclei was estimated by dividing the number of c-Fos+ nuclei also labeled with Gad1/2 staining versus the total number of c-Fos+ nuclei. All animals were killed for analysis 90 min after the assay or optical stimulation. Optical stimulation was delivered for 5 min in mice while remaining in their home cages (ChR2: 473 nm, 8 pulses of 5-ms pulse-width, at 30 Hz, delivered every 5 s, ~15–20 mW).

**Slice preparation and electrophysiology.** For optogenetic slice electrophysiology, mice (age 10–12 weeks) were killed for analysis by i.p. injection of sodium pentobarbital (200 mg/kg). Brains were quickly removed and placed in an oxygenated ice-cold cutting solution containing (in mM): 2.5 KCl, 1.25 NaH2PO4, 20 HEPES, 2 thiourea, 5 Na-ascorbate, 92 NMDG, 0.5 CaCl2 and 10 MgSO4. Brain slices (~180–200 µM) were made using a Leica VT1200 vibratome. For recordings in the IPN and VTA, coronal brain slices (~200 µm) containing these regions were obtained using a vibratome (VT1200, Leica). For verifying optogenetic control of mHb→IPN connections in ChatCre mice, hybrid brain sections containing both regions were prepared. Brain slices were incubated in oxygenated cutting solution at 34 °C for 20 min. Slices were transferred into oxygenated artificial cerebrospinal fluid (ACSF) at room temperature for recording. ACSF solution contains (in mM): 125 NaCl, 2.5 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.4 CaCl2, 26 NaHCO3 and 11 D-glucose. Single slices were transferred into a recording chamber and continually superfused with oxygenated ACSF. The junction potential between the patch pipette and bath ACSF was nullified just before obtaining a seal. Action potentials were recorded at 32 °C using the whole-cell configuration of a patch-clamp amplifier (Multiclamp 700B; Molecular Devices). Action potentials were obtained by an episodic or gap-free acquisition mode using Clampex software (Molecular Devices). Signals were filtered at 1 kHz using the amplifier's four-pole, low-pass Bessel filter, digitized at 10 kHz with a Digidata 1440A interface (Molecular Devices) and stored on a personal computer. Pipette solution contained (in mM) 121 KCl, 4 MgCl2, 11 EGTA, 1 CaCl2, 10 HEPES, 0.2 GTP and 4 ATP. For optical recordings, eYFP+ neurons or eYFP signal surrounding soma (for presynaptic stimulation) were identified under fluorescence microscopy. Light pulses at 593 nm (for NpHR) or 473 nm (for ChR2, 20 Hz) were applied to neurons under current clamp or voltage clamp using LEDs of the appropriate wavelength (ThorLabs). For pharmacological experiments to block optogenetically evoked DAergic responses, SCH39166 (10 µM) was added to the bath solution.

**Statistics.** Data were analyzed by means of two-tailed unpaired t test, one-way or two-way ANOVA or repeated-measures (RM) ANOVA as indicated, using Graphpad (Graphpad Software Inc.). Bonferroni post hoc test was used when appropriate for one-way and two-way ANOVA. Unpaired t tests were used for choice models to calculate differences between two groups in terms of familiar and novel stimuli investigation, preference ratio, total investigation time and total distance traveled. The t test was also applied for anxiety tests, c-Fos expression analysis between two groups and for the CPP experiment, to calculate the effects of two different conditions. Two-way RM ANOVA was used for single interactions with familiar and novel stimuli across consecutive days when comparing two groups of mice, with day and group as main factors. One-way ANOVA was used for analysis of c-Fos expression in more than two groups of mice and in the choice experiments comprising three groups of animals. To analyze the changes in AP upon light delivery we used one-way RM ANOVA. Each data set was tested for normal distribution and equal variances before analysis, and statistical significance was established at *P* < 0.05. No statistical methods were used to predetermine sample sizes, but animal numbers were determined based on previous studies using similar endpoints33. All statistical methods were used to pre-determine sample sizes, but animal numbers were determined based on previous studies using similar endpoints33. All data are expressed as mean ± s.e.m. A Life Sciences Reporting Summary is available.

**Data availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request.

38. Shevtsova, Z., Malik, J.M., Michel, U., Bähr, M. & Kügler, S. Promoters and serotypes: targeting of adeno-associated virus vectors for gene transfer in the rat central nervous system in vitro and in vivo. *Exp. Physiol.* **90**, 53–59 (2005).

39. Silverman, J.L., Yang, M., Lord, C. & Crawley, J.N. Behavioural phenotyping assays for mouse models of autism. *Nat. Rev. Neurosci.* **11**, 490–502 (2010).
Experimental design

1. Sample size
   Describe how sample size was determined.
   No statistical methods were used to predetermine sample sizes. Animal numbers were determined based on previous studies using similar endpoints.

2. Data exclusions
   Describe any data exclusions.
   We indicate that animals with incorrect cannula placement, virus mis-injections, and incorrect optic fiber placements were excluded from analysis.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   All experiments were done in triplicate and reliably reproduced.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   All experiments were conducted in at least three cohorts of animals (i.e. in triplicate) which were randomly allocated into experimental groups for each genotype and counterbalanced across cohorts.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   For behavioral analysis, experimenters were blind to experimental conditions. In addition, injection sites and viral expression were confirmed for all animals by experimenters blinded to behavioral outcome. Finally, animals showing incorrect cannula placement were excluded from analysis by experimenters blinded to treatment.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   n/a | Confirmed
   × | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   × | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   × | A statement indicating how many times each experiment was replicated
   × | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   × | A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   × | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
   × | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   × | Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

All software used for analysis is commercially available. For mouse tracking, EthoVision XT 11.5 (Noldus Apparatus) was used. For elevated plus maze and conditioned place preference, MED-PC IV software was used. Electrophysiology data were acquired using Clampex (in Pclamp 10.0, Molecular Devices). For slice imaging, data were acquired with Axiovision Rel., 4.6.1. Data were graphed and analyzed using Graphpad Prism 7.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Primary antibodies used: rabbit anti Fos 1:700 (Santa Cruz Biotechnology, SC-42); mouse anti-TH 1:500 (Millipore, MAB318); rabbit anti-GAD1/2 1:1000 (Sigma, G5163), mouse anti-GAD2 1:800 (Sigma, G1166); goat anti-ChAT 1:400 (Millipore, AB144P). Secondary antibodies used: (1:800; Life Technologies) donkey anti-rabbit 488 (A21206); donkey anti-rabbit 594 (A21207); donkey anti-mouse 594 (A21203); goat anti-mouse 488 (A11017); donkey anti-goat 594 (A11058). All antibodies used have been verified previously for the intended use by the manufacturer.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

All experiments were conducted in accordance with the guidelines for care and use of laboratory animals provided by the National Research Council, as well as with an approved animal protocol from the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School (UMMS). C57Bl/6J (#000664), GAD2-Cre (#10802), Chat-Cre (#006410), DAT-Cre (#006660) and Drd1a-tdTomato (#016204) mice were obtained from The Jackson Laboratory, bred in the UMMS animal facility and used in behavioral, optogenetic and biophysical experiments as indicated. Cre lines were crossed with C57Bl/6J mice and only heterozygous animals carrying one copy of the Cre recombinase gene were used for experimental purposes. For social experiments, juvenile stimuli always consisted of male C57Bl/6J mice (4-7 weeks old) bred in the UMMS animal facility. All mice were housed together and kept on a standard 12 h light/dark cycle (lights ON at 7 A.M.) with ad libitum access to food and water. Three to four weeks before experimentation, subject mice were kept under a reverse 12 h light/dark cycle (lights ON at 7 P.M.), and individually housed for at least 5 days before any behavioral testing.

All behavioral experiments were conducted during the active dark phase (8 A.M. to 6 P.M.) of mice aged between 9-12 weeks old. Animals were acclimated to the testing room for 30 min before any experiment, and all testing was performed under dim red light conditions. For experiments involving C57BL/6J and Drd1aTdtomato mice, animals were used only in one behavioral paradigm. For optogenetic experiments, animals used for both social and object interactions underwent a minimum of at least a 4-day wash-out period between photo-stimulations. All social behavior experiments were performed in male mice that interacted with male juvenile stimuli. Data for object interaction using the GAD2Cre line included both males and females as no sex differences were detected. All experiments were conducted in at least three cohorts of animals (i.e. in triplicate) which were randomly allocated into experimental groups and counterbalanced across cohorts.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Provide all relevant information on human research participants, such as age, gender, genotypic information, past and current diagnosis and treatment categories, etc. OR state that the study did not involve human research participants.