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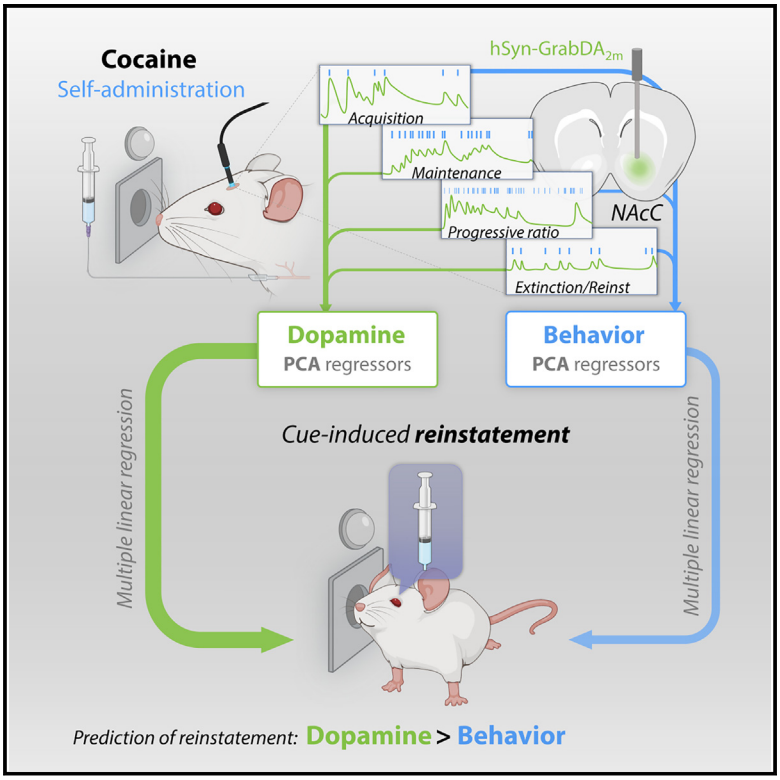
A multivariate regressor of patterned dopamine release predicts relapse to cocaine

Graphical abstract



Highlights

- Patterned NAc dopamine release predicts cue-induced cocaine reinstatement
- Considering the animal's sex improves dopamine-based predictions of cocaine-seeking behavior
- Male mice display greater NAc dopamine release in response to cocaine-predictive cues

Authors

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In brief

Luján et al. use a fluorescent sensor to record cocaine-evoked dopamine release events in the nucleus accumbens of mice during self-administration. They identify features of dopamine signaling that sufficiently predict cue-induced reinstatement of cocaine seeking, a model of drug relapse, and report sex-specific differences related to extinction resistance.

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A multivariate regressor of patterned dopamine release predicts relapse to cocaine

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SUMMARY

Understanding mesolimbic dopamine adaptations underlying vulnerability to drug relapse is essential to inform prognostic tools for effective treatment strategies. However, technical limitations have hindered the direct measurement of sub-second dopamine release *in vivo* for prolonged periods of time, making it difficult to gauge the weight that these dopamine abnormalities have in determining future relapse incidence. Here, we use the fluorescent sensor GrabDA to record, with millisecond resolution, every single cocaine-evoked dopamine transient in the nucleus accumbens (NAc) of freely moving mice during self-administration. We reveal low-dimensional features of patterned dopamine release that are strong predictors of cue-induced reinstatement of cocaine seeking. Additionally, we report sex-specific differences in cocaine-related dopamine responses related to a greater resistance to extinction in males compared with females. These findings provide important insights into the sufficiency of NAc dopamine signaling dynamics—in interaction with sex—for recapitulating persistent cocaine seeking and future relapse vulnerability.

INTRODUCTION

Cocaine is the most consumed illicit psychostimulant in the world, with a resurgence of cocaine use disorders within the last decade.[1] The increased risk of relapse associated with prolonged cocaine use is a major contributor to these sobering statistics. Given the limited efficacy of existing treatments, it is of capital importance to identify prospective biomarkers that can serve as accurate predictors of relapse to improve the diagnosis, prognosis, and treatment of cocaine use disorders.[2]

Increased vulnerability to relapse results from maladaptive potentiation of the mesolimbic dopamine system, which remains compromised even after long abstinence periods.[3,4] The ventral tegmental area (VTA) is the origination of what has been described as the "final common pathway" of reinstatement.[5] From there, dopaminergic projections to the nucleus accumbens (NAc), the medial prefrontal cortex, and the basolateral amygdala facilitate the initiation of drug-directed behaviors, ultimately leading to relapse.[4–9] Despite recent experiments reemphasizing the role of VTA dopamine neuron activation to promote relapse to reward-associated cues,[10] technical limitations have precluded the formulation of an unambiguous "dopaminergic hypothesis" of relapse[11,12] that does not introduce artificial manipulations to alter the mesolimbic dopamine system (optogenetics, chemogenetics, electrical stimulation, pharmacological agents, etc.). The recent emergence of dopamine-specific genetically encoded fluorescent sensors bridges this gap in knowledge by testing whether dopaminergic variables carry sufficient information to reproduce and predict cocaine relapse without artificially manipulating the anatomical substrate.

Similarly, temporal limitations have also hindered the ability to probe dopamine dynamics throughout the entire animal's history of cocaine taking—from initial consumption to relapse. It remains unclear at which stage sensitized dopaminergic signaling faithfully determines future enhanced risk to relapse.[13] In addition, potential sex differences in these longitudinal cocaine-evoked dopamine dynamics have yet to be elucidated.[14] This is of notable importance since human and animal studies reveal sex differences at every phase of drug addiction[15–18] (but also see Nicolas et al.[19]). Structural and functional differences in the dopamine systems of males and females may underlie these effects.[20–22] Surprisingly, no study has systematically explored sex-specific patterns of sub-second dopamine release in cocaine self-administering rodents.

Here, we utilize GrabDA-based fiber photometry to uncover sub-second cocaine-evoked dopamine responses in the NAc throughout the animal's entire history of contact with the drug. To refine the "dopaminergic hypothesis" of cocaine relapse,

we test whether cued reinstatement of cocaine seeking is reproduced from multivariate patterns of accumbal dopamine activity. Another correlate of enhanced risk of relapse after cocaine consumption—resistance to extinction—is also tested with our mathematical model. Furthermore, we uncover sex-specific trajectories of cocaine-evoked dopamine responses underlying male vulnerability to persistent cocaine seeking.

RESULTS

Longitudinal dopaminergic responses to voluntary, intravenous cocaine infusions in mice

An optical fiber was implanted into the NAc core to probe for changes in fluorescence induced by GrabDA$_{2m}$ (Figure 1A; STAR Methods). Then, animals (n = 12) were catheterized in the right jugular vein to allow for voluntary intake of intravenous cocaine (0.5 mg/kg/infusion [inf]). During 6–8 weeks of daily recordings, accumbal dopamine responses accompanying cocaine intake and drug-predictive cues were sampled throughout the acquisition, maintenance, extinction, and reinstatement of cocaine-seeking behavior. During acquisition, mice had to nose poke a porthole placed in one side of the chamber in order to receive a 2-s-long cocaine infusion paired with a compound cue (light + tone) (Figures 1B and 1C). Peri-event time histograms (PETHs), comprising trials from each experimental phase (averaged across sessions), served to characterize each subject's dopaminergic responses following cue onset and cocaine delivery. Significant GrabDA$_{2m}$ transients were determined using a bootstrapping confidence interval (CI) procedure (95% CI, 1,000 boot-straps)[23] (STAR Methods). A different set of animals (n = 12) followed the same training protocol but were reinforced with a sucrose pellet. Their dopaminergic responses served as the ground truth to illustrate the identifying features of cocaine-evoked dopamine transients in the NAc. During the first fixed ratio 1 (FR1) sessions, cocaine infusions elicited increases in extracellular dopamine release that were remarkably different from those following a sucrose pellet (Figure 1D). Cocaine infusions elicited steady, prolonged increases lasting a minimum of 30 s, reflecting the accumulation of extracellular dopamine due to the pharmacological action of cocaine at NAc dopamine transporters (DATs).[24–26] During this stage, food-paired cues elicited greater NAc dopamine release events compared with cocaine-paired cues, a difference that disappeared in the next stages. Figure S1 depicts the full extent of the GrabDA$_{2m}$ transients throughout an entire cocaine self-administration session, along with the modeled quantities of cocaine brain concentrations[27] (STAR Methods). Daily changes in dopamine release over the course of each cocaine intake phase were also examined. Our analyses indicate that cue-evoked NAc dopamine transient amplitudes did not significantly evolve on a session-by-session basis, although reward-evoked amplitudes decreased during FR3. Mice that acquired self-administration advanced to a progressive ratio (PR) test, an exponentially increasing schedule of reinforcement[28,29] (Figures 1F–1I). Cocaine, compared with sucrose pellets, yielded a prolonged elevation in reward-evoked dopamine transients (Figure 1J). 24 h after PR, animals underwent subsequent extinction sessions (Figure 1K) until seeking on the cocaine-paired porthole ceased. GrabDA$_{2m}$-averaged traces from the first (early extinction) and last extinction sessions (late extinction) were obtained and are plotted in Figures 1L and 1M, respectively. Bootstrap 95% CI procedure analyses of early and late extinction trials revealed a decrease in dopaminergic encoding of cocaine seeking in the active porthole. After the last extinction session, mice underwent a cue-induced reinstatement test (STAR Methods). Waveform and summary analyses confirmed the reappearance of the cue-evoked dopamine signal on the reinstatement test compared with the last extinction session (Figures 1N and 1O). Cocaine- and food-seeking mice exhibited similar cue-evoked dopamine events during reinstatement (Figure 1N, bootstrapped 95% CI). GrabDA$_{2m}$ transients of inactive nose pokes are shown in Figure S1.

Predicting reinstatement of cocaine seeking from multivariate patterns of dopamine responses

Prior to the emergence of genetically encoded, fluorescent dopamine reporters,[30,31] *in vivo* dopamine measures were constrained either by long-term stability (voltammetry) or by temporal and spatial resolution (microdialysis). These technical limitations may have hindered our ability to understand the relevance of sub-second dopamine signatures of cocaine seeking in the context of long-term drug addiction. Our first approach to uncover associations between cocaine-evoked dopamine release and reinstatement of cocaine-seeking behavior consisted of Pearson's correlation matrices. Behavioral measurements (number of nose pokes or infusions during each phase and number of days to extinction) were also correlated with the number of nose pokes on the reinstatement test. Figure 2 shows the Pearson's matrices resulting after correlating every dopaminergic (Figure 2A) or behavioral measurement (Figure 2B) obtained during the entire history of cocaine self-administration. Correlations with the number of active nose pokes during reinstatement are highlighted in both panels. As expected, there was a higher degree of correlation among behavioral measurements. Interestingly, dopamine amplitudes (averaged GrabDA$_{2m}$ $\Delta F/F_0$ Z scores) aligned to cue onset during FR1 ($r = 0.61$, $p = 0.047$) and PR ($r = 0.81$, $p = 0.003$) (depicted in Figure S1) were positively correlated with the incidence of reinstatement. This result highlights the importance that early dopamine responses may have in predicting renewed cocaine use.

Following this line of evidence, we next implemented a multiple linear regression (MLR) model to predict cue-induced reinstatement of cocaine-seeking behavior solely by using the dopamine responses observed. Given the number of independent variables available, we conducted a principal-component regression (PCR) to avoid overfitting our linear model with too many free parameters. PCR is a combination of MLR and principal-component analysis (PCA) in which the principal-component scores resulting from PCA are used as predictors on the MLR model. The resulting components obtained for the dopaminergic variables are shown in Figures 2C–2E. The three first principal components (Figure 2F) were selected for further analysis. The first principal component (DA_{PC1}) explained 34% of the total variance and was exclusively composed of cue-evoked dopamine release variables, therefore reflecting a stable signature of accumbal dopamine responses to drug-paired cue presentation. Principal components 2 and 3 (DA_{PC2}, DA_{PC3}) yielded a more multifaceted combination of

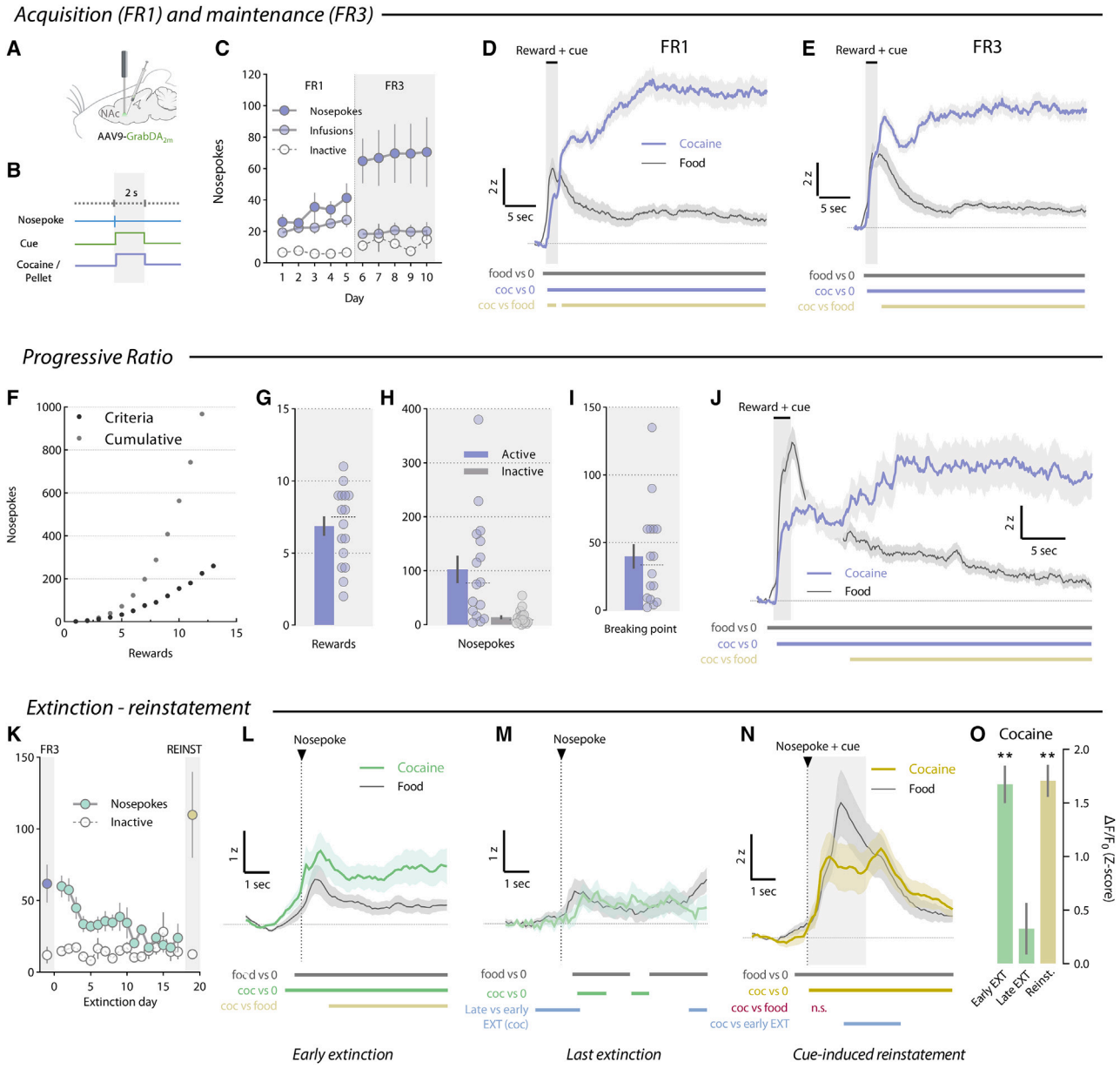

Figure 1. Sub-second dopamine responses in the NAc during cocaine-seeking behavior in mice

(A) GrabDA$_{2m}$ was transduced in the NAc core, where an optical fiber was implanted.

(B) Schematic representation of the operant task.

(C) Active and inactive nose pokes and infusions from the acquisition and maintenance phases of cocaine self-administration.

(D and E) GrabDA$_{2m}$ transients centered −2 s to +30 s around cue onset and obtained during FR1 and FR3.

(F) Schematic representation of the response requirement during PR.

(G–I) Number of rewards obtained, active/inactive nose pokes, and breakpoints exhibited on the PR test.

(J) GrabDA$_{2m}$ transients obtained during PR.

(K) Active/inactive nose pokes during extinction and cue-induced reinstatement. FR3-averaged responding is shown for reference. One-way repeated measures ANOVA reported a significant decrease of cocaine-seeking behavior across days ($F_{16,149} = 4.05$; $p < 0.001$).

(L and M) GrabDA$_{2m}$ transients from the first and last extinction sessions, centered −2 s to +5 s around every active nose poke.

(N) GrabDA$_{2m}$ transients from the cue-induced reinstatement session, centered −2 s to +5 s around cue onset.

(O) Summary of GrabDA$_{2m}$ $\Delta F/F_0$ *Z* scores during extinction and reinstatement (one-way ANOVA; $^{session}F_{2,1901} = 5.35$, $p < 0.01$; **$p < 0.01$ vs. late extinction). Colored bars below traces represent periods significantly different from 0, between reinforcers, or between sessions, as defined by bootstrapped 95% CIs. Data are presented as mean ± SEM.

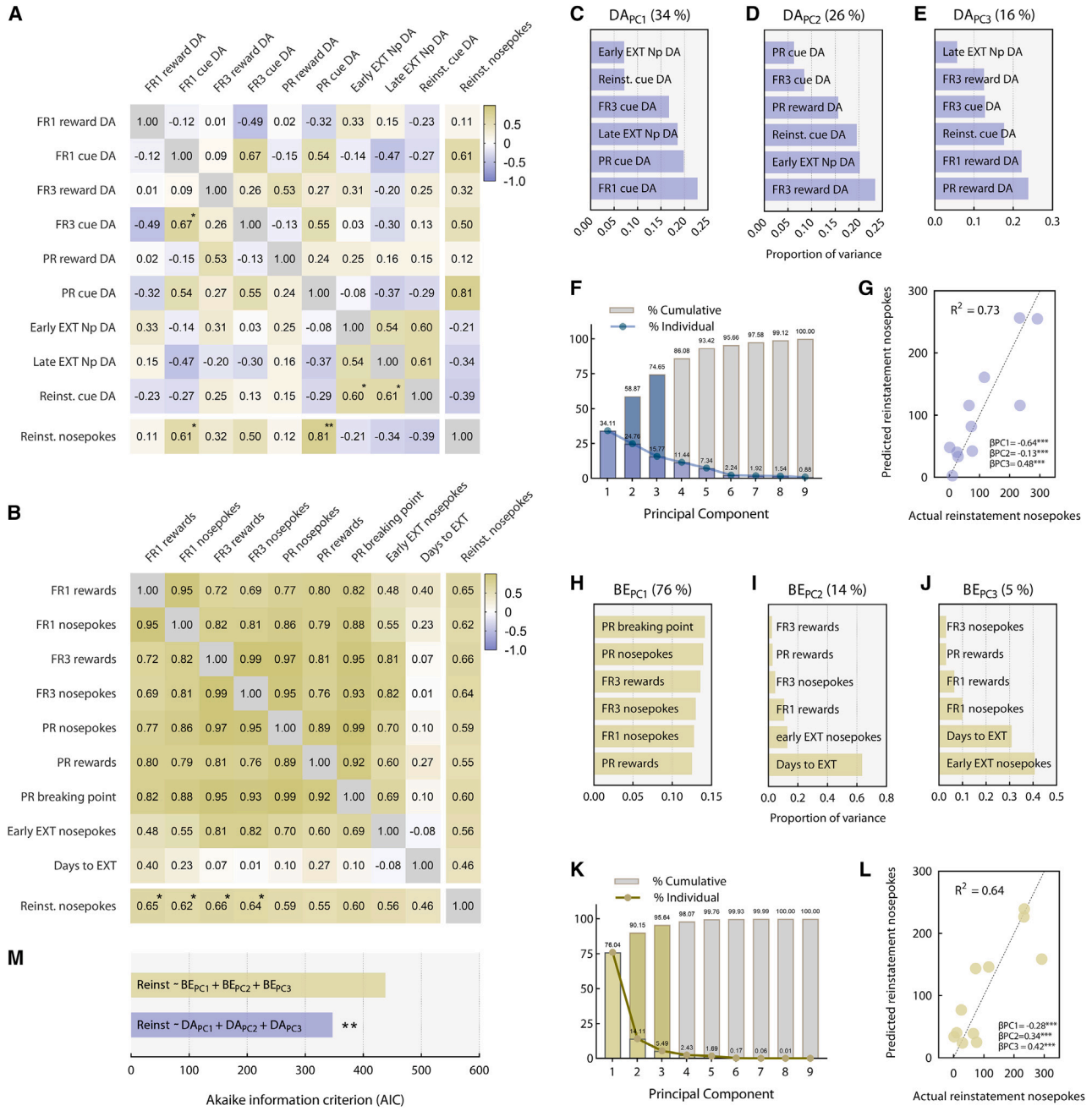

Figure 2. Low-dimensional dopamine signatures robustly predict reinstatement of cocaine-seeking behavior

(A and B) Pearson's correlation matrix obtained from GrabDA2m $\Delta F/F_0$ *Z* scores and behavioral measurements made during cocaine self-administration. Pearson's *r* values from each correlation are color coded and shown within each cell. Nose-poke correlations observed during the relapse test are shown separately. Statistically significant correlations (*$p < 0.05$, **$p < 0.01$) are marked with asterisks. For the sake of clarity, only significant behavioral correlations (*$p < 0.05$) during reinstatement are shown.

(C–E) Proportion of variance explained by operationalized dopaminergic variables of each principal component (DA$_{PC}$) obtained from principal-component analysis (PCA).

(F) Percentage of total variance (%) explained by each of the DA$_{PC}$ factors derived from PCA. Principal components selected for multiple linear regression (MLR) are colored in dark blue.

(G) Actual vs. predicted reinstatement nose pokes obtained from MLR with DA$_{PC1}$, DA$_{PC2}$, and DA$_{PC3}$ as covariates.

(H–J) Proportion of variance explained by behavioral variables of each principal component (BE$_{PC}$) obtained from PCA.

(legend continued on next page)

dopamine responses. The inclusion of cocaine-evoked dopamine variables (post-drug delivery, 2–30 s) among the top contributing parameters suggests a stable signature of reward-evoked dopamine responses. PCA factor loadings and subjects' scores are shown in Figure S2. Next, DA_{PC1}, DA_{PC2}, and DA_{PC3} were used as estimators in an MLR model (hereafter referred to as DA_{PC}) to predict the number of nose pokes observed during reinstatement (STAR Methods). Our results indicate that multivariate patterns of cocaine-related dopamine responses (DA_{PC1}, DA_{PC2}, and DA_{PC3}) can robustly predict reinstatement (R^2 = 0.73) (Figure 2G). The model also revealed that all DA_{PCs} contributed to the observed variance during reinstatement, suggesting that cue- and drug-evoked dopamine transients determine cue-induced reinstatement of cocaine-seeking behavior. An alternative model of reinstatement was constructed by PCR, this time including variables reflecting the progression of cocaine-evoked NAc dopamine transients throughout the course of self-administration. However, the resulting prediction of reinstatement nose pokes (R^2 = 0.73) did not differ from that of the DA_{PC} model described above, hence suggesting that the evolution of NAc dopamine transient amplitudes throughout sessions was not necessary to explain cued relapse (Figure S3). Considering the relatively small sample used, we sought to replicate these findings with an alternative approach. Using Bayesian Poisson regression (STAR Methods), we confirmed the model's prediction by showing that the posterior estimates of $DA_{PC1,2,3}$ β coefficients matched those of the MLR model (Figure S4).

To examine how informative these low-dimensional dopamine signatures were, we then compared the DA_{PC} model against another PCR model obtained from the behavioral measurements, as similarly seen in Flagel et al.[32] and Slosky et al.[33] Given the high degree of covariance between the behavioral variables, such a behavioral PCR should yield a reasonably good predictive model to compare. It is also safe to assume that nose-poking behavioral variables would be the best predictors of another nose-poking behavioral variable. To keep the number of free parameters consistent among models, we selected the three first components ($BE_{PC1,2,3}$) resulting from the initial PCA (Figures 2H and 2I). PCR yielded a considerably good prediction of reinstatement (R^2 = 0.64), to which all principal components contributed. Using the Akaike information criteria (AIC) (STAR Methods), we found that the DA_{PC} model fit reinstatement data better than the behavioral model (BE_{PC}) (ΔAIC = −90.24; p = 0.006) (Figure 2M). We performed additional PCR analyses to illustrate that the superiority of the DA_{PC} model did not depend on the method used to select principal components (Figure S5). Moreover, we iteratively tested how well the DA_{PC} model predicted other behavioral outcomes (such as FR1/3 responding, PR breakpoints, etc.). A comparison between the percentage of variance explained (R^2) of each dependent variable indicated that the best prediction of the DA_{PC} model was the reinstatement behavior. These results align with the "dopaminergic hypothesis" of cocaine relapse and prior ideas proposing dopaminergic signaling in the ventral striatum as a major hallmark of drug-induced adaptations and a robust predictor of future relapse behavior.[34–36] Moreover, we expand upon previous evidence by showing that low-dimensional features of the dopamine response space, pooling information from the first contact with the drug up until the reinstatement test, are sufficient to explain cocaine-seeking behavior in a relapse test.

Finally, we generated an equivalent DA_{PC} model using the dopaminergic and behavioral variables gathered from the mice that underwent food training. Their patterned dopamine responses ($DA_{PC1,2,3}$) were equally effective in predicting cue-induced reinstatement of food-seeking behavior (R^2 = 0.73; Figure S6). Delving further into the dopamine response commonalities between rewards, we report similar $GrabDA_{2m}$ transient rise times (STAR Methods) in response to food- and cocaine-paired cues. This similarity between reinforcers could explain why dopamine-based predictions of behavioral reinstatement are generalizable to drug and natural rewards.

Sex refines the dopamine-based prediction of reinstatement

To date, whether cocaine-evoked dopamine responses evolve differently through the stages of the addiction cycle in both sexes remains an open question of paramount relevance.[37] To explore this possibility, we examined whether sex played a role in dopamine-based predictions. If cocaine-evoked dopamine responses are influenced by sex, then considering this biological variable should refine the prediction of our DA_{PC} model. This was achieved by adding sex as an additional predictor, in combination with DA_{PC1}, DA_{PC2}, and DA_{PC3}, to the MLR model of reinstatement. Principal-component scores were similar between males and females (Figure 3A), meaning that cocaine-evoked dopamine responses did not diverge by sex when considering the animal's history of cocaine self-administration as a whole. Despite this, our analyses indicated that sex contributed (sex; β = 0.65, p < 0.001) and improved (R^2 = 0.82 > 0.73) the DA_{PC} model's prediction of relapse behavior (Figures 3B and 3C). Likelihood ratio testing indicated that the model composed of sex and the DA_{PC} variables was preferred over the DA_{PC}-only nested model (χ^2 = 90.33, p < 0.001). According to the AIC, the sex and DA_{PC} model represented a more parsimonious alternative, despite the additional free parameter (ΔAIC = −82.9; p < 0.001) (Figure 3D). These results reveal the impact of sex as a predictor of cocaine-evoked dopamine dynamics and drug-motivated behavior. Our modeling approach indicates that sex interacts with individual differences in sub-second dopamine responses to orchestrate future cocaine-seeking behavior during relapse.

Discrete sub-second dopamine dynamics and latency to extinction are influenced by sex

Low-dimensional features of cocaine-evoked dopamine responses provide information about future reinstatement behavior, especially when the subject's sex is considered. In support of this,

(K) Percentage of total variance (%) explained by each of the BE_{PC} factors derived from PCA. Principal components selected for MLR are colored in yellow.
(L) Actual vs. predicted reinstatement nose pokes obtained from MLR with BE_{PC1}, BE_{PC2}, and BE_{PC3} as covariates.
(M) Model comparison using AIC supports the superiority of the DA_{PC} model (ΔAIC = −90.24; **p = 0.006). Insets: the models' R^2 and β-parameter estimates. Asterisks indicate β significantly different from 0 (***p < 0.0001). Reinst, reinstatement; Np, nose poke; EXT, extinction; DA, dopamine.

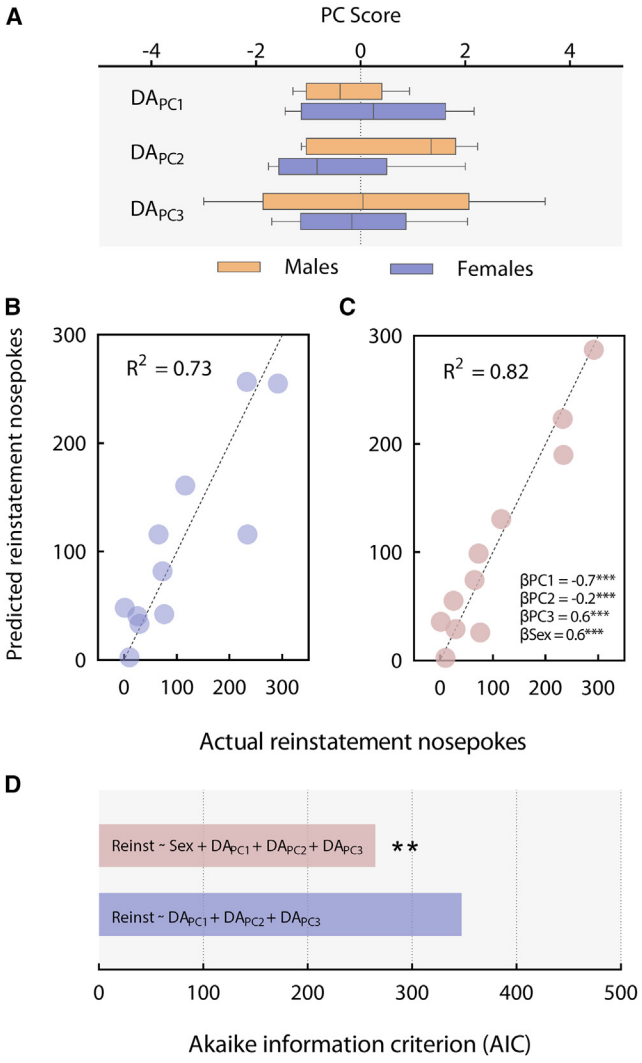

Figure 3. Inclusion of sex optimizes the prediction of reinstatement obtained by the DA_{PC} model

(A) $DA_{PC1,2,3}$ scores by sex (DA_{PC1}: $t_9 = 0.15$, p = 0.88; DA_{PC2}: $t_9 = 1.20$, p = 0.25; DA_{PC3}: $t_9 = 0.82$, p = 0.43) (males, n = 5; females n = 6).

(B) Actual vs. predicted reinstatement nose pokes obtained from an MLR model with DA_{PC1},DA_{PC2}, and DA_{PC3} as covariates.

(C) Actual vs. predicted reinstatement nose pokes obtained from MLR with sex, DA_{PC1}, DA_{PC2}, and DA_{PC3} as covariates. Inset: the models' R^2 and β-parameter estimates for sex and each DA_{PC}. Asterisks indicate β significantly different from 0 (***p < 0.0001).

(D) Based on AIC, the sex + DA_{PC} model is less likely to be excluded. Asterisks indicate significant likelihood ratio test (**p < 0.01) favoring the sex + DA_{PC} model.

we explored which discrete sex differences could be found in terms of behavior and cocaine-evoked dopamine events. Supplementary results show that both sexes consumed and pursued cocaine at similar levels (Figure S7), as previously documented.[22] Despite a common behavioral phenotype, males exhibited higher dopamine release at cue onset and cocaine infusion than females during FR1 and FR3 (Figures 4A–4F). The effect of sex during PR was diametrically opposed (Figures 4D–4F). Waveform analysis detected a significant increase in accumbal dopamine release in males during early extinction (Figures 4G and 4H) and reinstatement (Figure 4I), despite there being no differences in the number of nose pokes (Figure S7). Similarly, no behavioral sex differences were observed in mice that underwent food training. Notably, male mice needed more sessions to reach cocaine-seeking extinction criteria, therefore showing higher resistance to extinguish cocaine-seeking behavior (Figure 4J). Furthermore, we performed similar analyses in food-trained animals. Again, males displayed greater cue-evoked dopamine transients during the reinstatement test compared with females (Figure S7). These comparisons illustrate not only drug-specific sex differences in NAc dopamine responses during cocaine consumption but also a generalized elevation in cue-evoked dopamine in males during reinstatement, regardless of reinforcer.

Sex and patterned dopamine release predict the transition to extinction

We next sought to reproduce the slower transition to extinction observed in males with the low-dimensional dopamine signatures DA_{PC1}, DA_{PC2}, and DA_{PC3}. The proportion of females reaching extinction criteria across sessions was significantly higher at earlier time points than that of males (log rank Mantel-Cox test; $\chi^2 = 5.25$, p = 0.021) (Figure 4K). Then, we employed Cox proportional hazards regression (STAR Methods) to model the transition to extinguished cocaine-seeking behavior across days. Cox regressions are the MLR equivalents of survival curves. They allow fitting of survival data (in this case, transition to "extinguished" cocaine seeking) as a function of a given set of predictors. Here, sex and DA_{PC1}, DA_{PC2}, and DA_{PC3} were the main predictors. The Cox model accurately fit the observed extinction survival curves and recapitulated the slower extinction rates displayed by males (Figure 4L). Similarly, the Cox model composed of sex and the DA_{PC} estimators explained the transition to extinction significantly better than an empty model with no covariates (χ^2 = 12.42, p = 0.014) or an equivalent model without sex as predictor ($\chi^2 = 9.65$, p = 0.002). As a goodness-of-fit indicator, we used Harrell's *C* statistic, which ranks how well the model attributed higher hazard ratios (risk for extinction) to subjects with shorter observation times (early transition to extinction). Harrell's *C* ranges from 0.5 to 1, and scores over 0.7 are usually attributed to strong models.[38] The Cox regression model, with sex and the DA_{PC} predictors, yielded a strong prediction of the transition to extinction of cocaine-seeking behavior (*C* = 0.88). Hazard ratios (equivalent to MLR β-parameter estimates) were examined to determine the weight of each parameter in the model's prediction of the extinction survival curve. Based on this metric, sex (hazard ratio [HR] = 33.01) and DA_{PC1} (HR = 2.39), but not DA_{PC2} (HR = 0.88) or DA_{PC3} (HR = 0.58), contributed to explain the transition to extinguished cocaine seeking. The above results reveal sex-specific trajectories of drug seeking when access to the drug is withdrawn. Moreover, we document the importance of sex-specific dopamine signatures that are sufficient to accurately predict the number of sessions needed to cease cocaine-seeking behavior.

DISCUSSION

Cocaine hijacks dopaminergic signaling through DAT blockade,[39] triggering long-lasting neuronal maladaptations[40]

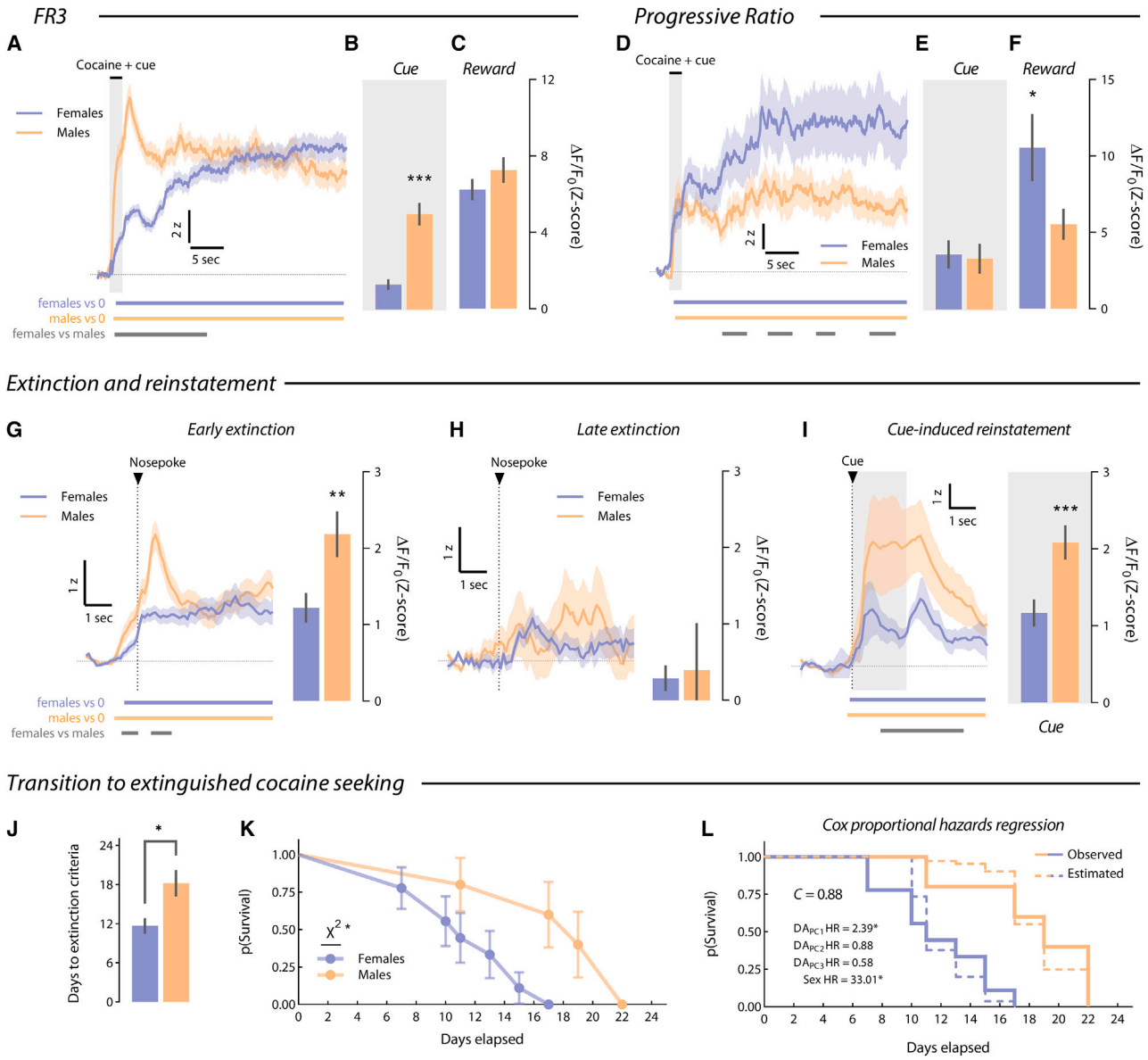

Figure 4. Sex differences in sub-second dopamine responses recapitulate male-specific delayed transition to extinction

(A) NAc GrabDA$_{2m}$ transients by sex obtained during FR3 sessions.

(B and C) GrabDA$_{2m}$ $\Delta F/F_0$ *Z* scores by sex from FR3 trials (B; 0–2 s, cue onset: t_{239} = 6.08, ***p < 0.001) (C; 2–30 s, cocaine delivery: t_{239} = 1.17, p = 0.240).

(D) GrabDA$_{2m}$ transients by sex obtained during PR.

(E and F) GrabDA$_{2m}$ $\Delta F/F_0$ *Z* scores by sex from PR trials (E; 0–2 s, cue onset: t_{76} = 0.21, p = 0.83) (F; 2–30 s, cocaine delivery: *t_{75}= *2.06,* p= *0.042*).

(G) Left: GrabDA$_{2m}$ transients by sex from the first extinction session. Right: GrabDA$_{2m}$ $\Delta F/F_0$ *Z* scores (0–5 s, nose poke) by sex from first extinction session (t_{642} = 2.75, **p = 0.006).

(H) Left: GrabDA$_{2m}$ transients by sex during the last extinction session. Right: GrabDA$_{2m}$ $\Delta F/F_0$ *Z* scores (0–5 s, nose poke) by sex from last extinction session (t_{135} = 0.21, p = 0.83).

(I) Left: GrabDA$_{2m}$ transients by sex during the cue-induced reinstatement test. Right: GrabDA$_{2m}$ $\Delta F/F_0$ *Z* scores (0–2 s, cue) by sex from reinstatement (t_{1121}= *3.01,* ***p = 0.002).

(J) Days required to reach extinction criteria by sex (t_{12} = 2.98, p = 0.011).

(K) Kaplan-Meier survival extinction curve of cocaine seeking by sex (probability of not achieving extinction criteria) (log rank Mantel-Cox test; χ^2 = 5.25, *p = 0.021).

(L) Cox proportional hazard regression containing sex and DA$_{PC1,2,3}$ as covariates. Solid lines represent the observed Kaplan-Meier survival curves of extinction by sex. Dashed lines represent the estimated survival curves as predicted by the Cox sex + DA$_{PC1,2,3}$ model. Colored bars below traces represent periods significantly different from 0 or between sexes, as defined by bootstrapped 95% CIs. Inset: Harrel's *C* statistic. Data are presented as mean ± SEM.

that curtail control of instrumental behavior.[41] Here, we take advantage of photometric techniques to parse out dopamine dynamics during cocaine seeking throughout the animal's entire drug experience. We report stable recordings of sub-second dopamine activity, from acquisition to the reinstatement of cocaine-seeking behavior. Prior studies have conducted similar long-term NAc recordings but were exclusively focused on escalation of intake,[42,43] were based on a within-session extinction procedure,[44,45] or were during incubation of craving.[46] Despite key methodological differences, NAc dopamine transients documented here are equivalent to those previously reported.[25,42–44,47]

Persistent risk of relapse is a defining feature of drug addiction and is a primary endpoint for treatment of substance use disorders.[48,49] Our results indicate that relapse correlates with the amplitude of cue-evoked NAc dopamine transients on the PR test. This evidence supports early work postulating that PR reinforcement schedules are well suited to infer maladaptive cue encoding.[28,29,50] Owing to the increasing availability of positron emission tomography (PET),[51] such a relationship could avail PET estimations of NAc dopamine on a PR schedule as powerful indicators of future relapse in concurrent cocaine users. Building on this finding, we sought to optimize the information gathered by our GrabDA$_{2m}$ measures to obtain a linear model of relapse. We found that cue-evoked dopamine responses clustered together in what can be viewed as a low-dimensional representation of the animal's dopaminergic reactivity to cocaine-paired cues (DA$_{PC1}$). Numerous computational accounts of drug addiction have stressed the importance of such dopamine-based representations,[52,53] which underlie decision-making impairments leading to exacerbated and uncontextualized drug seeking despite harmful consequences.[54,55] Finally, we showed that patterned NAc dopamine release (DA$_{PC1,2,3}$) accurately predicted cue-induced reinstatement behavior. Indeed, these dopamine signatures were more informative than a PCR model using the animal's behavior as the sole predictor, consistent with the notion that reinstatement to cocaine-seeking behavior is sufficiently explained by accumbal dopamine signaling and any maladaptive plasticity that accompanies it.[36] We found that not only could cocaine relapse be predicted with this dopamine-based model but also cue-induced reinstatement of food-seeking behavior. This finding aligns well with recent human and rodent studies depicting a common mesolimbic substrate shared by continued drug and natural reward seeking. Bobadilla et al.[56] found that cocaine- and sucrose-associated neuronal ensembles in the NAc shared ~30% of the constituent neurons. In humans, Koban et al.[57] recently showed that neural signatures of food-paired cues predicted food craving just as well as neural signatures of drug cues predicted drug craving.

Trajectories of drug use and abuse are markedly influenced by sex.[58] Preclinical studies have addressed these disparities with mixed findings.[19,37] Here, we tested the DA$_{PC}$ model of relapse to investigate possible sex differences present in our dataset. In support of prior reports[59–63] (but see Zhou et al.[64]), we could not find evidence in favor of increased female vulnerability to cue-induced reinstatement of food and cocaine seeking. Indeed, males displayed increased dopamine-related fluorescence during discrete phases of voluntary seeking and intake more readily (acquisition, maintenance, early extinction, and reinstatement), especially during cue presentation. Regarding this last detail, we speculate that fewer sex differences after cocaine delivery, compared with cue presentation, were present because dopamine release after cocaine infusion is vastly monopolized by a single variable, e.g., the pharmacological blockade of the DAT. This action leaves little room for other variables (that could be determined by sex) to be revealed during ongoing cocaine self-administration. Despite discrete differences, low-dimensional features of dopamine responses (DA$_{PC1,2,3}$) were equivalent between sexes. The cooccurrence of discrete dopamine differences during various phases of self-administration with a common overall dopamine response trait (DA$_{PC1,2,3}$) may seem contradictory. It is reasonable to theorize, however, that both sexes followed different trajectories, but are comparable as a whole, to ensure a common behavioral adaptation to a given environmental setting. Thus, sex does not determine a divergent behavioral adaptation between males and females but rather acts in concert with other individual differences to leverage a shared behavioral outcome. Accordingly, we show that when sex is incorporated into the DA$_{PC}$ regression model, an improvement of predictive performance is observed. Notably, the combination of sex and patterned dopamine release yielded a remarkably accurate prediction of reinstatement nose pokes, reproducing 82% of the observed variance with a relatively simple linear regression that did not require consideration of many previous dopaminergic algorithms[52,65] associated with motivated behavior.

We also observed a greater resistance to extinction in males. It is important to keep in mind that there is a considerable conceptual distance between the extinction procedure followed here and the abstinence period typically preceding relapse episodes in humans.[66] Nevertheless, investigating the behavioral and neural mechanisms of extinction can increase our understanding of drug-seeking reduction.[67] The resistance to cease cocaine seeking by male mice aligns well with human reports suggesting a higher propensity in men to transition from voluntary abstinence to cocaine use[18] and greater overdose rates.[68] In addition, we showed that slower extinction rates observed in males were accompanied by increased NAc dopamine release during cocaine seeking at the beginning of extinction training and during reinstatement. Importantly, sex and patterned dopamine responses accurately predicted the probability to extinguish cocaine seeking. Thus, our results demonstrate the predictive power of patterned dopamine responses evoked by drug-associated cues (DA$_{PC1}$) on the progression to reinstatement.

In conclusion, our experiments unambiguously show that it is possible to recapitulate reinstatement and extinction with a simple linear model that integrates the animal's longitudinal repertoire of cocaine-evoked dopamine responses in the NAc. A closer examination of the DA$_{PC}$ model reveals the particular importance of early dopamine responses (acquisition, PR) in the gating of future relapse behavior. Fiber photometry in self-administering mice allowed us to characterize sex-specific sub-second dopamine responses during the pursuit of cocaine. We postulate that this validated neuromarker, now accessible

through human PET studies, could be used to refine patient prognosis in research and clinical settings.

Limitations of the study

A limitation of the present study, inherent to rodent drug self-administration procedures,[69] is that cocaine was not available during the reinstatement test, unlike human relapse episodes. This divergence could explain why, within our conditions, dopamine release events during the relapse test had little impact on predicting the reinstatement of cocaine seeking. Related to the above, classical extinction protocols using rodents, like the one carried out here, are not designed to capture the rich nature of voluntary abstinence periods in humans.[70]

STAR★METHODS

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

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

M.A.L., J.F.C., and N.E.Z. were responsible for the concept and design of the study. M.A.L., B.L.O., R.Y.-M., S.A.E., and N.E.Z. performed the experimental studies. L.-Y.Z., J.M.W., and Y.L. were involved in the design and refinement of the methodological procedures. M.A.L., J.F.C., and N.E.Z. drafted the manuscript and participated in interpreting the findings. All authors critically reviewed and approved the final version for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. United Nations (UN). World Drug Report 2019: Stimulants. Sales No. E.19.XI.8. www.unodc.org/wdr2019.
2. Vafaie, N., and Kober, H. (2022). Association of drug cues and craving with drug use and relapse: a systematic review and meta-analysis. JAMA Psychiatr. *79*, 641–650. https://doi.org/10.1001/jamapsychiatry.2022.1240.
3. Nestler, E.J., and Lüscher, C. (2019). The molecular basis of drug addiction: linking epigenetic to synaptic and circuit mechanisms. Neuron *102*, 48–59. https://doi.org/10.1016/j.neuron.2019.01.016.
4. Volkow, N.D., and Boyle, M. (2018). Neuroscience of addiction: relevance to prevention and treatment. Am. J. Psychiatr. *175*, 729–740. https://doi.org/10.1176/APPI.AJP.2018.17101174.
5. Kalivas, P.W., and Volkow, N.D. (2005). The neural basis of addiction: a pathology of motivation and choice. Am. J. Psychiatr. *162*, 1403–1413. https://doi.org/10.1176/appi.ajp.162.8.1403.
6. Capriles, N., Rodaros, D., Sorge, R.E., and Stewart, J. (2003). A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) *168*, 66–74. https://doi.org/10.1007/s00213-002-1283-z.
7. Bossert, J.M., Marchant, N.J., Calu, D.J., and Shaham, Y. (2013). The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology (Berl) *229*, 453–476. https://doi.org/10.1007/s00213-013-3120-y.
8. McFarland, K., and Kalivas, P.W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J. Neurosci. *21*, 8655–8663.
9. McFarland, K., Davidge, S.B., Lapish, C.C., and Kalivas, P.W. (2004). Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. J. Neurosci. *24*, 1551–1560. https://doi.org/10.1523/JNEUROSCI.4177-03.2004.
10. Pascoli, V., Terrier, J., Hiver, A., and Lüscher, C. (2015). Sufficiency of mesolimbic dopamine neuron stimulation for the progression to addiction. Neuron *88*, 1054–1066. https://doi.org/10.1016/j.neuron.2015.10.017.
11. Koob, G.F. (2008). A role for brain stress systems in addiction. Neuron *59*, 11–34. https://doi.org/10.1016/j.neuron.2008.06.012.
12. Costa, K.M., and Schoenbaum, G. (2022). Dopamine. Curr. Biol. *32*, R817–R824. https://doi.org/10.1016/j.cub.2022.06.060.
13. George, O., Koob, G.F., and Vendruscolo, L.F. (2014). Negative reinforcement via motivational withdrawal is the driving force behind the transition to addiction. Psychopharmacology *231*, 3911–3917. https://doi.org/10.1007/S00213-014-3623-1.
14. Liu, Y., Jean-Richard-dit-Bressel, P., Yau, J.O.-Y., Willing, A., Prasad, A.A., Power, J.M., Killcross, S., Clifford, C.W.G., and McNally, G.P. (2020). The mesolimbic dopamine activity signatures of relapse to alcohol-seeking. J. Neurosci. *40*, 6409–6427. https://doi.org/10.1523/jneurosci.0724-20.2020.
15. Becker, J.B., and Koob, G.F. (2016). Sex differences in animal models: focus on addiction. Pharmacol. Rev. *68*, 242–263. https://doi.org/10.1124/pr.115.011163.
16. McHugh, R.K., Votaw, V.R., Sugarman, D.E., and Greenfield, S.F. (2018). Sex and gender differences in substance use disorders. Clin. Psychol. Rev. *66*, 12–23. https://doi.org/10.1016/j.cpr.2017.10.012.

17. Greenfield, S.F., Back, S.E., Lawson, K., and Brady, K.T. (2010). Substance abuse in women. Psychiatr. Clin. *33*, 339–355. https://doi.org/10.1016/J.PSC.2010.01.004.

18. Gallop, R.J., Crits-Christoph, P., ten Have, T.R., Barber, J.P., Frank, A., Griffin, M.L., and Thase, M.E. (2007). Differential transitions between cocaine use and abstinence for men and women. J. Consult. Clin. Psychol. *75*, 95–103. https://doi.org/10.1037/0022-006X.75.1.95.

19. Nicolas, C., Zlebnik, N.E., Farokhnia, M., Leggio, L., Ikemoto, S., and Shaham, Y. (2022). Sex differences in opioid and psychostimulant craving and relapse: a critical review. Pharmacol. Rev. *74*, 119–140. https://doi.org/10.1124/pharmrev.121.000367.

20. Orendain-Jaime, E.N., Ortega-Ibarra, J.M., and López-Pérez, S.J. (2016). Evidence of sexual dimorphism in D1 and D2 dopaminergic receptors expression in frontal cortex and striatum of young rats. Neurochem. Int. *100*, 62–66. https://doi.org/10.1016/J.NEUINT.2016.09.001.

21. Kritzer, M.F., and Creutz, L.M. (2008). Region and sex differences in constituent dopamine neurons and immunoreactivity for intracellular estrogen and androgen receptors in mesocortical projections in rats. J. Neurosci. *28*, 9525–9535. https://doi.org/10.1523/JNEUROSCI.2637-08.2008.

22. López, A.J., Johnson, A.R., Euston, T.J., Wilson, R., Nolan, S.O., Brady, L.J., Thibeault, K.C., Kelly, S.J., Kondev, V., Melugin, P., et al. (2021). Cocaine self-administration induces sex-dependent protein expression in the nucleus accumbens. Commun. Biol. *4*, 883. https://doi.org/10.1038/s42003-021-02358-w.

23. Jean-Richard-dit-Bressel, P., Clifford, C.W.G., and McNally, G.P. (2020). Analyzing event-related transients: confidence intervals, permutation tests, and consecutive thresholds. Front. Mol. Neurosci. *13*, 14. https://doi.org/10.3389/fnmol.2020.00014.

24. Cheer, J.F., Wassum, K.M., Sombers, L.A., Heien, M.L.A.V., Ariansen, J.L., Aragona, B.J., Phillips, P.E.M., and Wightman, R.M. (2007). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J. Neurosci. *27*, 791–795. https://doi.org/10.1523/JNEUROSCI.4152-06.2007.

25. Siciliano, C.A., and Jones, S.R. (2017). Cocaine potency at the dopamine transporter tracks discrete motivational states during cocaine self-administration. Neuropsychopharmacology *42*, 1893–1904. https://doi.org/10.1038/NPP.2017.24.

26. Oleson, E.B., Talluri, S., Childers, S.R., Smith, J.E., Roberts, D.C.S., Bonin, K.D., and Budygin, E.A. (2009). Dopamine uptake changes associated with cocaine self-administration. Neuropsychopharmacology *34*, 1174–1184. https://doi.org/10.1038/NPP.2008.186.

27. Pan, H.-T., Menacherry, S., and Justice, J.B. (1991). Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. J. Neurochem. *56*, 1299–1306. https://doi.org/10.1111/J.1471-4159.1991.TB11425.X.

28. Richardson, N.R., and Roberts, D.C. (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J. Neurosci. Methods *66*, 1–11. https://doi.org/10.1016/0165-0270(95)00153-0.

29. Roberts, D.C.S., Gabriele, A., and Zimmer, B.A. (2013). Conflation of cocaine seeking and cocaine taking responses in IV self-administration experiments in rats: methodological and interpretational considerations. Neurosci. Biobehav. Rev. *37*, 2026–2036. https://doi.org/10.1016/j.neubiorev.2013.04.017.

30. Sun, F., Zeng, J., Jing, M., Zhou, J., Feng, J., Owen, S.F., Luo, Y., Li, F., Wang, H., Yamaguchi, T., et al. (2018). A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice in brief the development of GPCR-activation-based-DA (GRAB DA) sensors enables measurements of dopamine dynamics in freely behaving animals with high spatiotemporal precision. Cell *174*, 481–496.e19. https://doi.org/10.1016/j.cell.2018.06.042.

31. Patriarchi, T., Cho, J.R., Merten, K., Howe, M.W., Marley, A., Xiong, W.H., Folk, R.W., Broussard, G.J., Liang, R., Jang, M.J., et al. (2018). Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science *360*, eaat4422. https://doi.org/10.1126/SCIENCE.AAT4422.

32. Flagel, S.B., Chaudhury, S., Waselus, M., Kelly, R., Sewani, S., Clinton, S.M., Thompson, R.C., Watson, S.J., and Akil, H. (2016). Genetic background and epigenetic modifications in the core of the nucleus accumbens predict addiction-like behavior in a rat model. Proc. Natl. Acad. Sci. USA *113*, E2861–E2870. https://doi.org/10.1073/PNAS.1520491113/SUPPL_FILE/PNAS.201520491SI.PDF.

33. Slosky, L.M., Pires, A., Bai, Y., Clark, N.B., Hauser, E.R., Gross, J.D., Porkka, F., Zhou, Y., Chen, X., Pogorelov, V.M., et al. (2022). Establishment of multi-stage intravenous self-administration paradigms in mice. Sci. Rep. *12*, 21422. https://doi.org/10.1038/s41598-022-24740-2.

34. Nutt, D.J., Lingford-Hughes, A., Erritzoe, D., and Stokes, P.R.A. (2015). The dopamine theory of addiction: 40 years of highs and lows. Nat. Rev. Neurosci. *16*, 305–312. https://doi.org/10.1038/NRN3939.

35. Lüscher, C. (2016). The emergence of a circuit model for addiction. Annu. Rev. Neurosci. *39*, 257–276. https://doi.org/10.1146/ANNUREV-NEURO-070815-013920.

36. Volkow, N.D., Wise, R.A., and Baler, R. (2017). The dopamine motive system: implications for drug and food addiction. Nat. Rev. Neurosci. *18*, 741–752. https://doi.org/10.1038/NRN.2017.130.

37. Becker, J.B., and Chartoff, E. (2019). Sex differences in neural mechanisms mediating reward and addiction. Neuropsychopharmacology *44*, 166–183. https://doi.org/10.1038/s41386-018-0125-6.

38. Hosmer, D.W., Lemeshow, S., and Sturdivant, R.X. (2013). Applied logistic regression. In Biometrics Wiley Series in Probability and Statistics (Wiley), p. 1632. https://doi.org/10.1002/9781118548387.

39. Collins, A.L., and Saunders, B.T. (2020). Heterogeneity in striatal dopamine circuits: form and function in dynamic reward seeking. J. Neurosci. Res. *98*, 1046–1069. https://doi.org/10.1002/JNR.24587.

40. Pascoli, V., Terrier, J., Espallergues, J., Valjent, E., O'Connor, E.C., and Lüscher, C. (2014). Contrasting forms of cocaine-evoked plasticity control components of relapse. Nature *509*, 459–464. https://doi.org/10.1038/nature13257.

41. Takahashi, Y., Schoenbaum, G., and Niv, Y. (2008). Silencing the critics: understanding the effects of cocaine sensitization on dorsolateral and ventral striatum in the context of an Actor/Critic model. Front. Neurosci. *2*, 86–99. https://doi.org/10.3389/NEURO.01.014.2008/BIBTEX.

42. Willuhn, I., Burgeno, L.M., Everitt, B.J., and Phillips, P.E.M. (2012). Hierarchical recruitment of phasic dopamine signaling in the striatum during the progression of cocaine use. Proc. Natl. Acad. Sci. USA *109*, 20703–20708. https://doi.org/10.1073/pnas.1213460109.

43. Willuhn, I., Burgeno, L.M., Groblewski, P.A., and Phillips, P.E.M. (2014). Excessive cocaine use results from decreased phasic dopamine signaling in the striatum. Nat. Neurosci. *17*, 704–709. https://doi.org/10.1038/nn.3694.

44. Stuber, G.D., Wightman, R.M., and Carelli, R.M. (2005). Extinction of cocaine self-administration reveals functionally and temporally distinct dopaminergic signals in the nucleus accumbens. Neuron *46*, 661–669. https://doi.org/10.1016/j.neuron.2005.04.036.

45. Saddoris, M.P., Wang, X., Sugam, J.A., and Carelli, R.M. (2016). Cocaine self-administration experience induces pathological phasic accumbens dopamine signals and abnormal incentive behaviors in drug-abstinent rats. J. Neurosci. *36*, 235–250. https://doi.org/10.1523/JNEUROSCI.3468-15.2016.

46. Burgeno, L.M., Farero, R.D., Murray, N.L., Panayi, M.C., Steger, J.S., Soden, M.E., Evans, S.B., Sandberg, S.G., Willuhn, I., Zweifel, L.S., and Phillips, P.E.M. (2023). Cocaine Seeking And Taking Are Oppositely Regulated By Dopamine. BioRxiv. https://doi.org/10.1101/2023.04.09.536189.

47. Pribiag, H., Shin, S., Wang, E.H.J., Sun, F., Datta, P., Okamoto, A., Guss, H., Jain, A., Wang, X.Y., de Freitas, B., et al. (2021). Ventral pallidum DRD3 potentiates a pallido-habenular circuit driving accumbal dopamine release

and cocaine seeking. Neuron *109*, 2165–2182.e10. https://doi.org/10.1016/j.neuron.2021.05.002.

48. Volkow, N.D. (2020). Personalizing the treatment of substance use disorders. Am. J. Psychiatr. *177*, 113–116. https://doi.org/10.1176/APPI.AJP.2019.19121284.
49. Food and Drug Administration. (2020). Opioid use disorder: endpoints for demonstrating effectiveness of drugs for treatment guidance for industry. https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs.
50. O'Connor, E.C., Chapman, K., Butler, P., and Mead, A.N. (2011). The predictive validity of the rat self-administration model for abuse liability. Neurosci. Biobehav. Rev. *35*, 912–938. https://doi.org/10.1016/J.NEUBIOREV.2010.10.012.
51. Kanthan, M., Cumming, P., Hooker, J.M., and Vasdev, N. (2017). Classics in neuroimaging: imaging the dopaminergic pathway with PET. ACS Chem. Neurosci. *8*, 1817–1819. https://doi.org/10.1021/acschemneuro.7b00252.
52. Mollick, J.A., and Kober, H. (2020). Computational models of drug use and addiction: a review. J. Abnorm. Psychol. *129*, 544–555. https://doi.org/10.1037/abn0000503.
53. Redish, A.D. (2004). Addiction as a computational process gone awry. Science *306*, 1944–1947. https://doi.org/10.1126/SCIENCE.1102384.
54. Simon, D.A., and Daw, N.D. (2012). Dual-system learning models and drugs of abuse. In Computational Neuroscience of Drug Addiction (Springer), pp. 145–161. https://doi.org/10.1007/978-1-4614-0751-5_5.
55. Volkow, N.D., Wang, G.-J., Telang, F., Fowler, J.S., Logan, J., Childress, A.-R., Jayne, M., Ma, Y., and Wong, C. (2006). Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J. Neurosci. *26*, 6583–6588. https://doi.org/10.1523/JNEUROSCI.1544-06.2006.
56. Bobadilla, A.C., Dereschewitz, E., Vaccaro, L., Heinsbroek, J.A., Scofield, M.D., and Kalivas, P.W. (2020). Cocaine and sucrose rewards recruit different seeking ensembles in the nucleus accumbens core. Mol. Psychiatr. *25*, 3150–3163. https://doi.org/10.1038/s41380-020-00888-z.
57. Koban, L., Wager, T.D., and Kober, H. (2022). A neuromarker for drug and food craving distinguishes drug users from non-users. Nat. Neurosci. *26*, 316–325. https://doi.org/10.1038/s41593-022-01228-w.
58. Riley, A.L., Hempel, B.J., and Clasen, M.M. (2018). Sex as a biological variable: drug use and abuse. Physiol. Behav. *187*, 79–96. https://doi.org/10.1016/j.physbeh.2017.10.005.
59. Kawa, A.B., and Robinson, T.E. (2019). Sex differences in incentive-sensitization produced by intermittent access cocaine self-administration. Psychopharmacology (Berl) *236*, 625–639. https://doi.org/10.1007/s00213-018-5091-5.
60. Weber, R.A., Logan, C.N., Leong, K.C., Peris, J., Knackstedt, L., and Reichel, C.M. (2018). Regionally specific effects of oxytocin on reinstatement of cocaine seeking in male and female rats. Int. J. Neuropsychopharmacol. *21*, 677–686. https://doi.org/10.1093/IJNP/PYY025.
61. Bechard, A.R., Hamor, P.U., Schwendt, M., and Knackstedt, L.A. (2018). The effects of ceftriaxone on cue-primed reinstatement of cocaine-seeking in male and female rats: estrous cycle effects on behavior and protein expression in the nucleus accumbens. Psychopharmacology (Berl) *235*, 837–848. https://doi.org/10.1007/S00213-017-4802-7.
62. Swalve, N., Smethells, J.R., Zlebnik, N.E., and Carroll, M.E. (2016). Sex differences in reinstatement of cocaine-seeking with combination treatments of progesterone and atomoxetine. Pharmacol. Biochem. Behav. *145*, 17–23. https://doi.org/10.1016/J.PBB.2016.03.008.
63. Fuchs, R.A., Evans, K.A., Mehta, R.H., Case, J.M., and See, R.E. (2005). Influence of sex and estrous cyclicity on conditioned cue-induced reinstatement of cocaine-seeking behavior in rats. Psychopharmacology (Berl) *179*, 662–672. https://doi.org/10.1007/s00213-004-2080-7.
64. Zhou, L., Pruitt, C., Shin, C.B., Garcia, A.D., Zavala, A.R., and See, R.E. (2014). Fos expression induced by cocaine-conditioned cues in male and female rats. Brain Struct. Funct. *219*, 1831–1840. https://doi.org/10.1007/S00429-013-0605-8.
65. Schultz, W. (2015). Neuronal reward and decision signals: from theories to data. Physiol. Rev. *95*, 853–951. https://doi.org/10.1152/physrev.00023.2014.
66. Venniro, M., Banks, M.L., Heilig, M., Epstein, D.H., and Shaham, Y. (2020). Improving translation of animal models of addiction and relapse by reverse translation. Nat. Rev. Neurosci. *21*, 625–643. https://doi.org/10.1038/s41583-020-0378-z.
67. Mcnally, G.P. (2014). Extinction of drug seeking: neural circuits and approaches to augmentation. Neuropharmacology *76 Pt B*, 528–532. https://doi.org/10.1016/j.neuropharm.2013.06.007.
68. Butelman, E.R., Huang, Y., Epstein, D.H., Shaham, Y., Goldstein, R.Z., Volkow, N.D., and Alia-Klein, N. (2023). Overdose mortality rates for opioids or stimulants are higher in males than females, controlling for rates of drug misuse: state-level data. Preprint at medRxiv. https://doi.org/10.1101/2023.01.20.23284833.
69. Sanchis-Segura, C., and Spanagel, R. (2006). Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. Addiction Biol. *11*, 2–38. https://doi.org/10.1111/j.1369-1600.2006.00012.x.
70. Venniro, M., Caprioli, D., and Shaham, Y. (2016). Animal models of drug relapse and craving: from drug priming-induced reinstatement to incubation of craving after voluntary abstinence. Prog. Brain Res. *224*, 25–52. https://doi.org/10.1016/BS.PBR.2015.08.004.
71. Institute for Laboratory (2011). Animal Research Division on Earth and Life Studies. Guide for the Care and Use of Laboratory Animals. https://doi.org/10.17226/12910.
72. Luján, M.Á., Alegre-Zurano, L., Martín-Sánchez, A., Cantacorps, L., and Valverde, O. (2022). CB1 receptor antagonist AM4113 reverts the effects of cannabidiol on cue and stress-induced reinstatement of cocaine-seeking behaviour in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry *113*, 110462. https://doi.org/10.1016/j.pnpbp.2021.110462.
73. Barker, D.J., Miranda-Barrientos, J., Zhang, S., Root, D.H., Wang, H.L., Liu, B., Calipari, E.S., and Morales, M. (2017). Lateral preoptic control of the lateral habenula through convergent glutamate and GABA transmission. Cell Rep. *21*, 1757–1769. https://doi.org/10.1016/J.CELREP.2017.10.066.
74. Cunningham, J.P., and Yu, B.M. (2014). Dimensionality reduction for large-scale neural recordings. Nat. Neurosci. *17*, 1500–1509. https://doi.org/10.1038/nn.3776.
75. Pacheco, J., Casado, S., and Porras, S. (2013). Exact methods for variable selection in principal component analysis: Guide functions and pre-selection. Comput. Stat. Data Anal. *57*, 95–111. https://doi.org/10.1016/j.csda.2012.06.014.
76. D'Angelo, L., and Canale, A. (2022). Efficient posterior sampling for Bayesian Poisson regression. https://doi.org/10.1080/10618600.2022.2123337, 1–19. 10.1080/10618600.2022.2123337.
77. Ibrahim, J.G., Chen, M.-H., and Sinha, D. (2014). B ayesian survival analysis. In Wiley StatsRef: Statistics Reference Online (John Wiley & Sons, Ltd). https://doi.org/10.1002/9781118445112.stat06003.
78. Kim, Y., and Kong, L. (2015). Estimation of C-index for cox proportional hazards model with censored biomarker covariate subject to limits of detection. J. Biopharm. Stat. *25*, 459–473. https://doi.org/10.1080/10543406.2014.920859.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
pAAV-hsyn-GRAB_DA2m	Gifted by Dr. Yulong Li	Addgene #140553-AAV9
Chemicals, peptides, and recombinant proteins		
Ketamine HCl	Millipore-Sigma	1356009
Xylazine HCl	Millipore-Sigma	X1251
Bactroban	GlaxoSmithKline	R11145
Cocaine HCl	NDSP	9041–001
Experimental models: Organisms/strains		
C57bl/6J	Jackson Laboratories	000664
Software and algorithms		
Synapse Software	Tucker-Davis Technologies website	v98
FP signal MATLAB processing script	GitHub	github.com/djamesbarker/FiberPhotometry
GraphPad Prism	GraphPad website	9.1
BPR package for R	GitHub	github.com/laura-dangelo/bpr
MATLAB	MATLAB website	2020b
RStudio	Posit website	2021.08.0
Bootstrapping CI script	GitHub	github.com/philjrdb
Other		
Sweetened grain pellets	Bio-Serv	F05684
Optical fiber	Thorlabs	CFM15L4
i.v. cannula	PlasticsOne	313-002BM-10
Operant chambers	Med Associates	ENV-307A-CT
Microinfusion pump	Med Associates	PHM-100A
Liquid swivel	Instech Laboratories	375/25
470nm LED source	Thorlabs	M470F3
405nm LED source	Thorlabs	M405FP1
Fluorescence minicube	Doric Lenses	FMC4
Photoreceiver	Doric Lenses	Newport2151
FP real-time processor	Tucker-Davis Technologies	RZ5P

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Joseph F. Cheer (jcheer@umaryland.edu).

Materials availability

The study did not generate any new unique reagents or materials. The materials and reagents employed herein can be accessed commercially without availability restrictions.

Data and code availability

- Access to the original data will be provided upon email request to the lead contact.
- This paper does not report original code.
- 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

Animals

For the cocaine experiment, adult (3-month-old) female and male C57BL/6J mice were used (n = 20). Prior to fiber implantation surgery, mice were group-housed in plastic cages with ad libitum access to food and water. Following surgery, animals were singly-housed. All rodent holding rooms were maintained at 24°C and 40–50% humidity under a 12-h light/dark cycle with lights on at 07:00 h. Research facilities were certified by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Maryland School of Medicine and the University of California, Riverside School of Medicine and in accordance with the Guide for the Care and Use of Laboratory Animals.[71] Animals were transduced with a genetically-encoded fluorescent dopamine sensor[30] ($GrabDA_{2m}$) and implanted with optical fibers in the core subregion of the NAc on postnatal day (PND) 70. pAAV-hsyn-GRAB_DA_{2m} was a gift from Yulong Li (Addgene viral prep #140553-AAV9). On PND 100, they underwent the second surgical procedure, in which they were implanted with intravenous catheters and trained to self-administer cocaine. Then, mice underwent *in vivo* fiber photometry recordings during cocaine self-administration. A separate group of mice (n = 12, 6/sex) performed the exact same experiments described below but were instead reinforced with sweetened grain pellets (Bio-Serv, F05684).

METHOD DETAILS

Viral delivery and optical fiber implantation

Animals were anesthetized using isoflurane in O_2 (4% induction and 2% maintenance) and then placed in the stereotaxic apparatus. To express $GrabDA_{2m}$ in the NAc, AAV9-hSyn-DA-sensor 4.4 (10^{13} genome copies (gc)/ml)) was unilaterally injected using the following coordinates: antero-posterior, 1.1 mm; medio-lateral, 1 mm; and dorsoventral, −3.8 mm; relative to bregma (brain surface). By a microsyringe pump, AAV9-hSyn-DA-sensor 4.4 (500nL) was slowly infused through a sharp glass pipette into the target brain area at rate of 100 nL/min. Immediately following virus infusion, an optical fiber (diameter, 400μm; NA, 0.5; Thorlabs) embedded within a ceramic ferrule was implanted with its tip targeting 0.1mm above the above-mentioned NAc DV coordinates. Ceramic ferrules were secured to the skull using dental cement with the help of two skull-penetrating screws. Mice were gently removed from the stereotaxic instrument and placed over a heat pad in their home cages. With daily monitoring for wound healing, mice were singly housed and allowed to recover for 4 weeks.

Intravenous catheterization surgery

After 4 weeks of recovery and viral expression, $GrabDA_{2m}$ fluorescence was tested during environmental exploration. Only those animals showing fluctuation in fluorescence intensity were selected for catheterization of the jugular vein (n = 17). Surgical implantation of the catheter into the jugular vein was performed following anesthesia with a mixture of Ketamine hydrochloride (100 mg/kg) and Xylazine hydrochloride (10 mg/kg), injected in a volume of 0.1mL/10 g body weight, i.p. Indwelling i.v. silastic catheters (0.3mm inner diameter, 0.6mm outer diameter) were implanted 1.3cm into the right jugular vein and anchored with suture.[72] The remaining tubing ran subcutaneously to the cannula (PlasticsOne), which exited at the midscapular region. All incisions were sutured and coated with antibiotic ointment (Bactroban, GlaxoSmithKline). After surgery, animals were allowed to recover for 3 days prior to initiation of self-administration sessions. To maintain patency, catheters were flushed daily with heparinized saline (30 USP units/ml).

Intravenous cocaine self-administration

After surgery recovery, mice were trained in operant chambers (Model ENV-307A-CT, Med Associates) equipped with two holes, one randomly selected as the active hole and the other as the inactive. Cocaine 0.5 mg/kg/infusion was delivered in a 20-μL injection over 2-s via a syringe mounted on a microinfusion pump (PHM-100A, Med-Associates) through a single-channel liquid swivel (375/25, Instech Laboratories) connected via tygon tubing to the chronic indwelling catheter. All FR1 and FR3 sessions started with a cocaine priming infusion. When mice responded on the active hole, the stimulus lights (located above the nosepoke hole) and tone were presented for 2-s and a cocaine infusion was delivered automatically over these 2-s. Each infusion was followed by a 10-s time-out period in which a nosepoke on the active hole had no consequences but was recorded.

Acquisition and maintenance of operant cocaine taking

Mice were trained to nosepoke in order to receive 0.5 mg/kg cocaine infusions or food pellets under a fixed ratio 1 reinforcement schedule (FR1) in 2h-long daily sessions. For the cocaine-trained group, no food self-administration pre-training was conducted. The food-trained group of mice were food-restricted and maintained at ∼90% their initial body weight throughout the whole procedure, from acquisition to reinstatement. Mice were moved to an FR3 reinforcement schedule when the following criteria were met on 2 consecutive FR1 sessions: *a*) ≥ 65% of responses were received at the active hole; and *b*) a minimum of 15 responses on the active hole. After meeting criteria, animals (cocaine: n = 15; food: n = 12) underwent 5 more FR3 sessions.

Progressive ratio test session

After five FR3 sessions, subjects (cocaine: n = 15; food: n = 12) were tested in a PR schedule, wherein the response requirement to earn a reinforcer escalated according to the following series: 1-2-4-6-9-12-15-18-27-32-40-60-73-90-135-178. The PR session ended when mice were not able to earn the response requirement in 1-h and was performed only once. After the PR test, catheter

patency was determined by i.v. infusion of 0.1 mg/kg thiopental (5 mg/ml). Catheter patency was confirmed when prominent signs of anesthesia appeared within 3s of the infusion. Two mice did not show prominent signs of anesthesia and were removed from the study.

Extinction and reinstatement of cocaine seeking

After PR testing, mice (cocaine: n = 13; food: n = 12) underwent operant extinction of cocaine- or food-seeking behaviors. During these sessions, nosepokes in the active hole produced neither reinforcement nor cue presentation. Extinction sessions (2-h long) were conducted once a day, 5 days/week until reaching the extinction criteria in two consecutive days (performing less than 15 total responses or <40% of the mean nosepokes exhibited during FR3 testing). Animals that did not consistently respond to the active porthole (>5 nosepokes) during the first two days of extinction were removed (n = 2) from the study. Twenty-four hours after, mice (n = 11, 6 females and 5 males) underwent a cue-induced reinstatement session, in which they were confined to the operant chambers for 2-h. During the reinstatement session, mice did not receive cocaine infusions or food pellet delivery but nose-poking in the active hole resulted in cue light and tone presentation and, for the cocaine-seeking group, activation of the cocaine microinfusion pump under an FR1 schedule. In the food-seeking group (n = 12, 6 females and 6 males), the pellet dispenser was activated following nose-poking on the active hole, but no food pellets were delivered.

Fiber photometry recording

Fiber photometry of GrabDA$_{2m}$ signals was conducted in all cocaine self-administration sessions, from acquisition to reinstatement. Two fiber-coupled LEDs producing 470nm (M470F3, ThorLabs) and 405nm (M405FP1, ThorLabs) lasers were used as the excitation source. The LED beams were reflected and coupled to a fluorescence minicube (FMC4, Doric Lenses). A 2 m-long optical fiber (400μm, Doric Lenses) was used to transmit light between the fluorescence minicube and the implanted fiber. To allow for concurrent drug i.v. self-administration and fiber photometry, the drug tubing (running from the liquid swivel to the animal's catheter) hung parallel to the optic fiber and no protective sleeve was used. The unprotected tubing had a length ∼15 cm longer than the straight distance between the back of the animal and the liquid swivel. This allowed the tubing to coil around the optic fiber over the course of the 2 h-long sessions without compromising the mobility of the animal. Video S1 illustrates the setup used and the animal's mobility during simultaneous dopamine recordings and voluntary cocaine taking. The LED intensity was measured at the tip of optical patch cable and adjusted to ∼ 5μW and ∼10μW for the 405nm and 470nm LEDs, respectively. GrabDA$_{2m}$ fluorescence was collected by the optical fiber, passed through the fluorescence minicube, and projected onto a photoreceiver (Newport 2151, Doric) where light intensity was converted into current signal. A RZ5P real-time processor (Tucker-Davis Technologies, TDT) was used to convert the current signal to voltage signal, which was processed through a low-pass filter (6Hz, sixth order Butterworth filter) to allow filtering of noise at higher frequency. Finally, the voltage signal and transistor–transistor logic (TTL) signals coming from the operant chambers were sampled at 6kHz and recorded using the Synapse software. Cue presentation onset, cocaine infusion or pellet delivery offset, and nosepoke onset times in the active and inactive holes were recorded as independent TTLs.

QUANTIFICATION AND STATISTICAL ANALYSIS

Fiber photometry signal processing and PETH creation

GrabDA$_{2m}$ signals were processed using the MATLAB script developed by Barker et al.[73] Noise-related changes in fluorescence across the whole experimental session were removed by scaling the isosbestic control signal (405nm) and regressing it onto the dopamine-sensitive signal (470nm). This regression generated a predicted model of the noise that was based on the isosbestic control. Dopamine-independent waveforms on the 405nm model were then subtracted from the raw GrabDA$_{2m}$ signal to remove movement, photo-bleaching, and fiber-bending artifacts (Figure S1). PETHs were constructed using 100-ms bins surrounding the event of interest. PETH time windows for FR1, FR3 and PR recordings spanned -2s–30s, centered around cue presentation TTLs. Time windows for extinction and reinstatement recordings spanned -2s–5s, centered around nosepoke or cue presentation TTLs, respectively. Each bin of the PETH was z-scored by subtracting the mean fluorescence found in the -2s to −0.5s time window preceding each trial and dividing by the s.d. across those windows (n = number of trials). In the FR1, FR3, PR and reinstatement phases of the experiment, z-scores obtained during the first 2 s following cue presentation (0-2s, aligned to cue onset) were considered to reflect cue-evoked dopamine-based fluorescence. When cocaine was available, z-scores of the remaining period (2-30s, aligned to cocaine delivery offset) were interpreted as reward-evoked dopamine-based fluorescence. For the extinction recordings, the 2 s following each nosepoke (0-2s) were interpreted to reflect nosepoke-related dopamine-based fluorescence. Cocaine-related GrabDA$_{2m}$ signals were relatively stable across days and only changed across experimental phases (Figures S1 and 1). Therefore, for graphing and analytical purposes, GrabDA$_{2m}$ traces were averaged across the different sessions that constituted an experimental phase. Food- and food-paired cue-evoked dopamine transients (Figures 1 and S6) were obtained and analyzed using the same fiber photometry signal processing methods. To compare rise times between rewards, we fit GrabDA$_{2m}$ trials (z-scores; -2s–2s, centered around cue onset) to GraphPad Prism built-in sigmoidal function. Extra sum-of-squares F tests were used to determine if the same hillslope term of the sigmoidal function significantly fit both datasets (cocaine and food pellets). To clarify, hillslopes were favored over $t_{1/2}$ analyses, since the former measure the steepness of GrabDA$_{2m}$ transients and do not depend on the rise onset time.

Significant dopamine transients while cocaine was available often exceeded the 30-s time window used on the PETHs. To visualize the full extent of the dopamine transients, we plotted a session-wide GrabDA$_{2m}$ recording from a representative cocaine FR1 session

with overlayed infusion timestamps (Figure S1). Figure S1 shows that cocaine-evoked GrabDA$_{2m}$ transients can last for several minutes before decaying to baseline. Inter-response intervals followed a similar temporal distribution, indicating that only a residual number of extra responses fell within the 30-s time window used for the PETH analyses. Delving further into this temporal coincidence, we modeled predicted quantities of cocaine brain concentrations (C_{brain}) using the equation $A(e^{-\alpha t} - e^{-\beta t})$, as characterized by Pan et al.,[27] where A = 4.82 (a multiplicative factor including the cocaine dose), $\alpha = 0.01$, $\beta = 0.0095$ and t being the time in minutes since the previous cocaine infusion. Except for the initial 5 min of the session, corresponding to the "loading phase" of consumption, cocaine-taking responses coincided with the decrease in cocaine content in the brain (C_{brain}). $\Delta F/F_0$ values were expressed as z-scores.

Statistical tests and GrabDA$_{2m}$-based predictive models

We analyzed the results of single factor, two-group, parametric variables (amplitude of evoked GrabDA$_{2m}$ z-scores and nosepoke comparisons between sexes) with unpaired Student's *t*-tests (two-tailed). Parametric measures resulting from the combination of two factors (PR z-scores by sex) were analyzed with two-way ANOVAs. When an experimental condition followed a within-subject design (e.g., trial, session) an ANOVA with repeated measures was calculated. In addition to *Z* score summary analyses, GrabDA$_{2m}$ fluorescence values were compared using waveform analyses, hence providing temporally-defined significance. Waveform analyses were based on the bootstrapping CI procedure developed by Jean-Richard-dit-Bressel et al.[23] Significant transients within the PETH were defined as periods (with a minimum length of 0.5-s) whose bootstrapped 95% CI did not contain 0 (baseline) or the other group's waveform. Bootstrapped CI were obtained by randomly re-shuffling (1000 boot-straps) trial z-scores. The bootstrap distribution was then expanded by a factor of $\sqrt{n/(n-1)}$ to adjust for narrowness bias.[23]

We then estimated Pearson's correlation coefficients between dopaminergic and behavioral variables, with an emphasis on reinstatement incidence, to uncover initial relationships. These variables were defined as averaged number of nosepokes or averaged cue-, reward- or nosepoke-evoked z-scores obtained during FR1, FR3, PR, extinction, and reinstatement recordings.

To generate a dopamine-based MLR model of reinstatement nosepokes, we followed a PCR strategy that allowed us to minimize the number of free parameters while pooling information from nine different dopaminergic variables. This was independently applied to both the cocaine- and food-seeking mice cohorts. In addition, PCR enabled an unbiased approach in which no prior hypothesis was needed to select dopaminergic variables to fit into the linear regression. Only animals tested throughout the acquisition, maintenance, PR, extinction, and reinstatement phases of the experiment were included on the analysis (n = 11; 6 females, 5 males). First, a low-dimensional representation of all the dopaminergic variables was obtained by PCA[74] after scaling the data to have a mean of 0 and s.d. of 1. The computed principal components compiled multivariate patterns of NAc dopamine responses to cocaine throughout the animal's entire history of drug exposure. The resulting loading scores are shown in Figure S2. Each variable's loading determined whether that variable increased or decreased a subject's DA_{PC} factor score. Of note, not all dopamine variables loaded in the same direction. This is important to discern how each variable related to reinstatement (predicted lower or higher levels of nosepoking). Second, we selected the first three principal components (referred to as DA_{PC1}, DA_{PC2}, and DA_{PC3}), which explained nearly 75% of the observed dopaminergic variance, to serve as covariates in an MLR model of reinstatement nosepokes. The same procedure was used to obtain a dopamine-based prediction of food-seeking reinstatement (n = 12; 6 females, 6 males). We replicated our main results with two other principal component selection methods[75] (Figure S5). The "Kaiser rule" method selected four principal components with an eigenvalue higher than 1. The "Elbow rule" method selected the first five principal components before an apparent dip in the explanatory power of the next factor. Given that the number of nosepokes during reinstatement is a count of events (with no possible negative or decimal values) and that its distribution violated the normality assumption (tested with three different statistics; Shapiro-Wilk, W = 0.248, p = 0.048; Anderson-Darling, A2* = 0.712, p = 0.044 and Kolmogorov-Smirnov, KS distance = 0.218, p = 0.046), we fit the MLR model using a Poisson's regression. The dopamine-based model (DA_{PC}) of reinstatement behavior became defined by $\ln(\hat{Y}) = \beta_0 + \beta_1(DA_{PC1}) + \beta_2(DA_{PC2}) + \beta 3_3(DA_{PC3})$. Similarly, we obtained the same Poisson's MLR model of reinstatement nosepokes using principal components derived from the behavioral variables shown in Figure 2B as covariates (BE_{PC}). To compare goodness-of-fit between models we used the small-sample corrected Akaike information criterion (AIC). AIC is an information theory-derived metric used to compare models. AIC values the resulting percentage of variance explained (R^2) but also the total amount of predictors used (degrees of freedom). A model that is predictive (high R^2) and parsimonious (few predictors) will have a lower AIC score and therefore ranked as the best model. The difference between AIC was calculated by $\Delta AIC = 2\ln(LR) + 2\Delta df$, where LR was the negative log likelihood ratio value of the model and *df* the number of free parameters. The probability of the alternative model (BE_{PC}) being more likely to be correct than the reference model (DA_{PC}) was determined by $p = 1 - (e^{0.5\Delta AIC}/1 + e^{0.5\Delta AIC})$. Nested models (e.g., $DA_{PC} \pm$ sex) were compared using both ΔAIC and likelihood ratio methods.

To replicate our MLR results, we performed an additional Bayesian Poisson inference procedure[76] to predict reinstatement nosepokes using DA_{PC1}, DA_{PC2} and DA_{PC3} as regression coefficients. First, we used Markov chain Monte Carlo (MCMC) method to generate 10000 samples (first 2500 iterations discarded as burn-in) that followed the posterior distributions of reinstatement nosepokes the observed data. Then, we ran a Metropolis-Hastings algorithm that iteratively sampled the β coefficients from the simulated samples until getting the β parameters that most closely regressed the observed data. The logarithm of the pseudo-marginal likelihood (LPML)[77] and R^2 between the observed and a randomly sampled distribution of reinstatement values were used as goodness-of-fit measures. Bayesian Poisson regression was performed under the "bpr" R package (Windows 11).

To characterize sex differences in the progression to extinguished cocaine-seeking behavior, Kaplan-Meyer survival curves were computed. Then, multivariate patterns of NAc dopamine release (DA_{PC1}, DA_{PC2} and DA_{PC3}) and sex were used as covariates to recover the observed Kaplan-Meyer survival curves. This was achieved using a semi-parametric Cox proportional hazards regression. A Cox regression model is the equivalent of an MLR that allows fitting of survival time curves. The values to fit are the hazard ratios of each animal on each day. These hazard ratios (h) estimate the probability of a given subject to experience a transition change (from "not extinguished" to "extinguished" cocaine seeking). Therefore, the Cox model is defined by $h(t) = h_0(t)\ \mathbf{exp}\ (x_{DA_{PC1}} \cdot \beta_{DA_{PC1}} + x_{DA_{PC2}} \cdot \beta_{DA_{PC2}} + x_{DA_{PC3}} \cdot \beta_{DA_{PC3}} + x_{sex} \cdot \beta_{sex})$, where $h(t)$ is the estimated hazard at time t, $h_0(t)$ is the baseline hazard when all the predictors are equal to zero, x_i are the values of the predictor variables, and β_i the parameter coefficients. Harrell's C statistic, equivalent to the area under the ROC curve for logistic regressions, was used to define the goodness-of-fit for the Cox model of extinction.[78]