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Nucleus accumbens dopamine release reflects the selective nature of pair bonds

Highlights

- Partner preference in an existing bond does not require D1- or D2-class signaling
- Social seeking in voles requires dopamine D1-, but not D2-, class receptor activity
- Accumbal dopamine release is greater for pair-bonded partners than unknown voles
- Long-term separation erodes enhanced partner-associated dopamine release

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In brief

Pierce et al. delineate a dopaminergic mechanism underlying partner seeking and selective affiliation in monogamous relationships. They show that dopamine signaling is required for social seeking in prairie voles and that accumbal dopamine release is enhanced during partner seeking and interaction, which erodes after long-term partner separation.

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Nucleus accumbens dopamine release reflects the selective nature of pair bonds

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SUMMARY

In monogamous species, prosocial behaviors directed toward partners are dramatically different from those directed toward unknown individuals and potential threats. Dopamine release in the nucleus accumbens has a well-established role in social reward and motivation, but how this mechanism may be engaged to drive the highly divergent social behaviors directed at a partner or unfamiliar conspecific remains unknown. Using monogamous prairie voles, we first employed receptor pharmacology in partner preference and social operant tasks to show that dopamine is critical for the appetitive drive for social interaction but not for low-effort, unconditioned consummatory behaviors. We then leveraged the subsecond temporal resolution of the fluorescent biosensor, $GRAB_{DA}$, to ask whether differential dopamine release might distinguish between partner and novel social access and interaction. We found that partner seeking, anticipation, and interaction resulted in more accumbal dopamine release than the same events directed toward a novel vole. Further, partner-associated dopamine release decreased after prolonged partner separation. Our results are consistent with a model in which dopamine signaling plays a prominent role in the appetitive aspects of social interactions. Within this framework, differences in partner- and novel-associated dopamine release reflect the selective nature of pair bonds and may drive the partner- and novel-directed social behaviors that reinforce and cement bonds over time. This provides a potential mechanism by which highly conserved reward systems can enable selective, species-appropriate social behaviors.

INTRODUCTION

Optimally navigating social interactions is critical for survival and reproduction. Across species, dopamine plays an important role in navigating social relationships. Dopamine is released in the nucleus accumbens during social interaction, and manipulations that increase or decrease dopaminergic activity within this region promote or impair social interactions, respectively.[1–3] Yet studies to date have examined real-time dopamine dynamics exclusively in laboratory species that do not form selective pair bonds.[1,2,4,5] Thus, a central question remains of how differences in dopaminergic signaling directed to a pair-bonded partner or novel individual may contribute to selective pair bonds and ultimately enable species-appropriate behaviors.

Prairie voles are monogamous rodents that form lifelong pair bonds. The formation of these bonds is facilitated by mating, an event that triggers dopamine release in the nucleus accumbens and results in a preference to affiliate with a specific partner, as well as aggression toward novel voles of either sex.[6,7] Both of these behavioral features of pair bonds have been shown to depend on dopaminergic signaling.[8–12] Blockade of dopamine D2- but not D1-class receptors during the initial mating period impedes the formation of a selective partner preference, although the same manipulation does not affect preference in established bonds.[9,12] Conversely, D1-class receptors mediate agonistic behaviors; activation of these receptors increases selective aggression in bonded voles,[8,13] and their activation can also impair bond formation.[9] Plasticity within dopaminergic systems has also been implicated in bond formation and maintenance. Dopamine D1-class receptors are upregulated,[8,13] and release dynamics are sensitized in established bonds.[13] Electrical stimulation of the striatum leads to enhanced accumbal dopamine release in bonded compared with sexually naive voles.[13]

While these results indicate that dopaminergic systems play a highly conserved role in social reward, a major outstanding question is how shared neuromodulatory mechanisms can be differently engaged to create species-typical social drives. We performed a series of experiments examining the role of dopamine in partner seeking and preference expression, respectively. We found that dopamine receptor blockade did not disrupt partner preference in voles with an established bond but that D1-class receptors instead modulate effortful seeking of social interaction. These findings are consistent with a broad role of

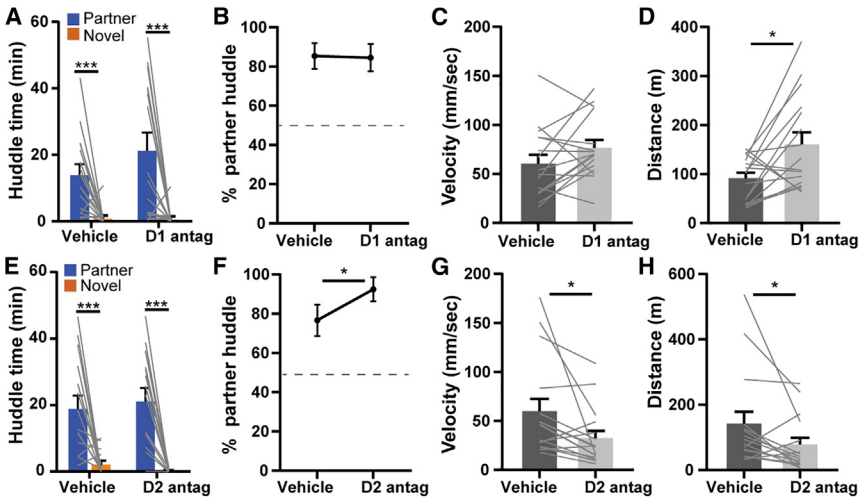

Figure 1. Systemic dopamine receptor blockade does not impede partner preference in established pair bonds

(A–D) D1 antagonism (SCH-23390 0.5 mg/kg) did not disrupt partner preference (A), percent partner huddle (B), or velocity (C), but it did increase distance traveled (D) during the first hour of the partner preference test.

(E–H) D2 antagonism (eticlopride, 2 mg/kg) did not disrupt partner preference (E) but did increase percent partner huddle (F). D2 antagonism decreased velocity (G) and distance traveled (H). Error bars show SEM. n = 16. *p < 0.05; ***p < 0.005. See also Figure S1 and Data S1.

dopamine D1 systems in appetitive aspects of motivation.[14–17] Reasoning that release dynamics may provide a level of specificity masked by the receptor blockade, we tested the hypothesis that accumbal dopamine systems differentiate between interactions with a bonded partner and an unknown conspecific. We found that pair-bonded partners elicit enhanced dopamine release during partner seeking and during subsequent social interactions, consistent with a reward-valuation role for dopamine in pair bonding (i.e., by assigning motivational valence) and providing a potential mechanism by which the highly conserved mesolimbic system can be engaged to elicit species-typical and selective social behaviors. Consistent with this hypothesis, we also observed an erosion of partner-enhanced dopamine release and partner-directed behaviors, following bond devaluation via prolonged separation.

RESULTS

All sample sizes and comprehensive statistical results, including effect size estimates, are reported in Data S1.

Systemic dopamine receptor blockade does not impair expression of an existing partner preference

Prior reports indicate that D1- and D2-class signaling is not required for expression of partner preference in voles with an existing pair bond.[12] Using the same antagonists in doses consistent with prior studies,[12] we replicated these findings. To target D1-class receptors, we used the antagonist SCH-23390 hydrochloride, and for D2-class receptors, we used eticlopride hydrochloride.[18–20] We focused on the first hour of the partner preference test, during which the animals had the highest circulating levels of antagonist. This duration is also consistent with additional experiments outlined below. D1 blockade did not alter partner preference (Figures 1A and 1B; vehicle: one-way t test relative to 50% [no preference] $t_{(15)}$ = 5.339, p = 8.27E−5; DRD1 antagonist: one-way t test relative to 50% [no preference] $t_{(15)}$ = 4.936, p = 1.79E−4; vehicle vs. antagonist paired t test: $t_{(15)}$ = 0.099, p = 0.922). D1-class antagonist administration did not alter velocity but did increase total locomotion in the apparatus (Figures 1C and 1D; velocity: paired t test: $t_{(15)}$ = 1.833, p = 0.087; locomotion: paired t test: $t_{(15)}$ = 2.864, p = 0.012). D2-class antagonism also did not impair partner preference expression, but unlike D1 blockade, it led to an increase in percent partner preference relative to vehicle treatment (Figures 1E and 1F; vehicle: one-way t test relative to 50% $t_{(15)}$ = 3.308, p = 5.00E−3; DRD2 antagonist: one-way t test relative to 50% $t_{(15)}$ = 6.86, p = 5.41E−6; vehicle vs. antagonist paired t test: $t_{(15)}$ = 2.653, p = 0.018). This was accompanied by a decrease in velocity and total locomotion (Figures 1G and 1H; velocity: paired t test: $t_{(15)}$ = 2.523, p = 0.012; locomotion: paired t test: $t_{(15)}$ = 2.323, p = 0.017). Although locomotor differences at these doses were not observed in previously published studies,[12] our results could reflect differences in how locomotion was calculated and/or could be more evident during the first hour of the partner preference test.

We also assessed locomotor coordination via a rotarod apparatus (Figure S1A). We found that 1 but not 0.5 mg/kg of SCH-23390 hydrochloride decreased time spent on the rod (Figure S1B; Data S1). Neither of the doses tested for eticlopride hydrochloride (1.25 and 2 mg/kg) altered locomotor effects (Figure S1C; Data S1). This indicates that locomotor coordination is at least partly dissociable from total locomotion/velocity.

Systemic D1-class but not D2-class antagonism reduces the appetitive aspects of social motivation

To systematically examine the potential role of dopamine in different facets of pair bonding, we next implemented lever pressing and barrier climbing for social access, two tasks that have been extensively used to evaluate behavioral activation and seeking behavior, key properties of motivated behavior.[21–24] Female prairie voles are more adept at learning lever-pressing tasks, show more consistent behavior than males, and exhibit stronger partner- than novel-directed motivation.[25–27] This, combined with prior reports showing the necessity of dopamine signaling in female voles for bond formation,[11,12] led us to focus on females.

We tested the functional role of D1- and D2-class receptors in pair-bonded voles in two lever-pressing tasks and in a barrier-climbing task. In the first version of the lever-pressing task, voles were presented with a single lever, which delivered access to a pair-bonded partner through a slotted divider for 30 s (Figure S1D). The second version of the task was equipped with 2 levers, with each lever assigned to provide direct access to the

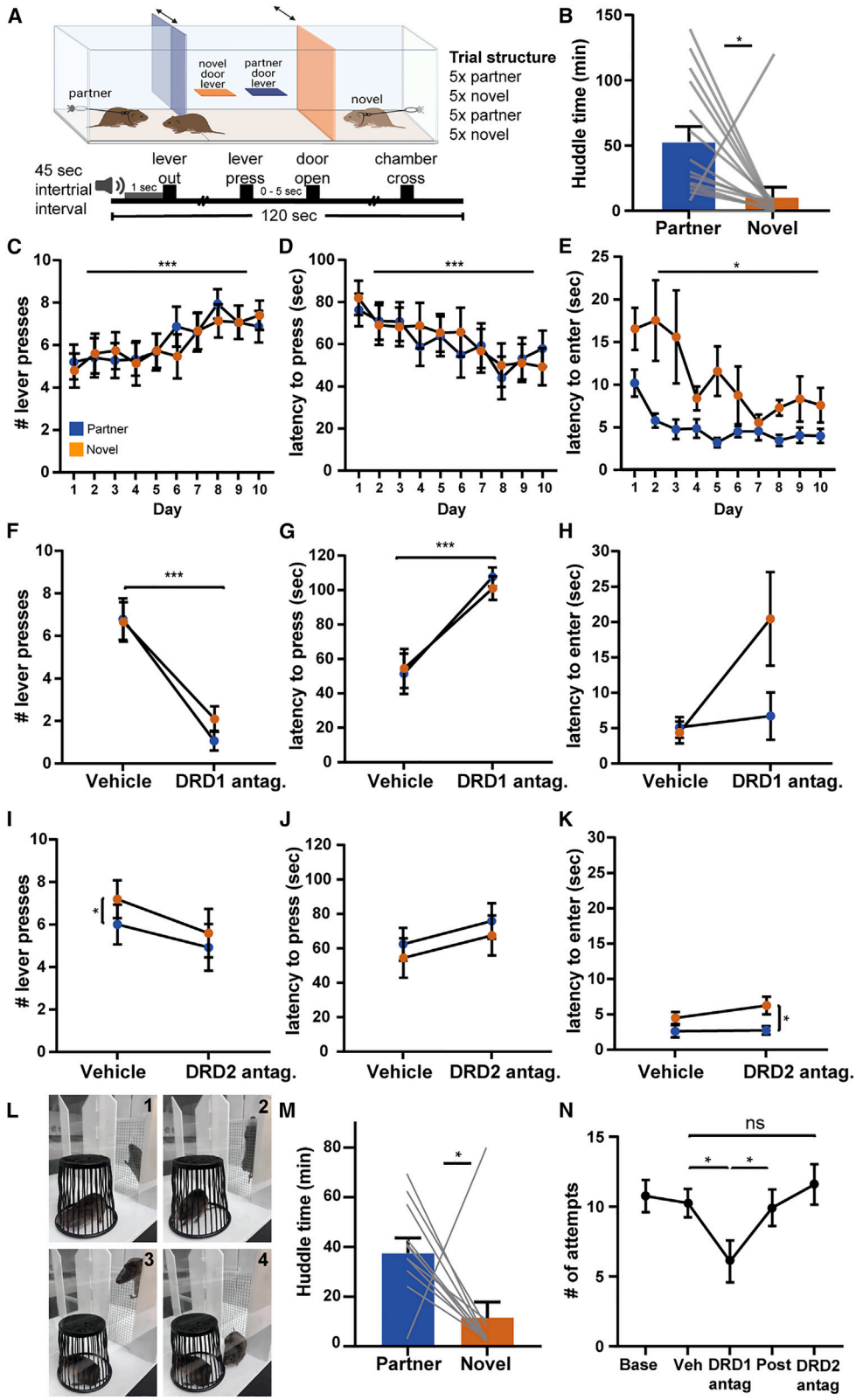

Figure 2. Systemic D1-class but not D2-class receptor signaling is required for social seeking

(A) Social operant chamber and operant trial structure.

(B) A partner preference test confirmed pair bond formation.

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partner or a novel vole, respectively, in 5-trial blocks (Figure 2A). Pair-bonded voles (Figure 2B) learned to press for social access in both paradigms. In the single-lever task, the number of lever presses increased across training days (Figure S1E; Data S1), and the latency to press decreased across training days (Figure S1F; Data S1). In the dual-lever task, we observed similar increases in lever pressing and decreasing latency to press as animals learned the task (Figure 2C; two-way RM-ANOVA: main effect of days $F_{(9,\ 126)} = 14.505$, $p = 0.002$; Figure 2D; two-way RM-ANOVA: main effect of days $F_{(9,\ 126)} = 27.947$, $p = 1.15E{-}4$). Consistent with subsequent cohorts (Figure 4), there was no difference in the number of lever presses or the latency to press for partner or novel access (Figure 2C; two-way RM-ANOVA: main effect of vole [partner vs. novel]: $F_{(1,14)} = 0.4707$, $p = 0.5039$; Figure 2D; two-way RM-ANOVA: main effect of vole [partner vs. novel]: $F_{(1,14)} = 0.3098$, $p = 0.5866$). We likewise found that the latency to enter the chamber decreased across training days and did not differ between partner and novel trials (Figure 2E; mixed-model ANOVA: main effect of vole [partner vs. novel]: $F_{(1,6)} = 1.69$, $p = 0.241$; main effect of days $F_{(9,\ 54)} = 7.119$, $p = 0.037$; interaction [vole × days]: $F_{(9,\ 54)} = 1.271$, $p = 0.303$). This is consistent with a prior report showing that voles will press equally for stranger and partner access when they are not forced to make a choice and if minimal effort is required for access.[25] This procedure ensured that we had enough trials to examine both partner- and novel-directed behaviors.

Once voles achieved consistent lever pressing, we asked whether systemic blockade of D1- or D2-class receptors altered lever pressing behaviors. In both tasks, administration of the D1-class antagonist, SCH-23390 hydrochloride, decreased lever pressing. We observed decreased lever presses and increased latency to lever press in the dual-lever task (Figures 2F and 2G; Data S1). In the single-lever task, lever pressing returned to pre-antagonist levels within 24 h post-administration (Figure S1G; Data S1). The effects generalized to both partner and novel voles. D1-class antagonist administration reduced pressing for the partner and novel voles (Figure 2F; two-way RM-ANOVA: main effect of partner vs. novel: $F_{(1,\ 13)} = 2.317$, $p = 0.1519$; post hoc Bonferroni: partner vehicle vs. partner antagonist: $t_{(13)} = 10.22$, $p = 7.69E{-}5$. Novel vehicle vs. novel antagonist: $t_{(13)} = 8.18$, $p = 2.984E{-}4$; Figure 2G; two-way ANOVA: main effect of partner vs. novel: $F_{(1,\ 13)} = 0.513$, $p = 0.488$. post hoc Bonferroni: partner vehicle vs. partner antagonist: $t_{(13)} = 10.34$, $p = 4.76E{-}4$. Novel vehicle vs. novel antagonist: $t_{(13)} = 8.57$, $p = 1.51E{-}3$). In contrast, administration of a D2-class antagonist did not reduce motivation in either task (single lever: Figure S1H; Data S1; dual lever: Figures 2I–2K; lever press: two-way RM-ANOVA: main effect of treatment: $F_{(1,\ 13)} = 1.661$, $p = 0.222$; latency to lever press: two-way RM-ANOVA: main effect of treatment: $F_{(1,\ 13)} = 1.51$, $p = 0.243$; latency to enter: mixed-model ANOVA: main effect of treatment: $F_{(1,\ 13)} = 1.881$, $p = 0.207$).

We also tested the role of D1- and D2-class signaling in a second task that leverages innate motivation to access a pair-bonded partner by climbing over a mesh barrier (Figure 2L).[28,29] Systemic administration of the D1-class antagonist, but not D2-class antagonist or vehicle, reduced attempts to climb the barrier to access a pair-bonded partner (Figure 2M; huddle time in partner preference test to verify pair bond: paired t tests: $t_{(11)} = 2.402$, $p = 0.0351$; % partner huddle one-way t test relative to 50% [no preference] $t_{(11)} = 2.51$, $p = 0.0290$ Figure 2N; one-way RM-ANOVA $F_{(4,\ 4)} = 4.158$, $p = 0.0061$. post hoc Sidak: D1 antag vs. veh: $p = 0.021$; D1 antag vs. post: $p = 0.0375$; D2 antag vs. veh: $p = 0.7508$). The convergence of our results across learned (lever pressing) and innate (climbing) tasks show that D1-class antagonism reduces social motivation in voles. Given that the same antagonist increased total locomotion in the partner preference test (Figure 1D), it is highly unlikely that reductions in climbing or pressing behavior are due to suppression of motor behaviors. This is further supported by the observation that D2-class antagonist administration reduced velocity and locomotion in the partner preference test (Figures 1G and 1H) but had no effects on lever pressing or climbing.

Dopamine dynamics reflect social operant learning

Based on the importance of D1 activity in social seeking behavior, we investigated the dynamics of dopamine release during these behaviors. We performed fiber photometry to measure $GRAB_{DA}$-mediated fluorescence as a proxy for dopamine release in the nucleus accumbens of voles engaged in operant responding and consumption of a social reward (Figures 3A–3E).[30] Voles initially learned to associate rewards with lever pressing through food delivery (Figures S2A–S2D) before being presented with two new, separate levers that provided transient access to a tethered partner or novel animal, respectively (Figures 3D–3F).

Dopamine dynamics are typically conserved across species; thus, we expected increased dopamine release for events predicting social access upon task learning.[2,31,32] We compared dopamine release on the first (day 1) and last (day 6) days of social operant access (Figure 3). Task learning was reflected in increased lever pressing across days (Figure 3G; one-way RM-ANOVA: $F_{(5,\ 10)} = 6.139$, $p = 0.0047$), a non-significant decrease in latency to press the lever (Figure3H; mixed-model ANOVA: $F_{(5,\ 10)} = 1.9$, $p = 0.178$) and decreased latency to enter the social chamber after the door opened (Figure 3I; mixed-model ANOVA: $F_{(5,\ 10)} = 8.217$, $p = 0.0018$). We found that dopamine release increased for operant events associated with social access on day 6 relative to day 1, consistent with prior reports

(C–E) The number of lever presses increased (C), and latency to lever press (D), and latency to enter the social chamber (E) decreased across training days.
(F–H) Compared with vehicle, systemic D1-receptor blockade reduced number of lever presses (F) and increased the latency to press for partner and novel access (G) but did not change the latency to enter the social chamber (H).
(I–K) Systemic DRD2 did not reduce the number of lever presses (I), latency to lever press (J), or latency to enter chamber (K).
(L) Time series images of vole climbing over barrier to get access to a partner vole (under cup).
(M) Animals performing the barrier task had a partner preference.
(N) Number of attempts to climb over the barrier was reduced by systemic D1 but not D2 antagonism. n = 15, operant task. Error bars show SEM. n = 12, barrier task. *$p < 0.05$; ***$p < 0.005$.
See also Figure S1 and Data S1.

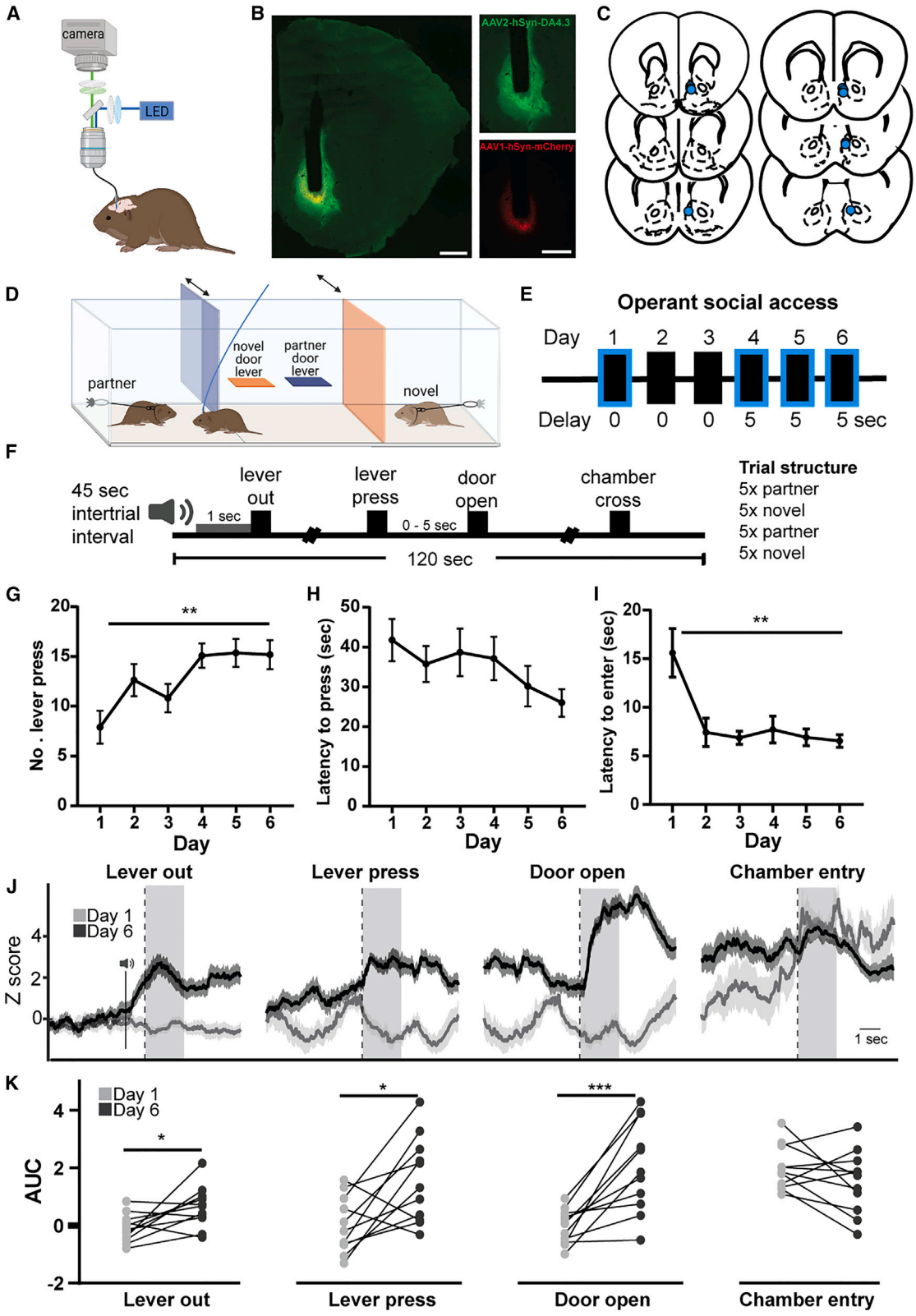

Figure 3. Dopamine dynamics reflect social operant learning

(A) Schematic of fiber photometry in a prairie vole.

(B) $GRAB_{DA}$ and mCherry expression in the nucleus accumbens shell with ferrule track. Scale bars, 500 μm.

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indicating that dopamine release increases as a function of learning and subsequent reward anticipation (Figures 3J and 3K; paired t tests: lever out $t_{(10)} = 2.716$, $p = 0.0217$; lever press $t_{(10)} = 2.948$, $p = 0.0146$; chamber open $t_{(10)} = 4.696$, $p = 0.0008$).[2,33] We did not observe any differences in dopamine levels associated with chamber entry as a function of task learning (Figures 3J and 3K; paired t test chamber entry $t_{(10)} = 1.474$, $p = 0.1711$), consistent with chamber entry as a highly salient event associated with social reward delivery.

Partner seeking elicits enhanced dopamine release, which is eroded by long-term bond disruption

We next asked whether dopamine dynamics distinguished between partner- and novel-associated operant events during interleaved 5-trial blocks in which lever pressing resulted in partner or novel access, respectively (Figure 4A). Because partner- and novel-directed pressing behavior did not differ (Figures 4C–4E; see Data S1), we asked whether dopamine dynamics could predict pressing behavior for the partner or a novel vole. We examined dopamine levels during the 1-s tone indicating the start of a trial and immediately prior to lever extension once the task was learned (Figures 4C–4E, boxed region). While tone-associated dopamine did not differ for partner and novel trials (Figure S3A; Data S1), we found tone-associated dopamine predicted whether the vole would press the lever for the partner (Figure S3B; Data S1), potentially reflecting a strong partner-reward association. This relationship between dopamine release and future lever pressing was not observed on novel trials (Figure S3C; Data S1), making it unlikely that the differences in DA release were tied to the motoric aspects of lever pressing. This was also limited to the 1-s tone; dopamine release after lever extension did not predict pressing behavior for either partner or novel voles (see Data S1). We next asked whether dopamine release differentiated partner and novel trials after lever pressing. Across the last 3 days of social operant access, we observed greater dopamine release for partner lever pressing and door opening, compared with the same events in novel trials (Figures 4F–4H; paired t tests: lever press $t_{(10)} = 2.791$, $p = 0.0191$; door open $t_{(10)} = 2.307$, $p = 0.0438$). Differences in dopamine release for partner and novel trials were not evident after lever extension or immediately after chamber entry (Figures 4F and 4G; paired t tests: lever out $t_{(10)} = 1.382$, $p = 0.1972$; chamber entry (0–2 s) $t_{(10)} = 1.515$, $p = 0.1606$).

To further explore the potentially unique features of pair bonding relative to other types of natural reward, we compared dopamine release during social operant and food operant access (Figure S2). Anticipation of food delivery (lever press and pellet dispense) and consumption of the food pellet resulted in significantly less dopamine release than lever pressing, door opening, and chamber opening, respectively, during partner trials. However, dopamine release during lever pressing for food and pellet dispensing were not significantly different than lever pressing and chamber opening for novel trials (Figure S2F; Data S1). Thus, partner anticipation and partner access elicit greater dopamine than at least two other motivating events—access to a novel vole and access to food.

Finally, as prior work has shown that pair bonds erode as a function of long-term separation,[34–36] we asked how long-term partner separation affected partner- and novel-associated dopamine dynamics. Prior to separation, we performed a partner preference test to confirm that experimental voles displayed a partner preference (Figure 4B; one sample t test relative to 50%: $t_{(10)} = 2.895$, $p = 0.016$). We then separated pairs for 4 weeks—sufficient time for a vole to be able to form a new bond that supersedes the prior bond.[34] We performed a single-probe operant test via 1 day of social operant access with trial structure identical to pre-separation tests (Figure 4I). We found that although experimental voles still pressed the lever, their performance on the task was reduced. There was a significant decrease in the number of lever presses (Figure 4C; two-way RM ANOVA; day 6 vs. post-separation: $F_{(1,10)} = 5.314$; $p = 0.044$; partner vs. novel: $F_{(1,10)} = 0.358$; $p = 0.563$; time point × conspecific interaction: $F_{(1,10)} = 0.004$; $p = 0.953$). While there was no change in the latency to lever press (Figure 4D; latency to press: two-way RM ANOVA; day 6 vs. post-separation: $F_{(1,8)} = 1.393$; $p = 0.272$; partner vs. novel: $F_{(1,8)} = 0.143$; $p = 0.715$; time point × conspecific interaction: $F_{(1,8)} = 0.134$; $p = 0.724$), there was an increase in the latency to enter the vole's chamber (Figure 4E; latency to enter chamber: two-way RM ANOVA; day 6 vs. post-separation: $F_{(1,8)} = 48.119$; $p = 1.2E{-}4$; partner vs. novel: $F_{(1,8)} = 0.024$; $p = 0.88$; time point × conspecific interaction: $F_{(1,8)} = 0.341$; $p = 0.575$). Surprisingly, we also saw a reversal in whether dopamine release predicted lever pressing, potentially reflecting a switch in reward away from the partner and toward a novel mating/bonding opportunity (Figures S3D–S3F; Data S1). Furthermore, after partner separation, lever pressing and chamber opening no longer elicited differences in dopamine release between partner and novel trials (Figure 4J; paired t tests: lever out: $t_{(10)} = 0.0379$, $p = 0.9705$; lever press: $t_{(7)} = 1.502$, $p = 0.1769$; chamber open: $t_{(7)} = 0.639$, $p = 0.5432$; chamber entry $t_{(7)} = 2.039$, $p = 0.0808$). To determine the underlying changes in dopamine dynamics that led to an erasure of partner-enhanced dopamine release, we compared pre- and post-separation dopamine release. We observed a consistent intra-animal decrease in operant-associated dopamine release upon lever presentation, door opening,

(C) Coronal atlas sections with locations of injection sites and ferrules (blue dots).
(D) Social operant chamber.
(E) Experimental timeline of operant social access. Blue outline indicates fiber photometry recording of dopamine levels. Delay (0 or 5 s) from lever press to chamber opening.
(F) Trial structure.
(G–I) The average number of lever presses increased (G), and latency to press (H) and latency to enter the social chamber (I) decreased across training days.
(J) Representative $GRAB_{DA}$ fluorescence on the first and last day of operant social access during lever extension (lever out), lever press, social chamber opening (door open), and while crossing into the social chamber as detected by an infrared beam break (chamber entry). The onset of each event is indicated by a dashed line.
(K) Area under the curve (AUC) for 2 s post-event (shaded regions of J) comparing the first and last training days. Error bars show SEM. n = 11. *p < 0.05; **p < 0.01; ***p < 0.005.
See also Figure S2 and Data S1.

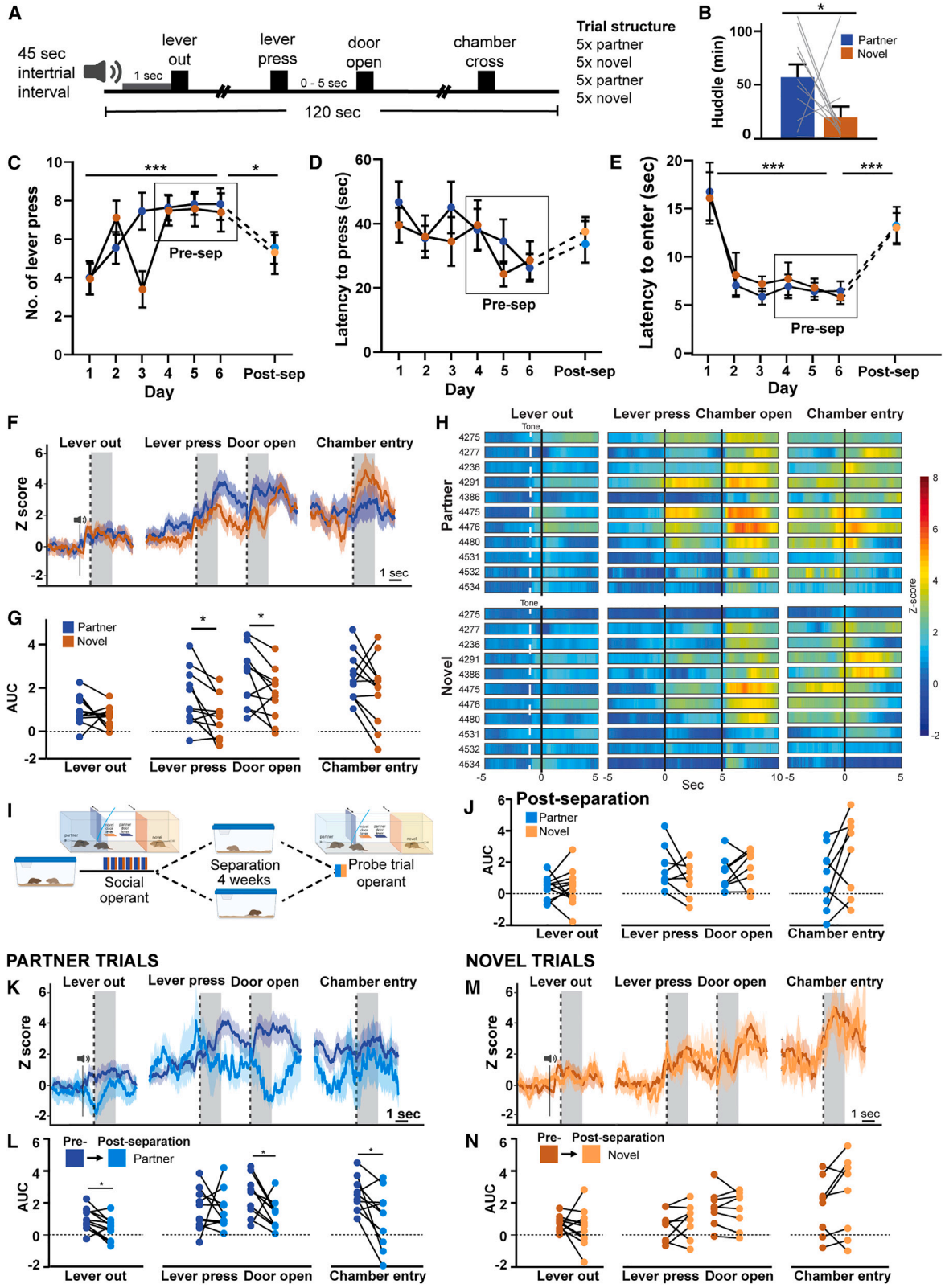

Figure 4. Partner seeking and access elicits enhanced dopamine release that erodes after partner separation

(A) Timing of a single trial. $GRAB_{DA}$-mediated fluorescence was recorded during interleaved blocks of partner and novel trials.

(B) Voles had a partner preference.

(legend continued on next page)

and chamber entry for partner trials (Figures 4K and 4L; paired t tests: lever out: $t_{(10)} = 2.88$, $p = 0.0164$; lever press: $t_{(9)} = 0.04279$, $p = 0.9668$; chamber open: $t_{(9)} = 2.403$, $p = 0.0397$; chamber entry: $t_{(9)} = 2.705$, $p = 0.0242$) but no change for novel trials (Figures 4M and 4N; paired t tests: lever out: $t_{(10)} = 0.717$, $p = 0.4898$; lever press: $t_{(7)} = 0.9486$, $p = 0.3744$; chamber open: $t_{(7)} = 0.5486$, $p = 0.6003$; chamber entry: $t_{(7)} = 1.479$, $p = 0.1826$). As the voles still showed increases in response to the tone/lever out, lever pressing, and door opening, this suggests that the partner-specific reduction in dopamine release was not simply a result of unlearning the task. Likewise, the lack of change in dopamine release during novel vole trials before and after separation indicates that decreased partner-associated dopamine release is not the product of technical considerations, such as a reduction in $GRAB_{DA}$ fluorescence.

Social behavior and dopamine dynamics that differentiate partner and novel interactions are eroded by long-term separation

Social interactions between partners and with novel voles differ dramatically. We next quantified social behavior and corresponding dopamine release in the operant task after the test animal entered the social chamber. In accordance with prior reports,[27] we found that pair-bonded voles are more affiliative toward their partner than toward a novel vole, displaying more cumulative bouts of direct investigative contact and huddling behavior (Figures 5C and 5G; log rank [Mantel-Cox] tests: direct contact investigation consisting of head, body, and anogenital sniffing: $\chi2 = 17.67$, $p \leq 0.0001$; huddle: $\chi2 = 16.25$, $p \leq 0.0001$).[7] In contrast, they show a greater number of cumulative bouts of non-contact investigation toward novel voles (Figure 5K; log rank [Mantel-Cox] $\chi2 = 6.565$, $p = 0.0104$). Physical interaction with partners—both via direct contact and huddling—produced greater dopamine release than the same behaviors directed toward a novel vole (Figures 5D, 5E, 5H, and 5I; body sniff: paired t test: $t_{(5)} = 8.974$, $p = 0.0003$; huddle: unpaired t test: $t_{(7)} = 3.268$, $p = 0.0137$). We did not observe differences in dopamine elicited by the partner or a novel vole during non-contact investigation (Figures 5L and 5M; paired t test: $t_{(10)} = 1.085$, $p = 0.3033$).

We next asked how long-term separation affected interaction behavior and associated dopamine release (Figure 5). Changes in social interaction behavior were largely consistent with a partial erosion of the pair bond. Overall, there was a greater duration of direct contact investigation of both the partner and novel (Figure 5B; two-way ANOVA: main effect of separation: $F_{(1,29)} = 9.936$, $p = 0.004$). After separation, voles still displayed an increased cumulative number of bouts of direct contact toward the partner, compared with the novel vole (Figure 5C; log rank [Mantel-Cox] test: $\chi2 = 7.303$, $p = 0.0069$). Separation did not significantly alter the duration of time spent huddling (Figure 5F; two-way ANOVA: main effect of separation: $F_{(1,11)} = 1.838$, $p = 0.2$), and voles still had a greater number of cumulative bouts of huddling with the partner, compared with the novel vole (Figure 5G; log rank [Mantel-Cox] test: $\chi2 = 10.66$, $p = 0.0011$). The duration of non-contact investigation decreased after separation (Figure 5J; two-way ANOVA: main effect of separation: $F_{(1,29)} = 5.646$, $p = 0.023$), and there was no difference in the number of bouts of non-contact investigation displayed toward the partner and novel vole (Figure 5K; log rank [Mantel-Cox] $\chi2 = 0.1704$, $p = 0.6797$). The overall larger number of bouts of direct-contact investigation and huddling toward the partner, compared with the novel vole, was retained, suggesting that the test animals remembered their partner, and partner-selective behaviors were not completely erased (Figures 5B and 5C). None of the partner-novel differences in dopamine release detected pre-separation were evident post-separation (Figures 5D, 5E, 5H, 5I, 5L, and 5M; paired t tests: direct contact $t_{(6)} = 0.218$, $p = 0.8347$; huddle: $t_{(4)} = 1.198$, $p = 0.2971$; non-contact investigation: $t_{(7)} = 0.7659$, $p = 0.4688$). Similar to the greater dopamine release observed in partner-seeking contexts, these data support the model that dopamine release potentially signals value/motivational valence in pair-bonded social contexts and that long-term separation reduces the value or valence of the partner.

DISCUSSION

Dopamine modulates reward, motivation, and learning across broad contexts and has been extensively implicated in social behavior across species. Here, we demonstrate that many broad dopaminergic functions are retained in monogamous prairie voles, including a role for dopamine D1-class signaling in appetitive social behaviors and conserved learning-related release dynamics. However, we also show that dopamine dynamics reflect the selective nature of pair bonds; partner-associated operant events and interactions led to greater accumbal dopamine release. Together, this suggests that dopamine plays a key role in mediating the appetitive aspects of pair bonding and provides a putative mechanism by which conserved neuromodulatory systems can contribute to species-appropriate and highly selective social behaviors.

We juxtaposed the well-established partner preference test with tasks that engage effort-driven seeking of social interaction. Reward acquisition in the partner preference test requires relatively little effort. In contrast, lever pressing and barrier climbing

(C–E) Number of lever presses increased (C), and latency to press (D) and to enter the social chamber (E) decreased across training days. Pressing behavior stabilized for the last 3 days of operant access (dotted box) and did not differ between partner and novel presses for any metric.
(F) Representative $GRAB_{DA}$ fluorescence in response to social operant events (days 4–6).
(G) Area under the curve (AUC) for 2-s post-event (shaded regions in F).
(H) Heatmap showing *Z*-scored fluorescence for operant events for each vole (partner trials top, novel trials bottom).
(I) Graphical image of operant testing schedule prior to partner separation and a single-probe operant trial after 4 weeks of partner separation.
(J) There are no differences in DA release for partner vs. novel operant events post-separation.
(K and L) Representative traces (K) and AUC graphs (L) showing that compared with pre-separation, dopamine release was reduced for partner-associated lever out, door opening, and chamber entry.
(M and N) Representative traces (M) and AUC graphs (N) showing that compared with pre-separation, dopamine release was unchanged for novel-associated operant events. All traces from vole 4291.Error bars show SEM. n = 11. *p < 0.05; ***p < 0.005.
See also Figures S2 and S3 and Data S1.

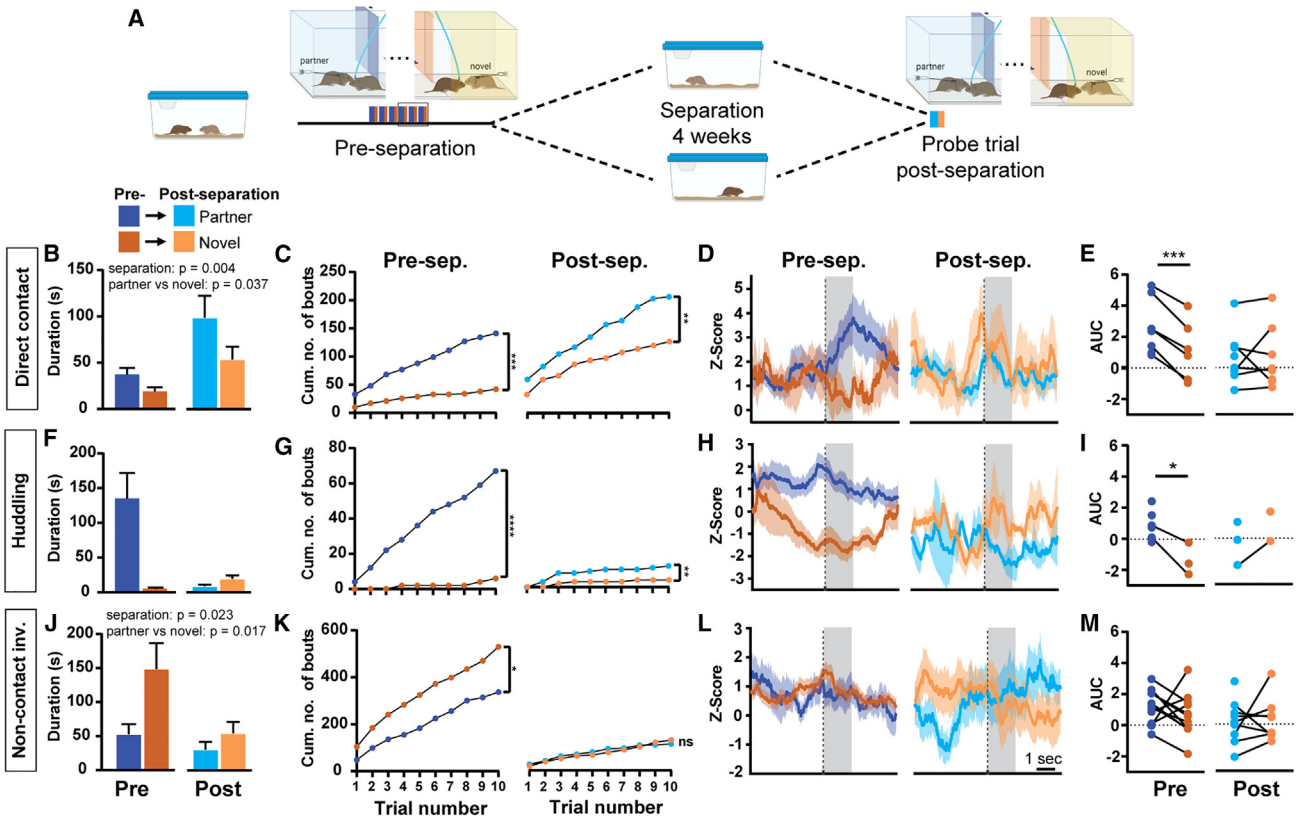

Figure 5. Social behavior and dopamine pre- and post-separation

(A) Social interactions were scored after chamber entry during social operant tests for the last 3 days prior to separation and after 4 weeks of partner separation.

(B–E) Direct contact investigation: there was more direct contact displayed toward partners, compared with novel voles, and after separation there was increased direct contact displayed toward partners and novels (B and C). There was greater dopamine release during partner direct contact investigation, compared with novel investigation, only prior to separation (D and E).

(F–I) Huddling: there was no statistical difference in the duration of time spent huddling with the partner and the novel vole, and no difference in time spent huddling prior to and after separation (F). Greater partner huddling was evident in increased cumulative number of bouts before and after separation (G). There was greater dopamine release during partner huddling, compared with novel huddling, only prior to separation (H and I).

(J–M) Non-contact investigation: there was more non-contact investigation toward the novel vole while paired, and separation reduced non-contact investigation displayed toward partners and novels (J and K). There were no differences in dopamine release, comparing partner and novel at either time point (L and M). Not all animals engaged in all behaviors, especially huddling, as reflected in the reduced number of dots and lack of connecting lines in some instances where an animal huddled only with the partner or with the novel vole. Error bars show SEM. Representative traces for (D), (H), and (L) are from animals 4277 (direct contact), 4476 (pre-sep) and 4236 (post sep) (huddling), and 4291 (non-contact inv.). n = 11. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.0005.

See also Data S1.

are classic tests for measuring the behavioral activation associated with the appetitive aspects of reward acquisition.[37,38] Within this framework, we found that systemic dopamine signaling is not required for expression of an existing partner preference (replicating[12]), but D1-receptor signaling is necessary for seeking social access, regardless of the identity of the social stimulus. Thus, our findings are consistent with prior work indicating that DA antagonism has a greater effect on appetitive/seeking behavior (i.e., "wanting") and less effect on consummatory behavior (i.e., "liking").[14] This also supports a broadly conserved role for D1 systems in appetitive aspects of diverse reinforcing experiences, including the necessity of D1-expressing accumbal neurons in operant self-administration for aggression in mice.[39]

Inspired by prior *ex vivo* work showing a potentiation of evoked dopamine release in pair-bonded voles,[13] we asked whether differences in release dynamics differentiate partner and novel voles. Voles exhibited the same amount of lever pressing during partner and novel trials but exhibited enhanced dopamine release during partner seeking and in anticipation of access, compared with the same events during novel vole trials. This finding effectively uncouples the dopamine-behavior relationship by showing that the same motoric behavior can result in differences in dopamine release when associated with access to different animals.

Unlike lever pressing, we found that social interaction behaviors were dramatically different when voles engaged with their partner compared with a novel vole. Voles engaged in substantially more direct contact investigation and huddling with their pair-bonded partner than that with a novel vole, which was accompanied by enhanced dopamine release during partner-directed behavior. In contrast, voles exhibited greater non-contact investigation of novel voles. This behavior enables assessment of a tethered animal without risking an aggressive encounter. There were no differences in dopamine when this

behavior was directed toward a partner or a novel vole. Together, this suggests that dopamine release distinguishes pair-bonded partners from novel voles both when seeking out potential social interaction and during the interactions themselves.

How might enhanced dopamine release activate downstream circuits to drive behavioral selectivity? Recent work in mice has shown that pharmacologically increasing dopamine in the striatum recruits more D1-type direct spiny projection neurons.[40] Enhanced dopamine release may therefore recruit D1 neurons and bias voles toward partner seeking behaviors. During social interaction, enhanced dopamine release may signify the relative valence and reward value of different social interactions with different voles, serving as a mechanism to reinforce partner-directed affiliation.

One of our most intriguing findings was an erosion of partner-directed behavior and partner-enhanced dopamine release after long-term separation. Consistent with a partial erosion of the bond, test animals show substantially fewer huddling bouts toward their partner and show overall increased direct contact investigation of both the partner and the stranger. They also exhibit an overall decrease in non-contact assessment behavior and no longer bias this behavior toward the stranger vole. Despite these large-scale shifts in social behavior, our test animals still exhibit more direct contact investigation toward their partner than a stranger, indicating some level of partner recognition. Given this, the lack of partner-enhanced dopamine during direct contact investigation and huddling following long-term separation is consistent with a devaluation of the bond rather than simply forgetting. The observed blunting of partner-elicited dopamine release following long-term partner separation may thus enable the formation of a new bond by decreasing the selectively rewarding nature or valence of a previous partner, nullifying the exclusivity of the bond. Additional experiments are needed to control for the passage of time and delineate the time course of dopamine erosion.

Why do novel voles also elicit dopamine release? Potential explanations for novel-vole-elicited dopamine are likely complex. Novel-associated dopamine release may encompass reward-related signaling tied to the potential for extra-pair copulations, which can increase fitness. Another possibility is that dopamine is released in response to a novel threat and a need to defend territories from intruders. The latter is consistent with prior work suggesting that dopamine signaling may mediate aggression and novelty or threat detection in addition to social reward.[3,8,33,41] Ultimately, testing these models, which posit differential roles for partner- and novel-elicited dopamine release, will require functional manipulation.

While the present study provides novel insights into the real-time dopamine dynamics that contribute to bond selectivity and species-appropriate social behavior, examining aggregate changes in fluorescence as a proxy for dopamine release has some limitations. Specifically, we do not know whether there exists spatial or synapse-level specificity with respect to partner- and novel-associated dopamine release. It also remains unclear whether dopamine-mediated effects on behavior are the result of different patterns of co-release of dopamine with glutamate or GABA or combined action with oxytocin or endogenous opioids. Finally, while intriguing, additional work is needed to clarify how reductions in partner-elicited dopamine release, following long-term separation, may functionally contribute to loss adaptation in voles.

It also remains unknown whether differential socially mediated dopamine release is evident in other brain regions in monogamous species. In monogamous zebra finches, which use vocal communication to elicit motivated responses, infusion of dopamine agonists into the auditory cortex enhanced preferences for less-preferred songs.[42] This suggests that dopamine in sensory-processing regions may shape the incentive salience of different types of social information, and enhanced partner-elicited dopamine release in various brain regions may coordinate different facets of bond-related preference behaviors.

In sum, we have shown that D1-class receptors are necessary for the appetitive aspects of social interaction and that accumbal dopamine release reflects the selective nature of pair bonds, with greater release associated with highly rewarding pair bond relationships. This work has important implications for human relationships, suggesting that dopamine may confer selectivity by predicting and reinforcing the rewarding aspects and motivational valence of partner interaction, thereby cementing relationships over time. The erosion of partner-associated dopamine release as a function of separation is consistent with a model in which pair bonds are assigned less motivational salience and/or reward following prolonged partner absence, thus providing a potential mechanism for overcoming loss. Altogether, this work suggests that real-time dopamine release dynamics are sensitive to experience and differentiate between relationship types, acting to shape real-time species-appropriate social decision-making and behavior.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.12.041.

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AUTHOR CONTRIBUTIONS

Z.R.D. and A.F.P. developed experimental design. A.F.P. executed experiments. Z.R.D. and A.F.P. analyzed and interpreted data and wrote the manuscript. D.S.W.P., G.D.C., and R.T.C. created the hardware and software for the operant chambers. D.S.W.P. provided feedback on experimental design and edited the manuscript. Y.L.W. developed, carried out, and analyzed the barrier-climbing task.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Viruses		
AAV1-hSyn-DA4.3 M205T	Plasmid gifted from Yulong Li[30]; Packaged at Vigene Biosciences	N/A
AAV2-hsyn-mCherry	Addgene	CAT# 114472-AAV2; RRID:Addgene_114472
Chemicals		
SCH-23390 hydrochloride	Tocris Bioscience	CAT# 0925
Eticlopride hydrochloride	Tocris Bioscience	CAT# 1847
Deposited data		
Data for figures	This paper	Github: https://github.com/donaldsonlab/Pierce2023_Currentbiology
Experimental models: Organisms/strains		
Prairie voles (Microtus ochrogaster)	University of Colorado-Boulder Colony	N/A
Hardware and software		
Operant Chambers Hardware	Brusman et al.[25] and this paper	Github: https://github.com/donaldsonlab/Operant-Cage/tree/main/V2
Operant Chambers Software	Brusman et al.[25] and this paper	Github: https://github.com/dprotter/RPi_Operant
Partner Preference and Barrier Climbing Chambers	This paper	Github: https://github.com/donaldsonlab/PPT-Chamber
Software for scoring Partner Preference Test behavior	CleverSys	N/A; RRID:SCR_017141
Software for hand scoring behaviors	Behavioral Observation Research Interactive Software (BORIS)[43]	N/A; RRID:SCR_021509
Acquisition of events from operant chamber and fiber photometry data	Bonsai[44]	N/A; RRID:SCR_021512
Fiber photometry equipment		
Fiber photometry system	Neurophotometrics	FP3002
Ferrules	Doric Lenses Inc.	MFC_200/230-0.37_5mm_ZF2.5_FLT Mono Fiberoptic Cannula **Fiber Photometry**
Patch cables	Doric Lenses Inc.	MFP_200/220/LWMJ-0.37_3m_FC-ZF2.5_LAF Mono Fiberoptic Patchcord - Low Autofluorescence
Other		
Dustless Precision Pellets Rodent Grain-Based Diet	Avantor/VWR	Cat# 89067-546
Rotarod	IITC Life Science Inc	Series 8

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Zoe R. Donaldson (zoe.donaldson@colorado.edu).

Materials availability

Operant chamber materials and chamber designs can be accessed at https://github.com/donaldsonlab/Operant-Cage/tree/main/V2. The apparatus was controlled via custom scripts and code (https://github.com/dprotter/RPi_Operant[45]; https://github.com/dprotter/RPi_Operant2[46]). Partner Preference Test and Barrier Climbing chambers designs can be accessed at https://github.com/donaldsonlab/PPT-Chamber.

Data and code availability

Data used in each figure panel have been deposited in GitHub: https://github.com/donaldsonlab/Pierce2023_Currentbiology and are publicly available as of the date of publication. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Prairie voles were bred in house, initially imported from colonies housed at Cornell University, Emory University, and UC Davis, all of which originated from wild animals captured in Illinois. Animals were maintained at a temperature of 23-26°C on a 14:10 light:dark cycle. All procedures occurred during the light phase. Animals were given water and rabbit chow *ad libitum* (5326-3 by PMI Lab Diet). Rabbit chow was supplemented with sunflower seeds, dehydrated fruit bits, and alfalfa cubes. Home cages were enriched with cotton nestlets and plastic houses. At postnatal day 21, animals were weaned and placed into standard static rodent cages (17.5 l. x 9.0 w. x 6.0 h. in.) at a density of 2-4 same sex prairie voles. All females were sterilized via tubal ligation between the ages of postnatal day 72 and 96. At the onset of opposite-sex pairing, voles were placed into smaller static rodent cages (11.0 l. x 6.5 w. x 5.0 h. in.) where they remained until they were separated from their opposite-sex partner and placed in clean small rodent cages. All voles were between 94 and 118 days old at the onset of pairing. Procedures were approved by the University of Colorado Institutional Animal Care Use Committee. Samples sizes for all experiments are represented by n values in the text and figure legends.

METHOD DETAILS

Surgical procedures

AAV infusion and ferrule implantation

Experimental animals underwent viral infusion and ferrule implantation surgery between 72 and 96 days of age. Voles were anesthetized with 1–3% isoflurane at an oxygen flow rate of 1L/min in a head-fixed stereotactic frame (David Kopf, Tujunga, CA). Body temperature was maintained at 37°C using a closed loop heating pad with a rectal thermometer (David Kopf, Tujunga, CA). Eyes were lubricated with ophthalmic ointment (Sterile Lubricant Eye Ointment). The fur was removed from the incision site using a shaver, and the wound area was disinfected with 70% isopropyl alcohol and betadine. Briefly, the scalp and any connective tissue was removed above the frontal and parietal skull plates. Two 0.5 mm guide holes were drilled—each in the parietal plates—and anchoring screws were rotated into place. The head was leveled in the anterior-posterior plane, and a 0.5 mm hole was drilled at +1.6 mm AP and +1 mm ML. A hole was drilled and a Nanoject syringe (Drummond Scientific, Broomall, PA) was lowered and 200 nL of AAV vector was injected at a rate of 1nL/sec unilaterally at -5.0, -4.9, and -4.8mm DV for a total of 600 nL. The following vectors and titers were used: AAV1-hSyn-DA4.4 M205T ($GRAB_{DA}$, plasmid gift from Dr. Yulong Li, packaged by Vigene) at $1.015X10^{13}$ GC/ml and AAV2-hsyn-mCherry (Addgene) at $3X10^{12}$ GC/ml. The latter AAV provided a red fluorescent signal used to normalize for motion artifacts.[47] The needle was left in place for 10 minutes following the last infusion. Then, a fiberoptic ferrule (0.2 mm diameter, 5 mm long; Doric Lenses Inc) was slowly lowered into position until reaching a final placement of -4.8 mm DV. The ferrule and screws were affixed to the skull with Loctite 454 cured with acrylic resin (Jet Liquid). The initial layer was covered with Loctite mixed with black carbon powder (Sigma). Extended Release Meloxicam (4 mg/kg), enrofloxacin (5 mg/kg), and saline (up to 3mL) were administered subcutaneously perioperatively for analgesia, to avoid bacterial infection at the wound site, and to prevent dehydration, respectively. Additionally, enrofloxacin (5 mg/kg) and saline (1mL) were administered subcutaneously for the three days following surgery. Animals were allowed to recover for at least 14 days prior to initiation of experiments. Ferrule placement and viral expression were confirmed posthumously (Figures 3B and 3C).

Tubal ligations

Females were tubally ligated to avoid confounds of pregnancy while keeping the ovaries hormonally intact. Tubal ligation was carried out during an independent surgery or under anesthesia during vector infusion and ferrule placement (described above). Briefly, hair was shaved at the incision site and the underlying skin was disinfected with betadine and 70% isopropyl alcohol A single incision was made in the midline of the back to provide access to the body cavity. The incision was pulled to one side until aligned above the ovary. A small incision was made into the body wall, the ovary was pulled through and bisected from the uterus via a cauterizer. The uterus and ovary were returned to the body cavity, the internal body wall was closed using an absorbable suture, the skin was pulled to the other side, and the procedure was repeated. The external incision was closed with staples that were removed 10-14 days later. Triple antibiotic ointment and lidocaine were placed on the closed wound.

Behavioral methods

Voles were tubally ligated, allowed to recover, and paired at least 14 days prior to the onset of operant training, barrier climbing, or partner preference testing, sufficient time for stable pair bonding to occur.[25,34]

Rotarod-based assessment of locomotor coordination

We used the series 8 Rotarod apparatus from IITC Life Science Inc to evaluate whether dopamine D1- or D2-class receptor antagonist administration affected motor ability and coordination. Animals were allowed to habituate to the testing room for two hours prior to daily rotarod training/testing. Voles were trained twice a day for 3 days by placing them on top of a 3.75-inch diameter drum. On the

first training day, a completed trial consisted of staying on the drums at a constant speed of 4 rpm for 60 seconds.[48] On all subsequent training and testing days the rotarod was set to increase continuously in speed from 4 to 6rpm over 60 seconds. Training was deemed successful if voles completed 2 trials per training day with a 20-minute break between trials. For testing, we measured the time spent on the drum before falling off or completing the trial. If animals completed the trial without falling off, they received a duration of 60 seconds for that trial; otherwise, they received the value of the duration of time they stayed on the rod before falling off. All trials on a given day were averaged for the reported duration of time spent on the rod. Voles received intraperitoneal injections of volume 0.1ml/10g body weight for vehicle and antagonist administration and were tested 3 times, 10-, 30-, and 50-minutes post-injection (see Supplementary Statistics Table for analysis by timepoint). Voles were tested in the following order with at least one day between tests: vehicle, 1mg/kg SCH23390, 0.5 mg/kg SCH23390, 1.25 mg/kg eticlopride, 2 mg/kg Eticlopride.

Operant training and timeline

Custom operant chambers were as previously described in Brusman et al.[25] Briefly, operant chambers contained 3 chambers separated by 2 motorized doors, one motorized pellet dispenser and trough, and 3 separate retractable levers (one for each type of reward). For experiments using partner-only presentation (Figure S1), only one social chamber was used, and a slotted divider was affixed next to the motorized door to allow social interaction while blocking social chamber entry. Chambers were constructed from a mix of laser cut acrylic and 3D printed ABS plastic. A bill of materials and chamber designs can be accessed at https://github.com/donaldsonlab/Operant-Cage/tree/main/V2.

The apparatus was controlled via custom scripts and code (https://github.com/dprotter/RPi_Operant) run on Raspberry Pi computers (Raspberry Pi Foundation). Servos were controlled via an Adafruit HAT (Adafruit 2327). Each apparatus was controlled by a corresponding Raspberry Pi. Food rewards were 20 mg pellets (Dustless Precision Pellets Rodent Grain-Based Diet; VWR 89067-546) delivered to a trough. Pellet dispensing and retrieval was detected by an IR beam break in the trough. Tones were generated via PWM on the Raspberry Pi (pigpio), and played through an amplified speaker (Adafruit 3885).

Voles were paired more than 14 days prior to the onset of operant training, sufficient time for stable pair bonding to occur.[25,34] We used three different training paradigms, each described below, adapted to the goals of each experiment. Voles were not food restricted. During partner separation, animals were socially isolated.

Food magazine training

Food magazine training was only included for $GRAB_{DA}$ experiments. Animals underwent 6 days of magazine training with 15 trials per day (Figure S2A), the goal of which was to learn associations between the lever, tone, and food reward. For each trial, a tone was played to indicate the start of the trial (5,000 Hz, 1s). The food lever was then extended for 2 seconds, a pellet cue (2,500 Hz, 1s) was played, and a single pellet was delivered to the trough. The lever was retracted 2 seconds later. If an animal pressed within the first 2 seconds of lever access, a pellet was immediately delivered. No more than 1 pellet was delivered per trial. Total trial time was 90s.

Operant food delivery

Operant food delivery was only performed for $GRAB_{DA}$ experiments. Animals underwent 20 trials per day for 8 days (Figure S2A). During the first two days (training), pellet delivery was not contingent on lever pressing. During each trial, a tone was played to indicate the start of the trial (5,000 Hz, 1s). The food lever was then extended for 30 seconds. After 30 seconds, the lever was retracted if the vole did not press the lever, a pellet cue (2,500 Hz, 1s) was played, and a pellet was delivered to the trough. If the vole pressed the lever within 30 seconds, a pellet cue (2,500 Hz, 1s) was played, and a pellet was immediately delivered to the trough. A single pellet was dispensed on every trial after 30s of lever presentation, but lever pressing elicited an immediate pellet dispense. During days 3-8 of training, pellet delivery was contingent on lever pressing. The lever was extended for a maximum duration of 120s. During each trial, a tone was played to indicate the start of the trial (5,000 Hz, 1s). After 120 seconds, the lever was retracted if the vole did not press the lever and no pellet was dispensed. If the vole pressed the lever within 120 seconds, a pellet cue (2,500 Hz, 1s) was played, and a pellet was delivered to a trough. To provide a window to observe anticipatory behavior and dopamine release, animals experienced a delay between lever pressing and food reward as follows: days 1-5: no delay, days 6-8: 5 second delay. The intertrial interval for all trials was 45 sec.

Operant social access

For single-chamber social operant training (Figure S1), animals had 20 trials per day where they were given the option to press a single lever to gain access to a partner through a slotted barrier. The trial structure was as follows: a tone was played to indicate the start of the trial (5,000 Hz, 1s) followed by the extension of a single lever. If the lever was pressed within 300 seconds, the lever was retracted, and a tone was played to indicate a successful lever press. Then a door opened for 30 seconds, allowing limited access to the partner through a slotted barrier. After 30 seconds a door closing tone was played (7,000 Hz, 1s) and the door was closed. If the lever was not pressed after 300 seconds, the lever was retracted. All trials had an intertrial interval of 45 seconds.

For dual chamber social operant experiments, pair bonded voles underwent 20 trials of social training per day, in which they were given access to 2 levers, one lever gave access to the partner and another lever gave access to a novel opposite sex vole. Experimental voles were given alternating sets of 5 trials for each lever, starting with the partner lever (i.e. 5x partner, 5x novel, 5x partner, 5x novel) (Figure 2A). The partner and novel stimulus animals were tethered at opposite ends of the apparatus and farthest from the doors in a similar fashion to the partner preference test (below). The tethering location of partner and novel voles remained consistent across days. To avoid a potential unintended bias in lever pressing, we assign the lever farthest from the door to provide access to the partner or novel, respectively (Figure 2A). A new novel vole was used each day of operant social access and during the probe trial after separation. On all training days, a tone was played to indicate the start of the trial (5,000 Hz, 1s). After 120 seconds, the lever was

retracted. If the lever was pressed within 120 seconds, social access was granted, and at the end of the trial, a door close tone was played (7,000 Hz, 1s) and the door was closed. If needed, subjects were manually returned to the center chamber immediately after the chamber closed. The duration of social interaction received was dependent on how quickly the vole pressed the lever. Each trial was a maximum of 120 seconds, and the amount of social interaction was 120 seconds minus the latency to press the lever. All trials had an intertrial interval of 45 seconds. In Figure 2, the door was opened with no delay following the lever press on days 1 - 8 and a 5 second delay on days 8 - 10. In Figures 3 and 4, the door was opened without any delay after lever press on days 1-3 and following a 5 second delay on days 4-6 and during the probe test after partner separation. For experiments with $GRAB_{DA}$ recording, animals received food magazine and operant food training prior to social operant training.

Barrier climbing task

The standard partner preference chamber was adapted so that a one-sided metal wire mesh barrier (5 mm holes, other side clear acrylic, Figure 2L) was placed between the middle and a side chamber with the mesh facing towards the middle chamber. The third chamber was blocked off by a solid acrylic barrier. Female subjects were placed in the middle chamber with access to the mesh side of the barrier, while their male partner was placed in the other chamber under a pencil cup. Before the mesh barriers were added, the subject was given a 2-minute habituation period where they could freely explore both chambers and their partner under the cup.

Climbing attempts were defined as instances when all the subject's feet left the ground while climbing the mesh barrier. Attempts were counted as successful when the animal landed on the other side of the barrier. Success rate was calculated as successes/attempts. After each successful attempt, the subject was given a 30 second period where they were able to interact with their partner under the cup before being returned to the middle chamber. Each session lasted 17 minutes, including the habituation period. All female subjects were initially given an 8.5 cm barrier (less than one body length). To avoid a potential ceiling effect, the barrier height was varied; upon reaching 90% success rate, we introduced a 17 cm barrier, and if the vole reached > 90% success rate again, we introduced a 34 cm barrier. Barrier height remained static while determining baseline crossing rate.

Partner preference test

Partner preference tests were performed as described in Scribner et al.[49] Briefly, both partner and novel animals were tethered to the end walls of three-chamber plexiglass arenas (76.0 cm long, 20.0 cm wide, and 30.0 cm tall). Tethers consisted of an eye bolt attached to a chain of fishing swivels that slid into the arena wall. Animals were briefly anesthetized with isoflurane and attached to the tether using a zip tie around the animal's neck. Two pellets of rabbit chow were given to each tethered animal and water bottles were secured to the wall within their access while tethered. After tethering the partner and novel animals, experimental animals were placed in the center chamber of the arena. At the start of the test, the opaque dividers between the chambers were removed, allowing the subject to move freely about the arena for three hours. Overhead cameras (Panasonic WVCP304) were used to video record eight tests simultaneously.

The movement of all three animals in each test was scored using TopScan High-Throughput software v3.0 (Cleversys Inc) using the parameters from Ahern et al.[50] Behavior was analyzed using a Python script developed in-house (https://github.com/donaldsonlab/Cleversys_scripts) to calculate the time spent huddling with the partner or novel. The partner preference score was calculated as (partner huddle time/[partner huddle time + novel huddle time]) × 100%. We report the analysis of preference score in the text using a one-way t-test relative to a null hypothesis of 50% (no preference). We also performed a paired t-test and/or RM-ANOVA comparing partner versus novel huddle times in the figure legends and Supplementary Statistics Table. The latter is less rigorous as it uses data that violates assumptions of independence as the test animal cannot interact with both the partner and novel simultaneously.

Pharmacological receptor blockade

To test the role of dopamine on behavior, we administered D1- or D2-class receptor antagonists during social operant, barrier climbing, and partner preference tests. To test the role of D1- and D2-class receptor inhibition on behavior, we administered intraperitoneally, 0.5mg/kg SCH-23390 hydrochloride, 2mg/kg Eticlopride hydrochloride (Tocris Bioscience), or saline, 5 minutes prior to test onset. To test for potential order effects, we counterbalanced dopamine class antagonists and vehicle administration across operant testing days shown in Figures S1G and S1H (vehicle data is always presented first in figures). There was no effect of testing day or drug order on the number of lever presses (reported in supplementary Statistics Table). Finally, we assessed effects only during the first hour of the partner preference test, comparable to the amount of time animals performed the operant task post-administration.

Fiber photometry

$GRAB_{DA}$-mediated measurement of nucleus accumbens dopamine during social operant

Subjects were habituated to the patch cable for 6 days in an open field chamber prior to the onset of operant training. Subjects were briefly anesthetized (<30s) to attach patch cables prior to recording and allowed 10 minutes to recover prior to operant testing.

Fluorescence was acquired using the Neurophotometrics (NPM) V2 system with 200uM core optical fibers purchased from Doric Lenses. Data was acquired using Bonsai.[44] During photometry recordings, light was delivered alternating between 470 nm, 560 nm, and 415 nm at a framerate of 180 frames per second. The LED power for each wavelength was set to 50uW at the optical fiber tip to reduce photobleaching. Signals were analyzed using a MATLAB script. To correct photobleaching and motion artifacts, the 560 nm signal was fit to the 470 nm, then this fit was subtracted from the 470 nm signal. Z-Scores were calculated as (fitted signal – baseline)/(baseline standard deviation) where the baseline for all events and behaviors was -8 to -3 seconds prior to lever extension (during the intertrial interval). The area under the curve was calculated as the average Z-Score values 2 sec after the event.

We time-locked operant events (extension of levers, lever pressing, chamber opening, chamber entry, pellet dispense, pellet retrieval) to the fluorescence signal using two microcontrollers. Bonsai cannot run on the Raspberry Pi 3 B+ operating system

that is used to control the operant chamber hardware, so we directed a Raspberry Pi to send a serial signal to an Arduino Uno microcontroller, which has communication functionality with Bonsai.

All behaviors that occurred after crossing into the chamber were hand scored using BORIS, a behavior event scoring software.[43] Behaviors performed by the subject directed towards the partner or novel on days 4-6 of social operant access were hand scored. We examined the following behaviors: non-contact investigation, head sniffing, body sniffing, anogenital sniffing, huddling, allogrooming, defensive posture, and attacking. Head, body, and anogenital sniffing were aggregated into a direct-contact investigation metric. Huddling and allogrooming were combined as highly prosocial behaviors. Non-contact investigation consisted of the test animal attending to the stimulus animal without physical touching, a type of assessment behavior with reduced risk of agonistic interaction. Animals displayed little to no defensive posture/attack, so these behaviors were omitted from subsequent analyses.

Brain collection

Upon completion of experimental sessions, voles were transcardially perfused with 4% paraformaldehyde in phosphate buffered saline. The head was removed with the ferrule intact and post-fixed for 24 hours in 4% paraformaldehyde before extracting ferrule and brain. The brain was equilibrated in 30% sucrose, sectioned in 50 μm slices using a sliding freezing microtome (Leica), and mounted on slides. Ferrule placement was drawn onto corresponding mouse atlas sections.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data are shown as means ± standard error of the mean (SEM). Statistical significance α was set as 0.05. All n values represent the number of animals. All statistical analyses were carried out using Graphpad PRISM 9.3.1 and SPSS 29.0.0.0. As appropriate, one- or two-way repeated measure ANOVAs were employed to examine the effects of day, stimulus animal (partner or novel vole), and/or antagonist administration on operant behaviors with specific tests indicated in the results section. Mixed-model ANOVAs were used when individual data points were missing (for instance due to failure to enter a social chamber). Z-scored fluorescence area under the curve comparisons for day 1 and day 6 (Figure 1) or partner and novel (Figures 2, 3, and 4) were analyzed using a paired or unpaired t-test (latter only Figure 5I). Differences in Z-scored fluorescence area under the curve elicited by food and social operant events were analyzed using a one-way repeated measures ANOVA with a Tukey's post-hoc test (Figure S2). Partner preference was analyzed using a one sample t-test relative to an expected null 50 percent preference (no preference) (Figures 1B, 1F, 2B, 2M, and 4B). Differences in durations of behaviors demonstrated towards partners and novels were analyzed using a 2-way ANOVA. Finally, cumulative bouts of behaviors demonstrated towards partners and novels were analyzed using a Log-rank (Mantel-Cox) test. See Data S1 for a full list of statistics information. Five animals were excluded from fiber photometry experiments due to placement outside of the NAc or misalignment of the ferrule with viral expression.