Cocaine place conditioning strengthens location-specific hippocampal inputs to the nucleus accumbens

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Conditioned place preference (CPP) is a widely used model of addiction-related behavior whose underlying mechanism is not understood. In this study, we used dual site silicon probe recordings in freely moving mice to examine interactions between the hippocampus and nucleus accumbens in cocaine CPP. We found that CPP was associated with recruitment of nucleus accumbens medium spiny neurons (MSNs) to fire in the cocaine-paired location, and this recruitment was driven predominantly by selective strengthening of hippocampal inputs arising from place cells that encode the cocaine-paired location. These findings provide *in vivo* evidence that the synaptic potentiation in the accumbens caused by repeated cocaine administration preferentially affects inputs that were active at the time of drug exposure. This provides a plausible physiological mechanism by which drug use becomes associated with specific environmental contexts.

INTRODUCTION

Cocaine addiction is a debilitating condition for which no highly effective treatments exist, in large part because the underlying mechanisms are not well understood. One of the simplest animal models of a cocaine addiction-related behavior is conditioned place preference (CPP), in which cocaine is repeatedly paired with a specific spatial location causing the animal to spend more time in that location during subsequent exploration. The association of spatial location and drug reward in CPP is believed to be related to clinically relevant drug seeking and relapse triggered by exposure to environmental contexts previously associated with drug use. However, despite its simplicity and relevance, the underlying mechanistic basis of cocaine CPP is still not understood.

The nucleus accumbens (NAc) is a part of the ventral striatum believed to play a central role in reward- and addiction-related behaviors including cocaine CPP. Medium spiny neurons (MSNs) in the NAc are known to fire preferentially near reward sites in over-trained animals[1-4], providing a potential substrate for location-reward association. Focal lesions[5] or D1 antagonist injections[6] in the NAc block CPP acquisition, and focal amphetamine injections into the NAc are sufficient to induce CPP[7], suggesting that this location-dependent NAc activity may drive CPP behavior.

A large glutamatergic inputs to the NAc arises from the CA1 and subiculum regions of the hippocampus (HPC)[8], which contain "place cells" that fire selectively in specific spatial locations[9, 10] or contexts[11]. Simultaneous HPC-NAc recordings in rats suggest that hippocampal inputs carry spatial information to the NAc[4, 12-14], and lesion disconnection experiments indicate that hippocampus-accumbens interactions are necessary for CPP[15]. However, the mechanism by which these inputs could mediate reward location-dependent MSN activity is not known.

One possibility is based on the well-established finding that hippocampal place cells are disproportionately likely to fire near reward locations[16, 17]. In this model (**Fig. 1a**), NAc MSN firing at reward locations passively reflects changes in hippocampal firing patterns. Focal injection of amphetamine into the hippocampus is sufficient to induce place preference, supporting this possibility[18]. However, cocaine exposure also potentiates synaptic inputs to the NAc[19, 20], and NMDA receptor blockade prevents CPP acquisition[21], suggesting that synaptic plasticity in the NAc may be necessary for CPP. Repeated cocaine exposure has been shown to increase the *average* strength of hippocampal synapses onto D1-positive MSNs in the NAc[22, 23], but other studies have shown that potentiation of *individual* synapses on D1-positive cells requires presynaptic activity[24]. This led us to consider a second model (**Fig. 1a**), in which we hypothesize that cocaine conditioning preferentially potentiates synaptic inputs to the NAc that were the most active at the time of cocaine exposure. For hippocampal inputs, this would correspond to synapses arising from place cells that encode the cocaine-paired location.

To determine whether place cell density increase in the rewarded environment alone underlies location-specific MSN firing or whether selective synaptic plasticity is the main contributor, we performed simultaneous dual site silicon probe recordings in the HPC and NAc of freely moving mice in a cocaine CPP paradigm. Our findings indicate that cocaine conditioning recruits location-specific MSN firing in the NAc that is driven by hippocampal inputs. Although cocaine conditioning somewhat increased the density of hippocampal place fields in the cocaine zone, we found that hippocampus-accumbens coupling was also strengthened and that this effect was a larger contributor to location-specific MSN firing than hippocampal place cell enrichment.

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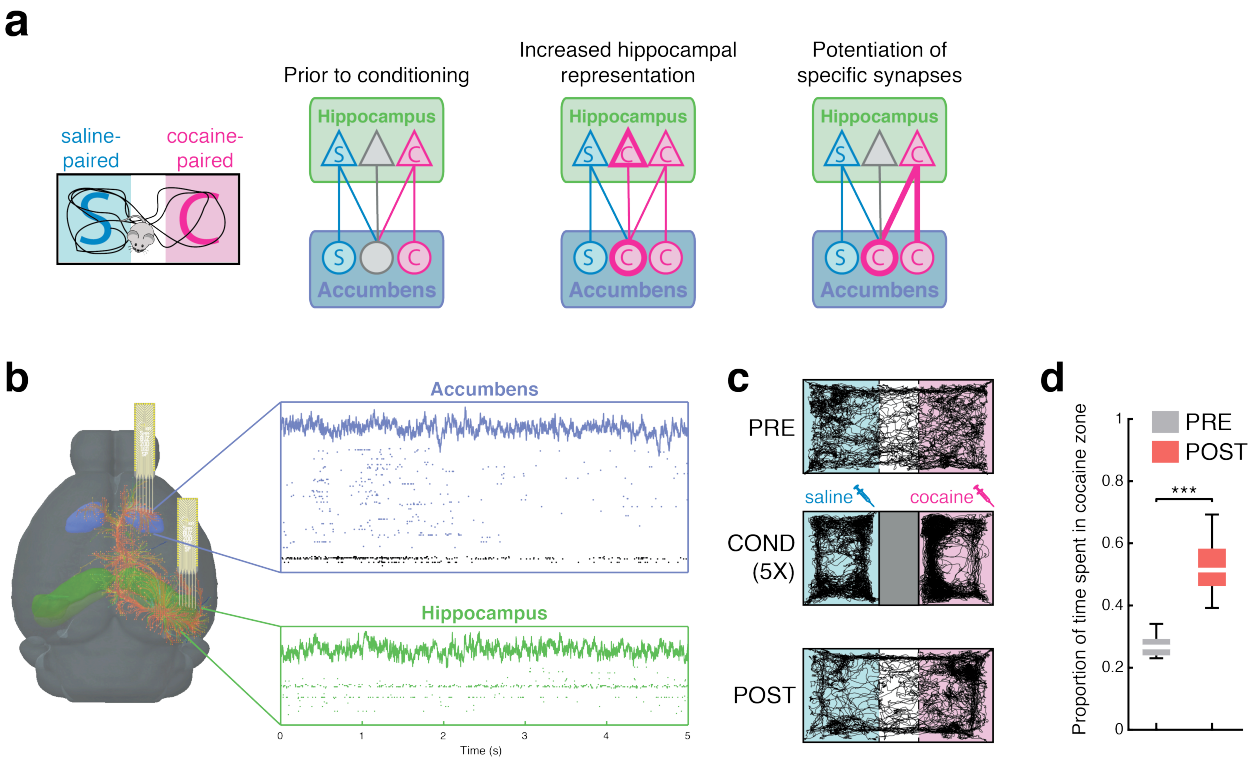

Figure 1: Cocaine conditioned place preference with dual-site silicon probe recording. a) Hypothesis 1: Increased density of place cells encoding the cocaine zone could support location-specific activity in the accumbens. Hypothesis 2: Preferential potentiation of synapses arising from cocaine zone-encoding place cells could support location-specific accumbens activity. **b)** Silicon probes were implanted in the hippocampus (green) and accumbens (blue), yielding LFP and single unit recordings. **c)** Mice (N = 6) were conditioned for five days with saline and cocaine 15 mg/kg IP. **d)** In POST sessions, animals exhibited a preference for the cocaine-paired zone (***$P < 10^{-5}$, Wilcoxon sign rank test).

RESULTS

Dual site silicon probe recording during cocaine conditioned place preference

To measure functional interactions between the hippocampus and NAc in cocaine CPP, we performed simultaneous silicon probe recordings in both structures during CPP behavior ($n = 6$ mice) (**Fig. 1b**). We used established waveform and bursting criteria to identify putative pyramidal cells (PYRs)[25, 26] in the hippocampus (n = 561 PYRs) and medium spiny neurons (MSNs) and interneurons (INs) in the NAc (n = 1293 MSNs, 313 INs; **Supplementary Fig. 1**)[27, 28]. Recordings were performed before (PRE) and after (POST) animals underwent cocaine place conditioning in a rectangular arena with a removable barrier (**Fig. 1c, Supplemental Fig. 2**). We recorded 1-3 daily 30-minute PRE sessions prior to five consecutive days of cocaine conditioning, then 1-5 daily 30-minute POST sessions. This conditioning paradigm induced robust CPP in POST sessions (PRE = 27% in cocaine zone, POST = 51%, Wilcoxon rank sum test, n = 12 PRE, 15 POST sessions, $P = 1.6 \times 10^{-6}$; **Fig. 1d**), which was attributable to an increase in the proportion of time spent immobile in the cocaine zone (**Supplementary Fig. 3**).

Cocaine conditioning selectively increases MSN firing in the cocaine zone

We found that after cocaine conditioning, NAc MSNs fire at higher rates when the animal is in the cocaine zone than the saline zone (Cocaine index = -0.009 ± 0.011 PRE, 0.094 ± 0.013 POST, n = 545, 748 MSNs, unpaired t-test, $P = 4.6 \times 10^{-9}$; **Fig. 2a**). This effect was not seen in NAc INs (n = 162, 151 INs, unpaired t-test, P = 0.88, **Fig. 2b**), and for hippocampal PYRs a trend was noted that failed to reach statistical significance (n = 184, 377 PYRs, P = 0.11, **Fig. 2c**). However, the density of PYR place fields was higher in the cocaine zone in POST sessions ($P = 2.6 \times 10^{-8}$, **Supplementary Fig. 4**). Further, we found that the strength of behavioral CPP expression in a given POST session is correlated with the extent of increased MSN firing in the cocaine zone during that session (n = 15 POST sessions, R = 0.62, P = 0.01, **Fig. 2d**). This correlation occurs only in POST sessions and was not observed in NAc INs (R = 0.3, P = 0.27; **Fig. 2e**) or hippocampal PYRs (R = 0.1, P = 0.72; **Fig. 2f**). These observations support our hypothesis that cocaine CPP involves similar reward location-dependent MSN activity to that seen with non-drug rewards and prompted us to explore whether hippocampal inputs provide the location information utilized by MSNs.

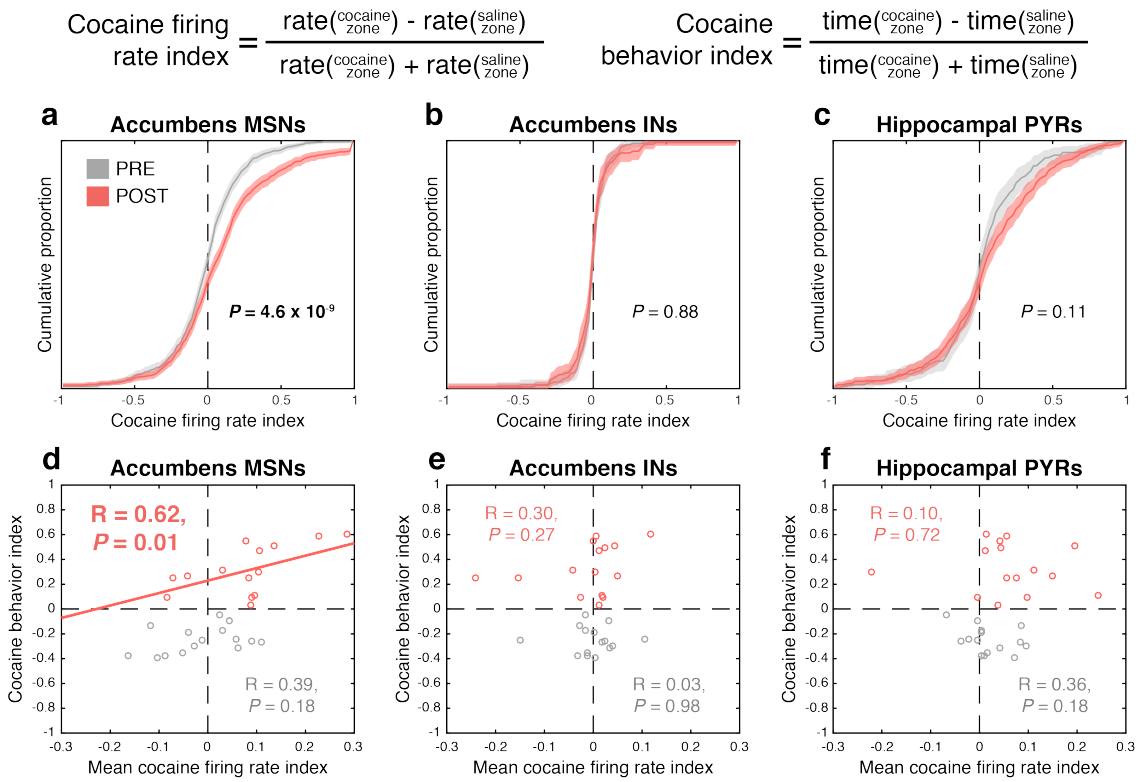

Figure 2: Cocaine conditioning selectively increases MSN activity in the cocaine zone. a) Accumbens MSNs fire preferentially in the cocaine zone after cocaine conditioning (P = 4.6 x 10^{-9}). Accumbens putative interneurons (INs, **b**) and Hippocampal PYRs (**c**) exhibit no shift after cocaine conditioning. **d)** Strength of behavioral CPP expression in each recording session is correlated with firing rate index in MSNs in POST (P = 0.01) but not PRE (P = 0.18) conditions. **e)** Behavioral CPP expression is not correlated with firing rate index in INs (P = 0.88 PRE, 0.27 POST). **f)** Behavioral CPP expression is not correlated with firing rate index in hippocampal PYRs (P = 0.18 PRE, 0.72 POST).

MSNs exhibit signatures of decoding spatial location and running speed from hippocampal inputs

Although our CPP protocol was designed to ensure that CPP was hippocampus-dependent[15, 29] (**Supplementary Fig. 2**), we first focused on analysis of spatial firing patterns of MSNs to verify that location-dependent MSN firing was driven by hippocampal inputs. Our initial analysis found that MSNs exhibit spatially biased firing patterns and also exhibit modulation by running speed (not shown). This complicates the analysis of MSN firing because CPP behavior fundamentally entails a strong correlation between movement and spatial location (**Supplemental Fig. 3**). We attempted to disentangle this issue using generalized linear models (GLMs) and model cross-validation, finding that the best model fit included separate terms for location and speed modulation (**Supplemental Fig. 5a-b**). The GLM method enabled us to analyze these two phenomena separately and determine that MSNs exhibit running speed modulation that is independent of location and stronger than HPC PYRs (n = 1293 MSNs, 561 PYRs, Wilcoxon rank sum test, P = 3.9 x 10^{-18}), which in turn are more strongly modulated than NAc INs (**Fig. 3b**, n = 561 PYRs, 313 INs, Wilcoxon rank sum test, P = 3.0 x 10^{-5}). After correcting for running speed, MSNs encoded approximately as much spatial information[30] as hippocampal PYRs (**Fig. 3c**, n = 1293 MSNs, 561 PYRs, Wilcoxon rank sum test, P = 0.20) and significantly more than INs (n = 1293 MSNs, 313 INs, P = 1.1 x 10^{-38}). Since the same MSNs tend to exhibit high spatial information and running speed modulation (**Fig. 3d**), we hypothesized that they may receive both spatial and speed information via inputs from the hippocampus. To test this, we used GLMs to predict MSN spike trains from combinations of hippocampal activity, location, and running speed (**Fig. 3e**). Using cross-validation, we found that adding hippocampal activity to a model containing no information other than baseline firing rate led to improvements in prediction quality. However, the improvement in prediction quality was smaller if we added hippocampal activity to a model that already contained information about location (**Fig. 3f**, n = 1203 MSNs, Wilcoxon signed rank test, P = 4.1 x 10^{-11}) or running speed (**Fig. 3g**, n = 1203 MSNs, Wilcoxon signed rank test, P = 6.3 x 10^{-23}). This is fully consistent with NAc MSNs decoding information about running speed and spatial location from hippocampal inputs. The prediction quality was highest when HPC led NAc by a time lag of ~30 ms (**Fig. 3h**), suggesting that this effect represented information transfer from HPC to NAc rather than both structures receiving this information from a common input.

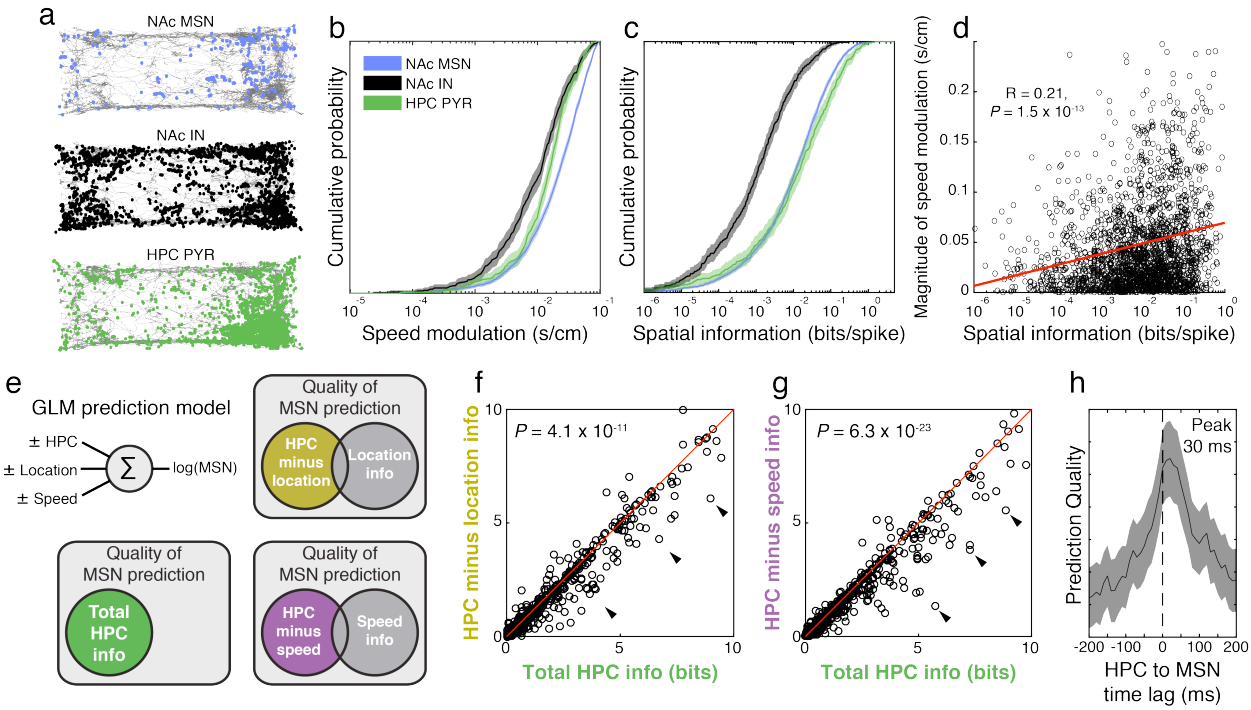

Figure 3: Accumbens MSNs decode spatial location and running speed from hippocampal inputs. a) Examples session illustrating spiking activity (dots) of accumbens MSNs, INs and hippocampal pyramidal cells (HPC PYRs) in the testing apparatus. The gray line indicates the movement trajectory of the mouse. **b)** After correction for spatial location, MSNs show greater modulation by running speed than PYRs (n = 1293 MSNs, 561 MSNs; P = 3.9 x 10^{-18} for MSN vs. PYR), and PYRs show greater modulation than INs (n = 561 MSNs, 313 INs, P = 3.0 x 10^{-5} for PYR vs. IN). **c)** After correction for running speed, MSNs and PYR activity carries similar amounts of spatial information (P = 0.20 for MSN vs. PYR), but MSN activity carries more spatial information than IN activity (P = 1.0 x 10^{-38}). **d)** MSNs with stronger running speed modulation also encode more spatial information (R = 0.21, P = 1.5 x 10^{-13}). **e)** GLMs enable prediction of individual MSN spike trains from combinations of PYR activity, location, and running speed as predictors. **f)** Adding hippocampal activity as a predictor to a model already containing explicit location information results in a smaller improvement in prediction quality (P = 1.2 x 10^{-9}) than for a model without explicit location information. This indicates that predicting MSN spike trains from PYR inputs implicitly decodes information about spatial location. **g)** Analogously, adding hippocampal activity as a predictor to a model containing explicit running speed information results in a smaller improvement in prediction quality (P = 3.8 x 10^{-21}) than for a model with no explicit speed information, indicating that predicting MSN spike trains from PYR inputs implicitly decodes information about running speed. **h)** MSN spike train prediction quality is highest when PYR activity leads MSN activity by ~30 ms, supporting the hypothesis that information is transferred from PYRs to MSNs.

Assembly prediction analysis reveals increased hippocampus-accumbens coupling after cocaine conditioning

We next addressed the question of whether hippocampal inputs to the accumbens are strengthened by cocaine conditioning. To test this, we performed an assembly prediction analysis using PYR assemblies as predictors in a linear model that predicts the activity of a single MSN assembly (**Fig. 4a, Supplemental Methods**). Consistent with our hypothesis that MSNs decode spatial information from hippocampal inputs, we found that the quality of the assembly prediction was correlated with the rate of spatial information encoded by the MSN assembly in POST, but not PRE, sessions (**Fig. 4b**). The spatial information rate was also higher in POST sessions (**Fig. 4c**, n = 34, 95 assemblies, Wilcoxon rank sum test, $P < 0.05$), suggesting that cocaine conditioning strengthens hippocampus-accumbens coupling.

To strengthen our finding that our assembly predictions were not due to hippocampus and accumbens being driven by common inputs or sensory cues in the CPP arena (**Fig. 3h**), we performed an analysis of sleep replay events. Replay events, which occur during hippocampal sharp-wave ripple oscillations, consist of temporally compressed firing of place cells sequences that encode recently visited spatial locations[31-33] and are generated locally in the hippocampus[34]. We used data from awake exploration of the CPP arena to fit the prediction weights of the GLM, then tested model predictions both on awake data withheld from the training set and on data collected during sleep ripples occurring before and after the animal explored the CPP arena. We found that during pre-exploration sleep, when replay events encoding locations in the CPP arena do not occur, the sleep ripple prediction quality was uncorrelated with wake prediction quality (**Fig. 4d**). In contrast, during post-exploration sleep the sleep ripple prediction quality was significantly correlated with wake prediction quality in POST sessions (**Fig. 4e**), indicating strengthened hippocampus-NAc coupling.

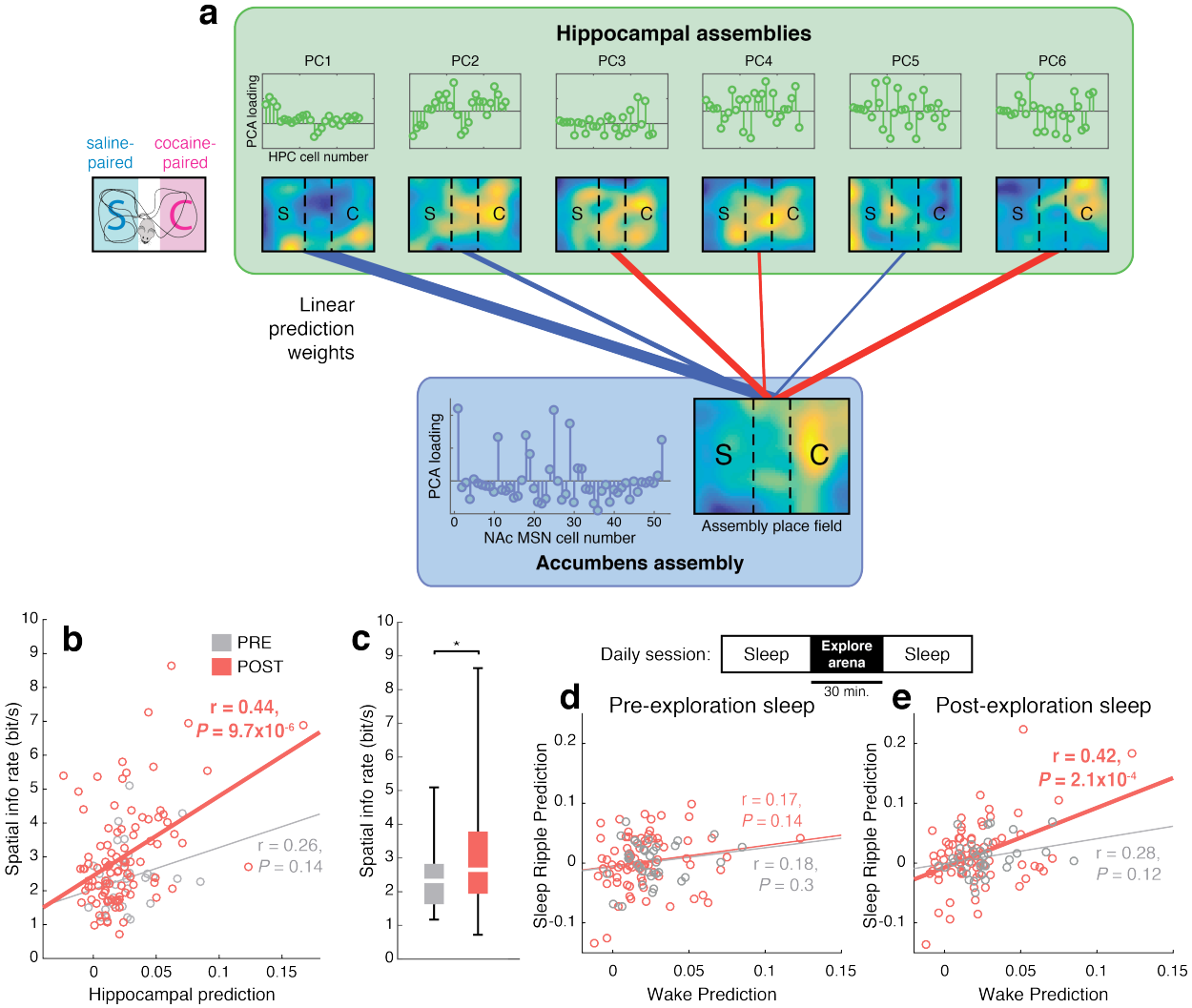

Figure 4: Cocaine conditioning increases the strength of functional coupling between hippocampal PYRs and accumbens MSNs. a) Activity of PYR assemblies predicts activity of MSN assemblies. **b)** The spatial information rate of an MSN assembly is correlated with the accuracy by which its activity can be predicted from PYR assemblies, in POST (R = 0.44, P = 9.7 x 10^{-6}), but not PRE (R = 0.26, P = 0.14) sessions. **c)** Spatial information rate of MSN assemblies increases after cocaine conditioning (*P < 0.05). **d)** Model predictions for MSN assemblies during awake locomotion are uncorrelated with model predictions during sharp wave ripples in pre-exploration sleep. **e)** Model predictions during awake locomotion are correlated with predictions during sharp wave ripples in post-exploration sleep in POST (P = 2.1 x 10^{-4}), but not PRE (P = 0.12), sessions. This suggests that cocaine conditioning strengthens coordinated hippocampus-accumbens replay.

Hippocampally-modulated MSNs are preferentially recruited to fire in the cocaine zone

We next turned to the question of whether strengthened hippocampal inputs mediate the recruitment of additional MSN activity in the cocaine zone. To this end, we used simultaneous LFP recordings in the hippocampus to determine how strongly each MSN is phase-locked to the hippocampal theta oscillation. Several prior studies indicate that phase-locking with hippocampal theta is a marker for MSNs receiving strong hippocampal inputs[12-14, 35]. We found that MSNs with high theta modulation encode more spatial information than MSNs with low theta modulation (**Supplemental Fig. 6a**) and are overrepresented among cells with either high and low cocaine indices (**Fig. 5a**, **Supplemental Fig. 6b**). Dividing the MSNs into high-theta and low-theta halves, we found that the high-theta half shifts after conditioning toward encoding the cocaine zone (PRE 0.0082 ± 0.013, POST 0.087 ± 0.017, n = 261, 351 MSNs; Unpaired t-test, P = 2.7 x 10^{-4}, **Fig. 5b**), while the low-theta half does not (PRE 0.0027 ± 0.016, POST 0.014 ± 0.016, n = 261, 351 MSNs; Unpaired t-test, P = 0.43, **Fig. 5c**). This suggests that strengthening of hippocampal inputs underlies recruitment of MSN firing to the cocaine zone.

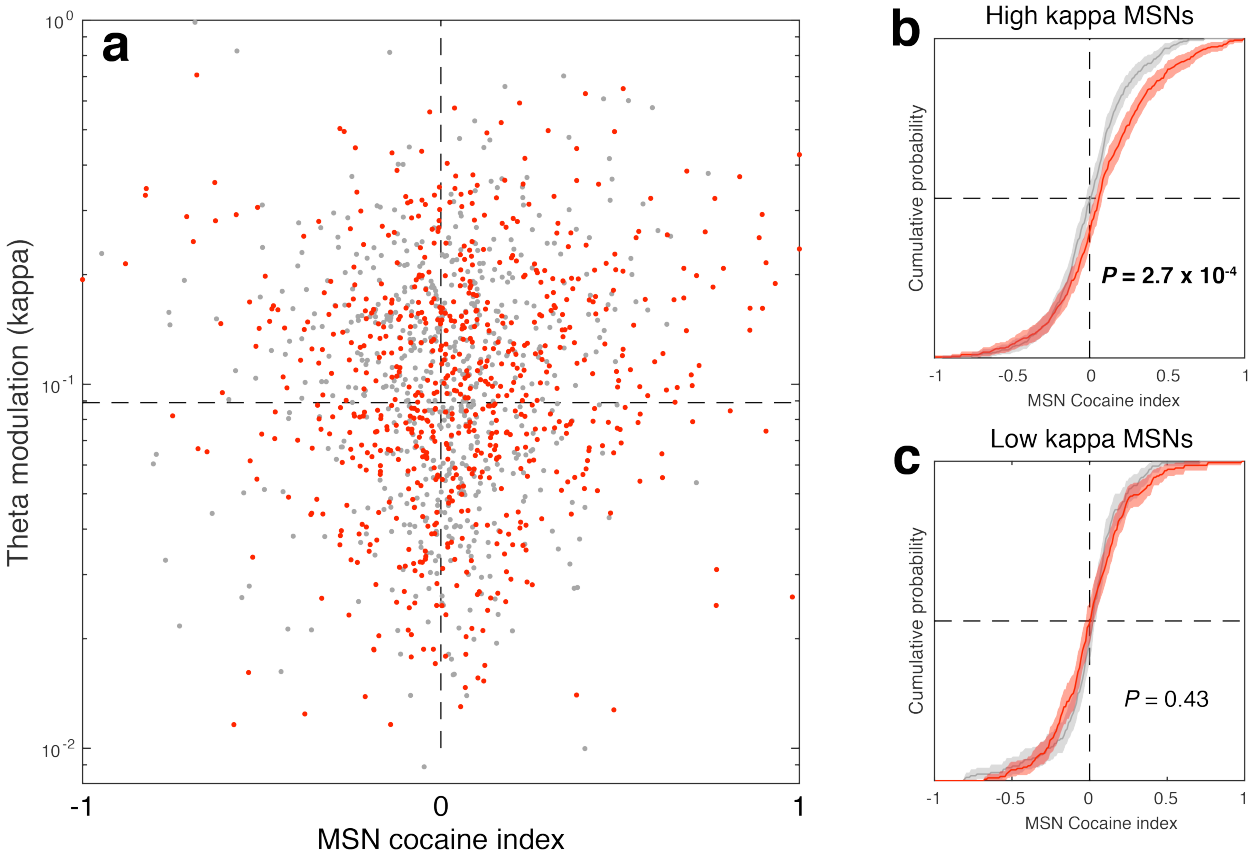

Figure 5: MSNs phase-locked to the hippocampal theta (~8 Hz) oscillation show greater recruitment by cocaine conditioning. a) MSN cocaine firing rate index varies as a function of phase-locking to hippocampal theta. Note highest density of red dots (MSN POST) in the upper right quadrant. **b)** MSNs that are strongly phase-locked to hippocampal theta show significant increases in activity in the cocaine zone after conditioning (P = 2.7 x 10^{-4}). **c)** MSNs that are weakly phase-locked to hippocampal theta do not show significant changes in cocaine zone activity after conditioning (P = 0.43).

Selective strengthening of hippocampal inputs is a larger contributor of MSN recruitment to the cocaine zone than increased density of place fields

Increased hippocampally-driven MSN activity in the cocaine zone could be due either to 1) increased hippocampal place field density in the cocaine zone or 2) selective strengthening of synapses arising from PYRs encoding the cocaine zone (**Fig. 1a**). Our results indicate that the MSNs shift their spatial tuning more than the PYRs (**Fig. 2**) and that hippocampus-accumbens coupling is strengthened (**Fig. 4**), both of which support the second model where changes in synaptic strength are responsible. However, PYRs show a nonsignificant trend toward additional firing in the cocaine zone (**Fig. 2c**) and exhibit a higher density of place fields in the cocaine zone after conditioning (**Supplemental Fig. 4**), which prompted us to address this question more rigorously. To this end, we fit a GLM to find connection weights that optimally predict MSN activity based solely on hippocampal activity (**Fig. 6a**). We then used this model with cross validation to generate predictions of each MSN's spike train, from which we calculated a cocaine index. Although the model contains no explicit location information, the cocaine indices predicted from hippocampal activity alone were significantly correlated with the observed cocaine indices (n = 531, 713 MSNs; PRE R = 0.45, P = 1.2 x 10^{-7}; POST R = 0.59, P = 6.8 x 10^{-68}; **Fig. 6b**), indicating that hippocampal activity is sufficient to predict MSN cocaine index.

To determine whether changes in PYR place fields were responsible for recruitment of MSN activity, we examined the PYR cocaine indices, which summarize the aspects of PYR spatial tuning that contribute to the MSN cocaine index. We compared the distribution of PYR cocaine indices with positive values to those with negative values, which represent PYRs encoding the cocaine and saline zones, respectively. There was no difference in PRE sessions (**Fig. 6c**) but in POST sessions the magnitude of the indices was larger for cocaine zone PYRs (n = 164, 205 PYRs, median 0.15 vs. 0.24; Wilcoxon rank sum test, P = 2.6 x 10^{-4}, **Fig. 6d**), confirming that place cells over-represent the cocaine zone. To determine whether this over-representation plays a direct role in the recruitment of additional MSN activity, we adjusted the spike trains of PYRs encoding the cocaine zone so that the distribution of cocaine zone PYR index magnitudes matched those of saline zone PYRs. By comparing predicted MSN cocaine indices before and after the adjustment, we were able to quantify the contribution of PYR place field over-representation to MSN location tuning. In PRE sessions, this adjustment caused no change in predicted MSN cocaine

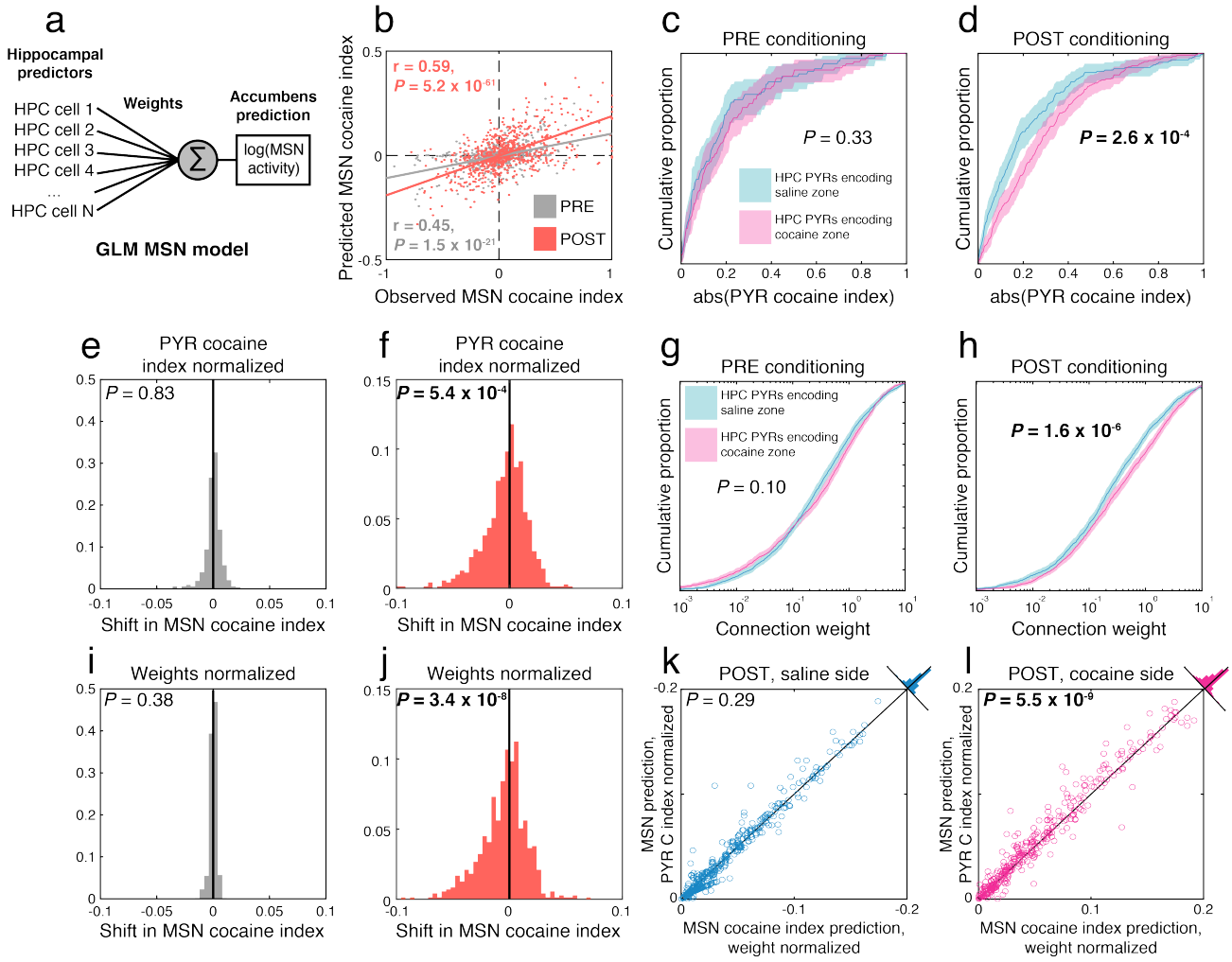

Figure 6: Changes in PYR place field density contribute significantly to MSN recruitment, but selective strengthening of hippocampal inputs is a larger contributor. a) A GLM that predicts MSN spiking activity from PYR spiking activity can estimate the contribution of changes in place field location or connection weights. **b)** Model-predicted MSN cocaine indices are significantly correlated with observed MSN cocaine indices (R = 0.45, P = 1.2 x 10^{-7} PRE; R = 0.59, P = 6.8 x 10^{-68} POST). **c)** The distribution of PYR cocaine index magnitudes for PYRs encoding the saline and cocaine zones is equal prior to conditioning (P = 0.33). **d)** After cocaine conditioning, PYRs encoding the cocaine zone have larger cocaine indices than PYRs encoding the saline zone (P = 2.6 x 10^{-4}). **e)** In PRE sessions, adjusting the cocaine indices of cocaine zone-encoding PYRs to match the distribution of saline zone-encoding PYRs does not change the predicted MSN cocaine indices (P = 0.83). **f)** In POST sessions, adjusting the cocaine indices of cocaine zone-encoding PYRs to match the distribution of saline zone-encoding PYRs causes a significant shift of predicted MSN cocaine indices away from the cocaine zone (P = 5.4 x 10^{-4}). This suggests that changes in the distribution of PYR cocaine indices induced by cocaine conditioning contribute to MSN recruitment. **g)** In PRE sessions, connection weights from cocaine zone-encoding PYRs and saline zone-encoding PYRs have equal distributions (P = 0.10). **h)** In POST sessions, connections from cocaine zone-encoding PYRs have stronger weights than connections from saline zone-encoding PYRs (P = 1.4 x 10^{-6}). **i)** In PRE sessions, adjusting connection weights from cocaine zone-encoding PYRs to match the distribution of weights from saline zone-encoding does not change predicted MSN cocaine indices (P = 0.39). **j)** In POST sessions, adjusting connection weights from cocaine zone-encoding PYRs significantly shifts predicted MSN cocaine indices away from the cocaine zone (P = 3.4 x 10^{-8}). **k)** For saline zone-encoding MSNs, adjusting PYR place field location or connection weights has equal effects on predicted MSN cocaine index (P = 0.29). **l)** For cocaine zone-encoding MSNs, adjusting connection weights has a larger effect on predicted MSN cocaine index than adjusting PYR place field location (P = 5.5 x 10^{-9}). This indicates that cocaine-induced changes in connection weights are a larger contributor to MSN recruitment to the cocaine zone.

indices (n = 531 MSNs, Wilcoxon signed rank test, P = 0.83, **Fig. 6e**), but in POST sessions the MSN indices showed a significant shift away from the cocaine zone (n = 713 MSNs, Wilcoxon signed rank test, $P = 5.4 \times 10^{-4}$, **Fig. 6f**).

We next performed the same analysis with PYR to MSN connection weights, comparing the weights arising from hippocampal PYRs that encode the saline vs. cocaine zones. In PRE sessions there was no difference (n = 2011, 2036 weights, Wilcoxon rank sum test, P = 0.10; **Fig. 6g**), but in POST sessions connection weights arising from cocaine zone PYRs were significantly larger (n = 2014, 1981 weights, Wilcoxon rank sum test, $P = 1.4 \times 10^{-6}$; **Fig. 6h**). To test whether this asymmetry in connection weights was responsible for the recruitment of MSN activity to the cocaine zone, we adjusted connection weights so that the distributions would be equal. In PRE sessions, this adjustment caused no change in MSN cocaine index (n = 531 MSNs, Wilcoxon signed rank test, P = 0.39, **Fig. 6i**), but in POST sessions the MSN indices showed a significant shift away from the cocaine zone (n = 713 MSNs, Wilcoxon signed rank test, $P = 3.4 \times 10^{-8}$, **Fig. 6j**).

Since both place field changes and synaptic weight changes contributed significantly to increased MSN activity in the cocaine zone, we compared the changes directly to determine which was a larger contributor. In POST sessions, the two effects contributed equally to the location tuning properties of MSNs encoding the saline zone (n = 355 MSNs, Wilcoxon signed rank test, P = 0.29, **Fig. 6k**). However, for MSNs encoding the cocaine zone, changes in connections weights were a larger contributor than changes in PYR place fields (n = 340 MSNs, Wilcoxon signed rank test, $P = 5.5 \times 10^{-9}$, **Fig. 6l**).

DISCUSSION

In this study, we found that cocaine place conditioning recruits NAc MSNs, and to a lesser extent hippocampal PYRs, to fire in the cocaine-paired zone. We also found that MSNs receive information about spatial location from PYRs, cocaine conditioning increases hippocampus-accumbens coupling, and MSNs modulated by the hippocampal theta rhythm show the strongest recruitment to the cocaine zone, all of which suggest that this effect is driven by hippocampal inputs to the NAc. Finally, we found that MSN recruitment is driven predominantly by preferential strengthening of hippocampal inputs encoding the cocaine zone.

Although extracellular recording is not able to make direct measurements of synaptic strength, repeated cocaine exposure has been shown to potentiate hippocampal inputs to NAc MSNs *in vitro*[22, 23], and dopamine-dependent synaptic potentiation in MSNs requires presynaptic activity[24]. This suggests that hippocampal synapses onto MSNs that arise from place cells encoding the cocaine zone would be preferentially potentiated, providing the most likely cellular substrate for the selective increase in hippocampus-NAc coupling that we observe. This also provides a possible explanation reconciling the conflicting results of Britt et al.[23], who found that cocaine selectively strengthened hippocampal inputs to the NAc, and MacAskill et al.[36], who found that cocaine selectively strengthened amygdalar inputs. Our results suggest instead that cocaine may preferentially strengthen the most active inputs, which could be predominantly hippocampal or amygdalar depending on the environmental conditions during cocaine exposure. It is worth emphasizing that our place conditioning paradigm (**Supplemental Fig. 2**) pairs cocaine with a spatial location defined relative to distal navigational cues, while proximal sensory cues were minimized and counterbalanced across cocaine/saline conditions. Under similar conditions, CPP has been shown to be dependent on dorsal hippocampus and not ventral hippocampus or amygdala[29, 37], but in CPP paradigms incorporating proximal sensory cues that differ between the cocaine and saline zones, circuits beyond dorsal HPC are likely recruited as well.

Our results appear to contradict those of German et al. [38], who recorded neurons in the NAc during CPP POST sessions in rats and found that NAc MSNs exhibit decreased firing rates in the drug-paired zone. A possible explanation for this difference is that their study used morphine, and ours used cocaine. An interesting paradoxical observation is that both morphine and cocaine produce CPP, self-administration, and other addiction-related behaviors, but cocaine exposure increases the density of dendritic spines in the NAc and morphine decreases it[39, 40]. This paradox was addressed recently by Graziane et al.[41], who found that cocaine exposure forms new spines selectively on D1-positive MSNs, while morphine exposure prunes spines selectively on D2-positive MSNs. Given the previous observation that cocaine selectively potentiates hippocampal inputs onto D1-positive MSNs in the NAc[22], it is tempting to speculate that the increased firing we observed in the cocaine-paired zone is attributable to D1-positive MSNs, while the decreased firing German et al. observed in the morphine-paired zone is attributable to D2-positive MSNs. A recent study by Calipari et al.[42] using fiber photometry provides some support for this hypothesis, but resolution of this issue will ultimately require future studies combining dual site unit recordings in the hippocampus and NAc with optogenetic tagging[43] of D1- or D2-positive MSNs.

Selective plasticity supports a plausible mechanistic model of cocaine CPP in which the NAc acts as an action-location-outcome associator[44, 45]. We hypothesize that the NAc integrates hippocampal inputs encoding a spatial location and prefrontal cortical inputs encoding an action plan to generate actions appropriate for a given spatial context. Under physiological conditions, performing an action in a specific location that generates a positive reward prediction error causes dopamine release in the NAc[46]. This would strengthen hippocampal synapses encoding the rewarded location, increasing the expected reward outcome associated with that action/location pairing. During cocaine place conditioning, NAc dopamine levels are artificially elevated, effectively rewarding all actions performed in the cocaine zone and increasing the strength of hippocampal inputs encoding that location. Upon exposure to the cocaine zone in POST sessions, the strengthened hippocampal inputs would drive increased firing in NAc MSNs, leading the animal to engage in activity in the cocaine zone rather than run to the saline zone. Determining whether this model is correct will require further studies involving multisite unit recording and manipulation of prefrontal cortex in CPP.

It is believed that drugs of abuse exert differential effects at various synaptic pathways in the brain – our results suggest that synaptic changes can be specific even at the level of functionally defined subsets of synapses within a single synaptic pathway. This reveals an additional layer of complexity that is not recognized in many circuit models of drug addiction and suggests that some of these models may require revision. Selective plasticity of specific corticostriatal synapses based on presynaptic firing properties has been

observed in other striatal regions[47] and may represent a canonical mechanism by which sensory and contextual information can bias action selection based on reward history.

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AUTHOR CONTRIBUTIONS

L.S. and G.B. conceived and designed the study. L.S., A.C., and D.C. performed experiments, and L.S., A.P., and A.C. analyzed the data. L.S. and G.B. wrote the paper.

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