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CHAPTER

Corticostriatal plasticity, neuronal ensembles, and regulation of drug-seeking behavior

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Abstract

The idea that interconnected neuronal ensembles code for specific behaviors has been around for decades; however, recent technical improvements allow studying these networks and their causal role in initiating and maintaining behavior. In particular, the role of ensembles in drug-seeking behaviors in the context of addiction is being actively investigated. Concurrent with breakthroughs in quantifying ensembles, research has identified a role for synaptic glutamate spillover during relapse. In particular, the transient relapse-associated changes in glutamatergic synapses on accumbens neurons, as well as in adjacent astroglia and extracellular matrix, are key elements of the synaptic plasticity encoded by drug use and the metaplasticity induced by drug-associated cues that precipitate drug-seeking behaviors. Here, we briefly review the recent discoveries related to ensembles in the addiction field and then endeavor to link these discoveries with drug-induced striatal plasticity and cue-induced metaplasticity toward deeper neurobiological understandings of drug seeking.

Keywords

Neuronal ensembles, Cocaine self-administration, Cued reinstatement, Nucleus accumbens, Glutamate, Synaptic plasticity, Synaptic potentiation, Spines

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1 INTRODUCTION: ENSEMBLES IN ADDICTION

According to classic theory, neuronal networks adapt during brain plasticity, modifying firing probability within the network (Hebb, 1949; Josselyn et al., 2017). As a result, all of the neurons included in a specific network will respond to the same stimulus. One of the first proofs of this theory was found in brain slices from the developing rat neocortex where measures of calcium signaling revealed functional domains formed by neurons activated in synchrony (Yuste et al., 1992, 1995). Concurrently, ensemble coding for location was measured in the hippocampus of rats in vivo (Wilson and McNaughton, 1993). A distributed coding model was first applied to the nucleus accumbens (NAc) by Pennartz et al. (1994), who implemented the theory to explain how a cue is associated with drug delivery. In this case, the cue induces activation of an interconnected network between the cortex, amygdala, and thalamus that converge to activate a neuronal ensemble in the NAc to induce long-term potentiation (LTP). More than 10 years later, Hope and colleagues established a causal link between an ensemble of neurons selectively activated by drugs, drug-associated cues and context, and the expression of cocaine-induced behavioral sensitization (Koya et al., 2009). The researchers made use of the specific pattern of neuronal activation of the immediate early gene c-fos during expression of context-specific sensitization to cocaine (Mattson et al., 2008) and induced expression of a β-galactosidase reporter only in that c-Fos-positive ensemble, which represents a surprisingly low 2%–3% of all NAc neurons. Subsequently, these investigators utilized the prodrug Daun02, which is converted to the Ca^{2+}-reducing agent daunorubicin only in the neurons that express β–galactosidase. Critically, using this method of selectively lesioning the ensemble coding the context–drug association, context-specific locomotor sensitization was abolished (Koya et al., 2009). Moreover, animals expressing sensitization to cocaine formed silent synapses specifically in the neurons comprising the ensemble activated by cocaine (Koya et al., 2012). In a follow-up experiment, only animals receiving cocaine in a context-dependent manner displayed sensitization to the context and had ensembles containing silent synapses, thus demonstrating that the ensemble selectively encodes the drug–context association (Whitaker et al., 2016).

This same approach has also been used in an increasing number of self-administration models to show the role of specific ensembles in operant responding for drugs. In addition to cocaine sensitization, context-induced reinstatement of cocaine seeking is also driven by a selective NAc ensemble that consists largely of medium spiny neurons and parvalbumin-positive interneurons (Cruz et al., 2014). Furthermore, ensembles in the ventral medial prefrontal cortex (mPFC) and orbitofrontal cortex were shown to encode the context-induced operant responding for heroin during relapse and after extended abstinence, respectively (Bossert et al., 2011; Fanous et al., 2012). Additionally, ensembles in the dorsal striatum were linked to voluntary abstinence from methamphetamine taking (Caprioli et al., 2017), ensembles in the central amygdala associated to craving alcohol and nicotine during

abstinence (de Guglielmo et al., 2016; Funk et al., 2016), and ventral mPFC ensembles were found to suppress ethanol seeking and drive or inhibit seeking of natural rewards depending on environmental contingencies (Pfarr et al., 2015; Suto et al., 2016; Warren et al., 2016). Importantly, all these studies report a very small number of activated neurons in a given brain region (e.g., <5%), indicating that highly specific addictive behaviors are regulated by small ensembles of neurons throughout the brain.

An interesting question is whether neuronal networks activated by cocaine are specific for drug-related behavior or whether they overlap with other engrams, such as those inducing behaviors driven by natural rewards. By studying the pattern of phasic neuronal firing in MSNs in the NAc, it was found that the selective encoding of natural rewards (food, water, or sucrose) involves largely distinct cells from those encoding cocaine, with only ~20% of neurons responding to both cocaine and sucrose or water reinforcements (Cameron and Carelli, 2012; Carelli et al., 2000). Remarkably, convergent findings using different biomarkers reveal that ~2%–5% of cells encode a putative cocaine engram, including deltaFosB immune labeling (Mattson et al., 2008), Daun02 inactivation of c-Fos-expressing neurons (Koya et al., 2009), and the number of NAc MSNs exhibiting phasic activity NAc MSNs (Cameron and Carelli, 2012). Furthermore, the same relative proportion of NAc cells in the cocaine engram is reported between passive drug–context associations during sensitization and active cocaine self-administration and reinstatement (Cameron and Carelli, 2012; Cruz et al., 2013; Koya et al., 2009).

2 CONSTITUTIVE CHANGES INDUCED BY DRUGS OF ABUSE

Abused drugs share the property of modifying the extracellular levels of three behaviorally important monoamines: noradrenalin, serotonin, and dopamine. This modulation is achieved either by blocking neurotransmitter plasmalemmal transporters (e.g., psychostimulants (Balster and Schuster, 1973, Crespi et al., 1997) or via disinhibition of synaptic release (e.g., opioids: Khachaturian and Watson, 1982). However, the release of dopamine in the NAc by all drugs of abuse (Di Chiara and Imperato, 1988) and the role of dopamine in reward prediction (Schultz, 1998) have placed dopamine as the most studied monoamine neuromodulator in the addiction field. Thus, dopamine release in the NAc and limbic cortical and allocortical regions is a necessary event for drug-mediated reward and has therefore been proposed to be required to establish learned associations between the rewarding effects of addictive drugs and the environment (Pascoli et al., 2015). Acute dopamine release during drug use reinforces learned associations between the environment and the drug to the extent that the environmental associations become provocateurs for initiating drug seeking and relapse. While acute dopamine release by drug-associated cues and context can contribute to initiating drug seeking (McFarland and Kalivas, 2001; McGlinchey et al., 2016; Phillips et al., 2003; See et al., 2001), particularly in limbic

cortical and allocortical regions, the constitutive synaptic plasticity and transient metaplasticity that create the high level of motivation to obtain drugs relative to other natural rewards have been demonstrated most convincingly at glutamatergic synapses in the NAc (Kalivas, 2009).

Morphological (Anderson and Self, 2017) and functional (Kourrich et al., 2015; Luscher, 2013; Luscher and Malenka, 2011) changes induced by chronic exposure to drugs of abuse have been extensively studied (Mulholland et al., 2016), and in Table 1 we have assembled the major constitutive changes induced in the NAc by cocaine and heroin, two drugs actively being investigated at the engram level. Remarkably, different drug types induce distinct constitutive modifications, such as opposing changes in spine head diameter and AMPA currents. However, both drug classes share a constitutive downregulation of the astroglial glutamate transporter, GLT-1, and a retraction of glial end feet from NAc synapses (Scofield et al., 2016). Given the overlap in the drug-seeking endophenotype produced by self-administration of opioids and psychostimulants, and the shared vulnerability to relapse in addicts, one interpretation is that the enduring synaptic changes in MSNs that are not shared between drug classes may be less important mediators of relapse than the shared changes in astroglia. In this respect, it would be particularly interesting to look specifically at astrocytes surrounding behaviorally relevant ensembles. We will describe in detail later how dysregulation of glutamate homeostasis in the core region of the NAc (NAcore) induces drug seeking and the findings, suggesting that a transient potentiation of glutamatergic synapses might be the common denominator driving seeking and relapse behaviors.

Table 1 Constitutive Changes in Glutamate Transmission Induced by Chronic Exposure to Cocaine and Heroin/Morphine

	Morphological Changes	Functional Changes		
	Spine Head Diameter	A/N Ratio	Plasticity	Glutamate Transporter
Cocaine	↑ Head diameter[a], longevity of the changes controversial, see[b]	↑[c]	↓ LTD[d] ↓ LTP[e]	↓ GLT-1 function[f]
Heroin/ Morphine	↓ Head diameter[g]	↓[h]	↓ LTD/LTP[i]	↓ GLT-1 function[j]

A/N, *AMPA/NMDA ratio.*
[a]*Shen et al. (2014a)* and *Stankeviciute et al. (2014).*
[b]*Anderson and Self (2017).*
[c]*Gipson et al. (2014)* and *Shen et al. (2014a).*
[d]*Huang et al. (2011), Kasanetz et al. (2010),* and *Moussawi et al. (2009).*
[e]*Moussawi et al. (2009).*
[f]*Fischer-Smith et al. (2012), Knackstedt et al. (2010),* and *Reissner et al. (2015).*
[g]*Shen et al. (2011).*
[h]*Shen et al. (2011).*
[i]*Shen and Kalivas (2013).*
[j]*Shen et al. (2014b).*

3 GLUTAMATE SPILLOVER AND TRANSIENT SYNAPTIC PLASTICITY, COMMON TO ALL DRUGS OF ABUSE

Glutamate release is increased in the NAc of cocaine-sensitized animals in response to a cocaine challenge (Pierce et al., 1996) and following presentation of a cue paired with noncontingent cocaine exposure (Hotsenpiller et al., 2001). A large body of work has established that elevated synaptic glutamate spillover from prelimbic cortical afferents is measured in the accumbens during drug seeking for cocaine, heroin, alcohol, or nicotine, but not sucrose seeking (Gass et al., 2011; Gipson et al., 2013b; LaLumiere and Kalivas, 2008; McFarland et al., 2003). Under normal conditions, synaptic glutamate spillover is minimized by the patterned expression of the glial glutamate transporter GLT-1 on astroglial end feet adjacent to the synaptic cleft. Thus, GLT-1 strongly controls basal extracellular glutamate by negatively regulating synaptic glutamate spillover. However, after cocaine, heroin, alcohol, and nicotine self-administration, GLT-1 expression and function are decreased (Ducret et al., 2016; Gipson et al., 2013b; Knackstedt et al., 2009, 2010; Sari et al., 2011; Shen et al., 2014b).

The strong association between relapse in animal models and extracellular glutamate levels in the NAc across drug classes (cocaine, heroin, nicotine, and alcohol) initiated many studies to understand the cellular mechanisms regulating extracellular glutamate levels and how these might be regulated by using addictive drugs. In addition to GLT-1, cocaine, but not other addictive drugs, reduces the cystine–glutamate exchanger (Baker et al., 2003). Cystine–glutamate exchange involves a one-to-one stoichiometric exchange of intracellular glutamate for extracellular cystine and is rate limiting in the synthesis of glutathione (Uys et al., 2011). Also, a variety of addictive drugs alter signaling through presynaptic metabotropic glutamate 2/3 autoreceptors (mGluR2/3) with a net result of increasing synaptic glutamate release probability in the NAc. Taken together, addictive drug use produces enduring changes in key proteins or signaling cascades that result in a net increase in synaptic glutamate spillover, with the most widely shared adaptation being downregulated GLT-1 (Kalivas, 2009; Fig. 1). Accordingly, compounds that negatively regulate glutamate spillover reduce drug seeking in animal models of relapse. Thus, stimulating mGluR2/3 to increase inhibitory presynaptic autoreceptor tone decreases cocaine, heroin, and ethanol seeking (Baptista et al., 2004; Bossert et al., 2006; Peters and Kalivas, 2006; Zhao et al., 2006). Stimulation of mGluR2/3 by glial glutamate release in the NAcore also reduces cued-induced cocaine reinstatement (Scofield et al., 2015). The antibiotic ceftriaxone restores the levels of GLT-1 and xCT (catalytic subunit of the cystine–glutamate exchanger) in NAc and normalizes the density of glial end feet adjacent to synapses, thereby inhibiting cue- and cocaine-primed reinstated drug seeking (Knackstedt et al., 2010; Scofield et al., 2016; Trantham-Davidson et al., 2012), nicotine seeking (Alajaji et al., 2013), and alcohol seeking (Weiland et al., 2015). Likewise, the acetylated amino acid *N*-acetylcysteine increases both xCT subunit and GLT-1 expression and prevents cocaine, nicotine,

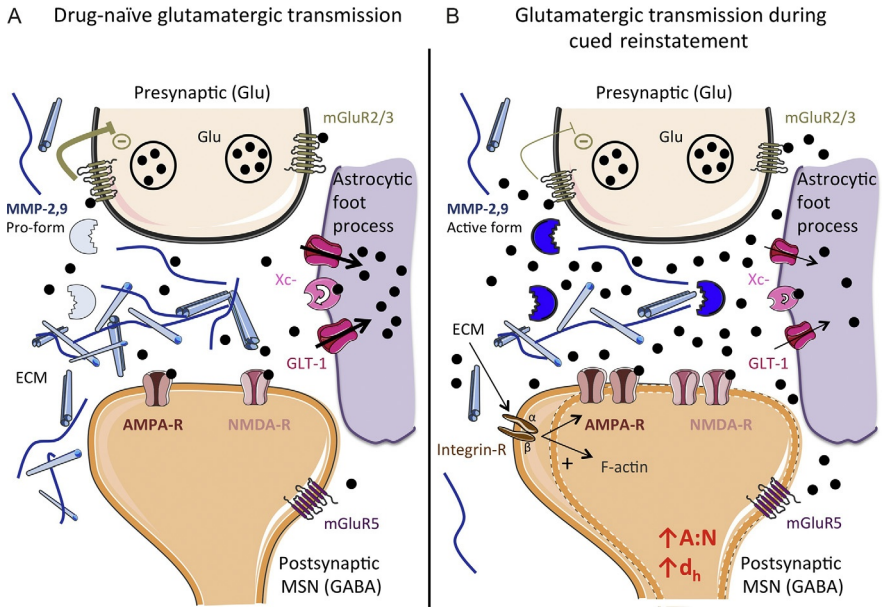

FIG. 1

Model of the tetrapartite synapse and how it is altered after withdrawal from addictive drugs. (A) Drug-naïve synaptic glutamate release probability is regulated by mGluR2/3 inhibitory autoreceptors, while the cystine–glutamate exchanger (Xc-) and glutamate transporter (GLT-1) expressed largely on astroglial cells regulate the elimination of glutamate and determine how much glutamate spills out of the synapse. The extracellular matrix maintains synaptic structure and mediates synaptic plasticity by activating MMPs and signaling to the postsynapse via integrins. (B) After drug self-administration and extinction training, presentation of the cues previously associated with the drug during acquisition of drug use induces a strong release of glutamate originating from prelimbic cortical afferents. Decreased function of mGluR2/3 and GLT-1 and withdrawal of astroglial end feet impair glutamate homeostasis and allow spillover of glutamate from the synaptic cleft. Extrasynaptic glutamate stimulates mGluR5 on nNOS interneurons (not shown), which activates matrix metalloproteases via nitrosylation. Catalytic signal transduction in the extracellular matrix by MMPs stimulates the expansion of postsynaptic spines and the insertion of AMPA receptors. *AMPA-R*, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; *ECM*, extracellular matrix; *GLT-1*, glutamate transporter 1; *Glu*, glutamate; *MMP*, matrix metalloprotease; *MSN*, medium spiny neuron; *NMDA-R*, *N*-methyl-D-aspartate receptor; *Xc-*, cysteine/glutamate exchanger.

alcohol, and heroin seeking (Baker et al., 2003; Madayag et al., 2007; Moussawi et al., 2009; Murray et al., 2012; Ramirez-Nino et al., 2013; Zhou and Kalivas, 2008). Using a selective protein knockdown strategy, the inhibition of cocaine reinstatement was shown to be mediated through the effect of *N*-acetylcysteine on GLT-1, not xCT (Reissner et al., 2015).

At the postsynapse, another glutamate metabotropic receptor, mGluR5, also plays a role in drug seeking, since its blockade though systemic or within the NAcore administration of antagonists prevents cued and primed reinstatement (Kenny and Markou, 2004; Olive, 2009; Wang et al., 2013). Since mGluR5 has predominantly perisynaptic localization, the efficacy of mGluR5 antagonists to inhibit drug seeking is hypothesized to arise from preventing the actions of synaptic glutamate spillover produced during a drug-seeking event, and at least for cocaine reinstatement, there is a critical involvement of mGluR5 expressed on accumbens interneurons that selectively express neuronal nitric oxide synthase (nNOS) (Smith et al., 2017).

Glutamate spillover into the NAcore during drug- or cue-induced reinstatement is paralleled by transient synaptic potentiation (t-SP) of medium spiny neurons. This pairing has been shown with many addictive drugs, including cocaine (Gipson et al., 2013a), heroin (Shen et al., 2011), and nicotine (Gipson et al., 2013b). t-SP is a transitory event that is measured by two main biomarkers of synaptic potentiation, i.e., (i) increases in spine head diameter and (ii) the AMPA/NMDA (A/N) ratio, an index of the strength of AMPA receptor-mediated transmission, at the excitatory synapses in the NAcore during the first 15 min of cue-induced reinstatement (Gipson et al., 2014). The increase in either A/N ratio or spine head diameter positively correlates with the intensity of cue-reinstated active lever pressing and, akin to synaptic glutamate spillover, is not seen during cue-induced sucrose seeking (Gipson et al., 2013a). Matrix metalloproteases (MMPs), a family of enzymes acting in the extracellular matrix, are essential to the induction of transient synaptic plasticity (Smith et al., 2014, 2015). In particular, activity of the gelatinase family of MMPs, MMP-2 and -9, is transiently increased during reinstatement induced by cues previously paired with cocaine, nicotine, and heroin, and inhibiting either MMP-2 or MMP-9 reduces the transient increases in both spine head diameter and A/N ratio, as well as cue-induced cocaine or heroin reinstatement (Smith et al., 2014). Moreover, intraventricular microinjection of a nonspecific MMP antagonist reduces cue-induced reinstatement of heroin seeking (Van den Oever et al., 2010). MMPs also appear to also be important in the transition to escalated ethanol self-administration (Smith et al., 2011), and MMP-9's role in spine remodeling was shown during electrically induced LTP in the hippocampus (Huntley, 2012; Wang et al., 2008). MMP activity signals to cells by catalytically creating ligands that bind to membrane receptors. The membrane receptor targets of MMP catalytic products that are required for t-SP are unknown, but one probable target is the adhesion molecule integrin. The β3 subunit of integrin is upregulated in the NAcore after cocaine self-administration and extinction (Wiggins et al., 2011), and modulating integrin receptor activation with RGD motif microinjections, a peptide ligand mimicking ECM binding, prevents ECM binding to integrin receptors and inhibits cocaine-primed reinstatement (Wiggins et al., 2011). Integrin stimulation induces activation of several kinases, among which integrin-linked kinase and the focal adhesion kinase (Niu and Chen, 2011) promote filamentous actin, a cytoskeletal protein critical for spine remodeling (Ghatak et al., 2013). Finally, we recently found that antisense knockdown of the β3 subunit inhibits t-SP and reinstated cocaine seeking (C. Garcia-Keller and P.W. Kalivas, unpublished observations).

Regardless of the signaling involved, MMP-2,9 activation is necessary to allow spine restructuring and t-SP in the NAcore initiated during cue-induced reinstatement (Smith et al., 2014). These results led to the idea of a critical role for the tetrapartite synapse in the NAcore in addiction-related mechanisms, including the canonical pre- and postsynaptic elements, astroglial end feet adjacent to the synaptic cleft, and the ECM surrounding the synapse, which is catalytically regulated by MMPs (Dityatev and Rusakov, 2011; Mulholland et al., 2016; Smith et al., 2015; Fig. 1).

In conclusion, there is strong evidence showing that presenting cues previously paired with drugs during self-administration induces release of glutamate from prelimbic cortex afferents in the NAcore. Due to drug-induced impairments of glutamate homeostasis (a combination of one or more: decrease of mGluR2/3 function, decrease in cysteine/glutamate exchanger and/or GLT-1 expression, and withdrawal of astroglial end feet from the synapse), glutamate release is not as tightly restricted to the synaptic cleft and spills more readily into the extracellular space. Given the capacity of mGluR5 antagonists in the NAcore to prevent reinstated drug seeking (Olive, 2009) and the location of these receptors largely outside of the synaptic cleft (Mitrano and Smith, 2007), mGluR5 is a likely target of glutamate spillover. Recently, it was shown that cue-induced cocaine seeking is critically dependent on mGluR5 located on a small population of NAc interneurons expressing nNOS, and that mGluR5 stimulation of nitric oxide production activates MMP-2,9 via nitrosylation (Smith et al., 2017). As described earlier, activated MMP-2,9 signals transient potentiation in NAcore MSNs. Later, we discuss how the induction of t-SP might expand the ensemble of neurons in the NAc that code cue-induced drug seeking, and thereby cause drug-associated cues to be more potent behavioral motivators than natural rewards.

4 COULD THE t-SP BE EMBEDDED IN A NEURONAL NETWORK SPECIFIC TO DRUG SEEKING?

The results described earlier on how t-SP drives drug seeking do not distinguish subpopulations of MSNs in the NAcore and in particular do not specifically identify an engram activated by reinstated drug seeking. Indeed, the A/N ratio and spine density measurements to date have been made indiscriminately from all the MSNs in the NAcore (Gipson et al., 2013a). Interestingly, according to the available studies on engrams and responses to drugs (Carelli et al., 2000; Koya et al., 2009), the percentage of neurons included in the engram responding to cocaine is estimated to be only 2%–5% of NAc cells. It is surprising that a robust and reproducible potentiation of both A/N and spine diameter can be measured in a large number of neurons throughout the NAc, both inside and outside of the cocaine engram. The fact that t-SP occurs in neurons that are not specific to the engram could argue for a transient enlargement of the engram in the NAcore involved in linking the drug-associated cue with the operant drug-seeking response (Fig. 2). We hypothesize that the network (or engram)

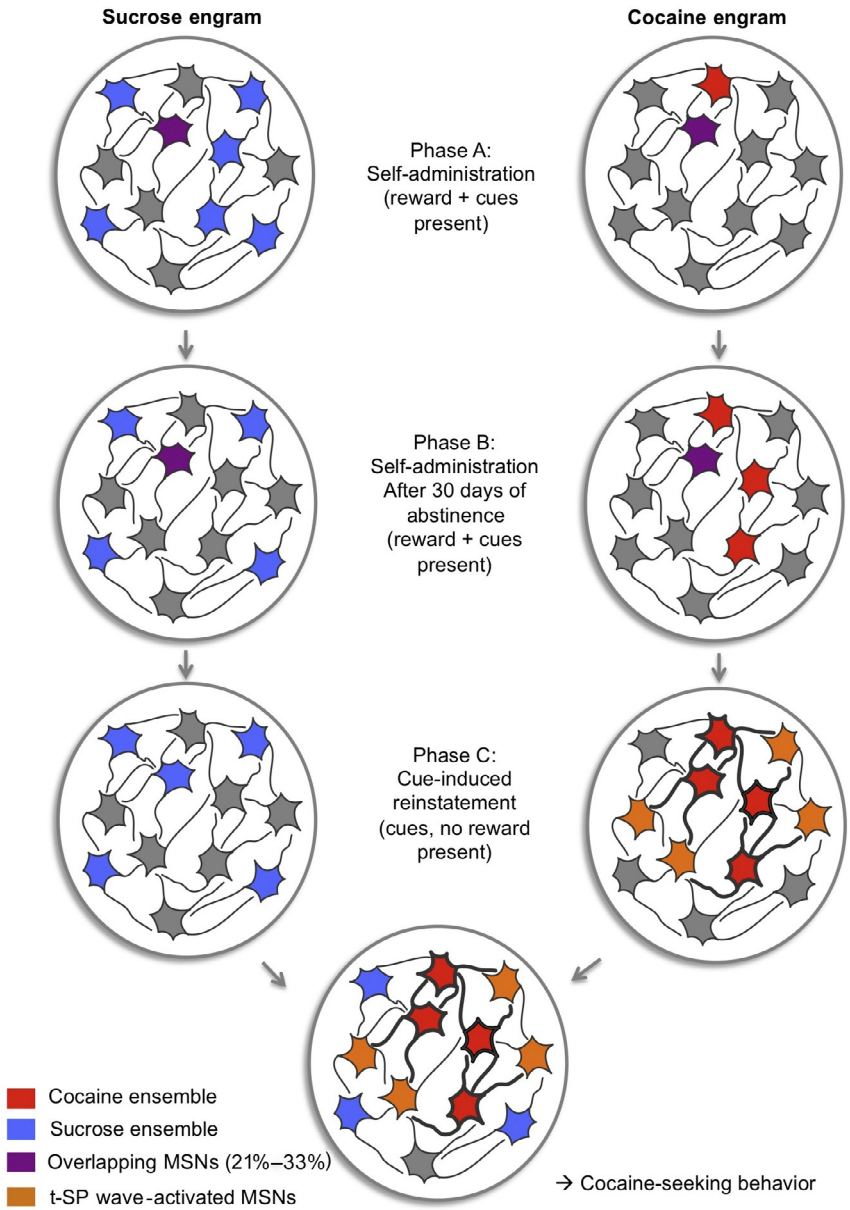

FIG. 2

Schematic of the wave of engram recruitment in the NAcore induced by a cocaine cue. We hypothesize that the cocaine/cue-associated ensemble, formed during cocaine self-administration, undergoes transient synaptic potentiation (t-SP) that spreads through the NAcore due to NO production and activation of MMPs (see Fig. 1). The local recruitment of a larger number of MSNs reduces the size of the ensemble activated by a sucrose cue, thereby promoting drug seeking over sucrose seeking. Cocaine-selective cells in *red*, sucrose-selective cells in *blue*, cells showing overlapping activity for cocaine and sucrose in *purple*, nonresponding cells in *gray*, and cells activated during reinstatement in *orange*. Phases A and B based on Cameron and Carelli (2012).

activated by drug-associated cues harbors synapses with poor glutamate homeostasis, resulting in the spillover of synaptically released glutamate. As a result, the original engram is enlarged during drug seeking through glutamate spillover inducing transient synaptic potentiation in near adjacent MSNs (represented as orange neurons in Fig. 2). This transient "potentiation wave" serves to either prevent further activation of the network by stimuli signaling alternate behaviors or, more simply, by recruiting a larger ensemble of NAcore neurons for the drug cue-initiated response (craving in humans), causing cues associated with biological stimuli that are not potentiated to become outcompeted.

Interestingly, measurement of the electrophysiological properties of engram neurons showed a depotentiation of these cells (decrease in A/N ratio and sEPSC frequency) after chronic cocaine exposure (Koya et al., 2012; Whitaker et al., 2016). However, these measures were obtained in animals repeatedly exposed to cocaine 90 min after a cocaine challenge and cocaine-paired context exposure, which may have resulted in these studies missing t-SP. The time course of t-SP in MSNs of reinstated cocaine withdrawn rats is <45 min for spine head expansion and <120 min for increased AMPA/NMDA (Gipson et al., 2013a). Also, LTD is observed 24 h after the last cocaine injection (Kourrich et al., 2007).

The concept of engram expansion receives behavioral support from a study showing that while rats prefer a food reinforcement over a cocaine reinforcement, in a cued reinstatement session where animals respond only to the reward associated cue, they respond quantitatively more to the cocaine cue (Tunstall and Kearns, 2016). In this study, animals presented with a choice between grain pellets or cocaine reward overwhelmingly chose food when the reward was present. After extinction training, animals underwent cued reinstatement, in the absence of rewards where they were presented with a choice between cocaine or food lever leading to either drug- or food-associated cue delivery. In this case, animals pressed the cocaine-associated lever at a high rate, hence seeking cocaine more intensely than food (Tunstall and Kearns, 2016). The authors conclude that after the drug-free period, the cocaine cue becomes more salient than the food cue. Based on the fact that glutamate spillover specifically occurs in synapses following cocaine and not sucrose self-administration (Gipson et al., 2013a) due to downregulated GLT-1, the induction of t-SP is initiated only in the cocaine engram. Thus, we propose that glutamate spillover and the widespread induction of t-SP represent the recruitment of additional synapses on neurons near adjacent to the cocaine engram neurons, and the recruitment of these neurons creates a stronger engram that mediates the strengthened behavioral response to a cocaine cue. Electrophysiological studies support the general idea that cocaine cues recruit a larger engram than natural reward-associated cues. For example, selective encoding of natural rewards (food, water, or sucrose) is different from the encoding for cocaine, with only 20% of measured cells responding to both types of reinforcers during the task, but after 30 days of cocaine abstinence, the percentage of cells overlapping increases to 33% as a result of the engram encoding cocaine reward having enlarged, and the food reward engram involving a smaller number of neurons (Cameron and Carelli, 2012; Carelli et al., 2000; Fig. 2). The relative enlargement of neuronal coding for the cocaine cue is consistent with the incubation of drug craving seen after cocaine withdrawal

(Grimm et al., 2001). Another study used prolonged access to cocaine self-administration, and in vivo electrophysiological recordings in the NAc were performed during escalation of cocaine intake, after 30 days of forced abstinence, and during cocaine reexposure (Guillem et al., 2014). The authors concluded that the incubation of cocaine seeking observed after the abstinence phase was significantly and selectively correlated to an increase in the proportion of neurons that fired phasically during cocaine seeking. Finally, a reanalysis of our earlier data (Gipson et al., 2013a) shows that if we arbitrarily define recruitment of neurons to an engram when the neurons have an A/N that is two standard deviations above the mean A/N value, reinstatement of lever pressing induced by a sucrose cue does not enlarge the engram size over 6%, while cue-induced cocaine seeking potentiates cells creating an engram constituting 18% of the MSNs recorded (Fig. 3). Concurrent with this observation, relapse to a cocaine

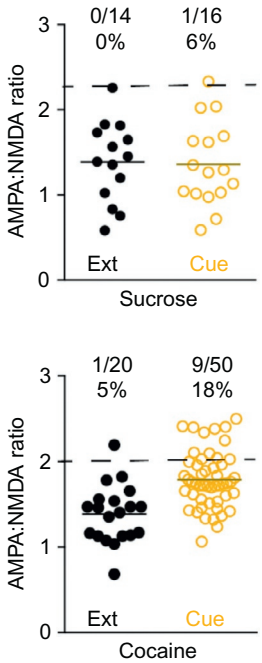

FIG. 3

AMPA:NMDA ratio measured in rats that underwent sucrose self-administration (sucrose) or cocaine self-administration (cocaine). In the extinction group (Ext), rats underwent an extinction session 24 h before taking NAcore tissue slices and measuring A/N; in the cue group, animals were tested after 15 min of cue-induced reinstatement. *Numbers on top* of the data recapitulate the number of cells with ratios two standard deviations above the mean ratio over total number of cells measured, as well as the corresponding percentages.

These data were originally published in Gipson, C.D., Kupchik, Y.M., Shen, H., Reissner, K.J., Thomas, C.A., Kalivas, P.W., 2013. Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. Neuron 77, 867–872.

context increased the number of c-Fos-positive neurons in the NAc by a similar magnitude compared to extinction baseline (Cruz et al., 2014).

A final consideration is that MSNs in the NAcore are divided into two subtypes, depending on the expression of dopamine D1 or D2 receptors that have opposing roles in cocaine taking and reinstatement (Bock et al., 2013; Heinsbroek et al., 2017). This raises the question that ensembles triggered by drug–associated cues might be limited to D1 or D2 MSNs. The data presented in Fig. 4 show that while both D1 and D2 MSN spine heads are enlarged in cocaine-extinguished animals compared to yoked saline controls, the transient increase in spine head diameter

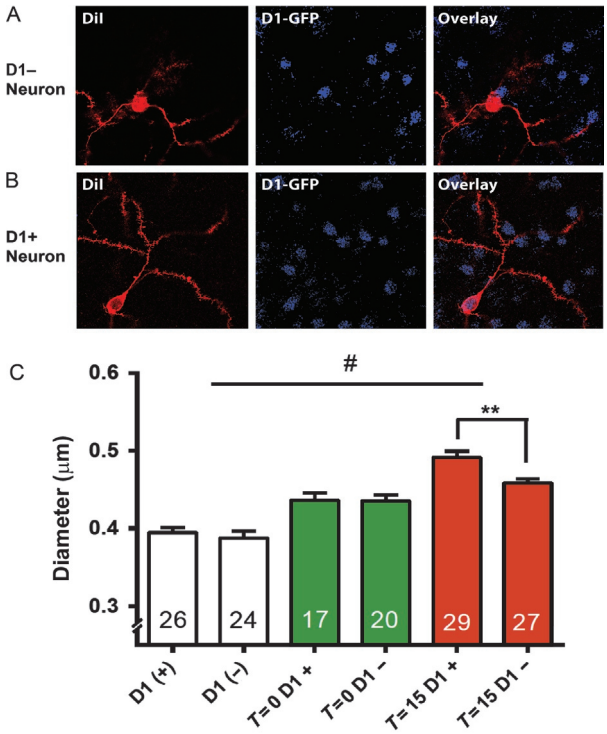

FIG. 4

DiI labeling coupled with immunohistochemistry enhanced labeling of GFP allowed visualization of (A) putative D2 neurons (D1 negative, D1–) and (B) D1-positive neurons in the NAc. (C) Spine head diameter on D1+ and D1– neurons. After extinction from cocaine self-administration ($T=0$, *green bars*), a potentiation in d_h is observed in both D1+ and D1– neurons compared to saline controls (*white bars*). During cue-induced reinstatement ($T=15$, *red bars*), d_h is elevated specifically on D1+ dendritic spines. *N* shown in *bars* is the number of neurons quantified, and the data were analyzed using a two-way ANOVA $F(1137)=4.613$, $P<0.05$ (main effect); ** D1 vs D2 $P<0.001$; # between groups $P<0.001$).

of NAcore MSNs produced during cue-induced drug seeking is significantly greater in D1 than in D2 MSN spines. These data were collected in D1-eGFP reporter mice that underwent standard intravenous saline or cocaine (0.8 mg/kg/infusion) self-administration, followed by extinction training, and subsequent cue-induced reinstatement (specific protocol described in Heinsbroek et al., 2017). Cells were diolistically labeled, and spine head diameter quantified as described by (Shen et al., 2008).

5 CONCLUDING REMARKS

Changes in NAc plasticity after drug exposure are critical to seeking behaviors. Particularly, the transient deregulation of glutamate homeostasis observed in NAcore tetrapartite synapses (constituted by pre- and postsynaptic elements, astroglial end feet, and extracellular matrix encompassing the synapse) has been shown to be necessary to initiate drug seeking during cue-induced reinstatement. Although dissecting the different types of neurons in the NAc and their respective projections has moved the addiction field forward (Lenz and Lobo, 2013), a growing movement campaigns for a shift from focusing on the neuron as the brain unit to integrated neuronal networks (Cruz et al., 2013; Yuste, 2015). We hypothesize here that transient glutamate overflow occurs in the NAcore during reinstatement within the engram specific to drug-seeking behavior, which was formed during drug self-administration. We speculate that this engram, constituted at first of a small number of neurons, is enlarged during reinstatement by synaptic glutamate spillover that produces an mGluR5-dependent NO activation of MMPs to induce t-SP in MSNs. This pathological process results in a prepotent behavioral response for drug-associated cues that inhibits the initiation of competing behavioral responses by stimuli associated with behaviors other than drug seeking. The development of novel tools that employ the expression of immediate-early genes like c-fos (Cruz et al., 2015; Reijmers et al., 2007) and recent advancements in narrowing the window of cell tagging around a specific behavior using the TRAP technology (Guenthner et al., 2013) allows for the study of specific cells activated during a behavior and will lead to a better understanding of how engrams steer behavior.

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