

Viewpoints

Silent Synapses in Cocaine-Associated Memory and Beyond

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Glutamatergic synapses are key cellular sites where cocaine experience creates memory traces that subsequently promote cocaine craving and seeking. In addition to making across-the-board synaptic adaptations, cocaine experience also generates a discrete population of new synapses that selectively encode cocaine memories. These new synapses are glutamatergic synapses that lack functionally stable AMPARs, often referred to as AMPAR-silent synapses or, simply, silent synapses. They are generated *de novo* in the NAc by cocaine experience. After drug withdrawal, some of these synapses mature by recruiting AMPARs, contributing to the consolidation of cocaine-associated memory. After cue-induced retrieval of cocaine memories, matured silent synapses alternate between two dynamic states (AMPA-absent vs AMPAR-containing) that correspond with the behavioral manifestations of destabilization and reconsolidation of these memories. Here, we review the molecular mechanisms underlying silent synapse dynamics during behavior, discuss their contributions to circuit remodeling, and analyze their role in cocaine-memory-driven behaviors. We also propose several mechanisms through which silent synapses can form neuronal ensembles as well as cross-region circuit engrams for cocaine-specific behaviors. These perspectives lead to our hypothesis that cocaine-generated silent synapses stand as a distinct set of synaptic substrates encoding key aspects of cocaine memory that drive cocaine relapse.

Introduction

Substance use disorder (SUD) is an acquired behavioral state that develops through repeated substance experience. While having substantial pathologic features, the development and maintenance of SUD share many key plasticity mechanisms and exhibit memory features that are commonly observed in learning and memory processes, including memory acquisition, consolidation, retrieval-induced destabilization, and reconsolidation (Torregrossa and Taylor, 2013; Everitt and Robbins, 2016). Indeed, SUD has been conceptualized as an extreme form of memory such that the underlying plasticity substrates can be targeted to decrease drug seeking and taking (Hyman et al., 2006).

In an attempt to identify neural underpinnings of SUD, intensive prior studies have identified several critical forms of drug-induced neural adaptations that promote the development of drug-associated behaviors (Wolf, 2016; Wright and Dong, 2020). Despite much exciting progress, our understanding has been partially limited to changes occurring in neuronal and synaptic populations that are broadly implicated in reward response, but not necessarily unique to SUD. For example, it has been shown that, following cocaine self-administration, synaptic potentiation is detected within the majority of hippocampal projections to medium spiny neurons (MSNs) that express D1 dopamine receptors (D1-MSNs) in the NAc, and this potentiation promotes cocaine seeking after cocaine withdrawal (Pascoli et al.,

2014; Zhou et al., 2019). However, potentiating the majority of hippocampal inputs to NAc D1-MSNs also promotes seeking of nondrug rewards (LeGates et al., 2018). Are there any neuronal substrates or synaptic alterations that are unique to drug experience?

Recent studies have identified a population of immature glutamatergic synapses, namely, AMPAR-silent synapses, which are generated *de novo* within the NAc after cocaine administration (Y. H. Huang et al., 2009; J. Wang et al., 2021a) (Fig. 1). After drug withdrawal, these synapses exhibit three major dynamic changes corresponding to the consolidation, retrieval-induced destabilization, and reconsolidation of cocaine memories (Lee et al., 2013; Ma et al., 2014; Wright et al., 2020). Although new synapses are constitutively generated through metabolic turnover or by other experiences, here we discuss the possibility that cocaine-generated synapses represent a discrete population of neuronal substrates that encode cocaine-associated memories. We will make a conceptual generalization by exploring how a unique set of nascent synapses is generated by a particular experience to contribute to the formation of neuronal engrams and unique circuit activity patterns corresponding to this particular experience.

Memory encoding: role of synapses and synaptogenesis

A prominent goal of SUD neuroscientists is to determine the highly specific neuronal substrates that underlie drug-associated memories. Hope arises from the Engram Hypothesis, which proposes that a given memory is encoded by a select set of persistent structural and functional changes in the brain, termed the engram (Semon, 1921; Josselyn and Tonegawa, 2020). Synaptic connections between neurons have long been hypothesized to encode such memory traces through experience-induced plasticity (Ramon y Cajal, 1894; Hebb, 1949). Experience-induced synaptic plasticity can be expressed as changes in the weight of preexisting synapses without changing circuit connectivity or,

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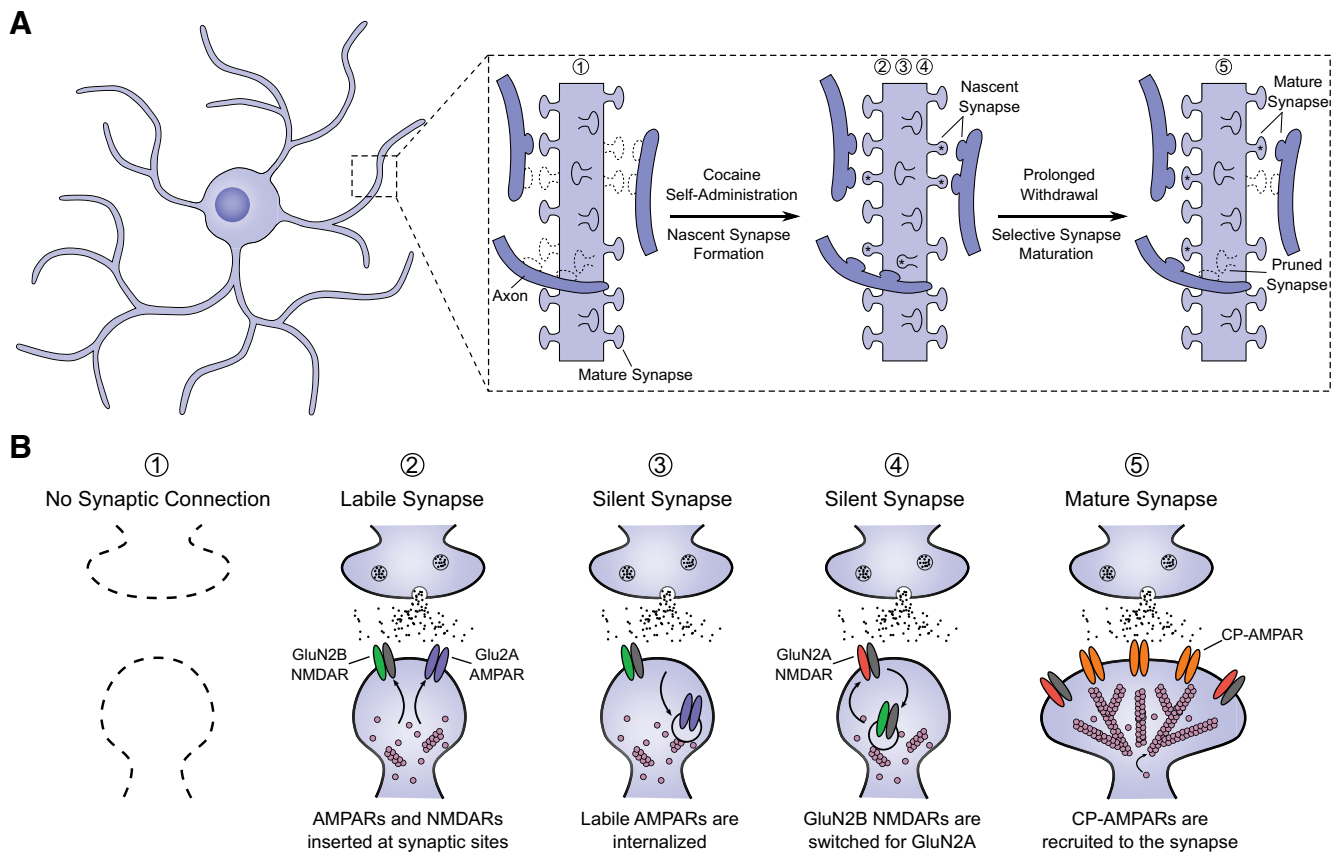


Figure 1. Cocaine-induced generation of silent synapses in the NAC. **A**, Schematic depiction of synaptogenesis in an MSN within the NAC during and following cocaine self-administration. During self-administration training, nascent, immature synapses are generated along the dendritic arbor, forming new connections. During withdrawal, some of these synapses mature into functional synapses, whereas others are presumably pruned away. Numbers correspond to the stages depicted in **B**. **B**, Illustration of the stages of cocaine-induced synapse generation and maturation. During cocaine self-administration, synaptogenic processes are triggered. This leads to the generation of new synapses, which contain both GluN2B-containing NMDARs and GluA2-containing AMPARs. These AMPARs, however, are highly labile and are soon internalized, resulting in silent synapses containing only NMDARs. During early withdrawal, GluN2B-containing NMDARs are replaced with GluN2A-containing NMDARs. This is critical for the subsequent functional maturation of the synapse mediated by the recruitment of CP-AMPA to the synapse.

alternatively, addition/elimination of synapses that results in circuit rewiring (Chklovskii et al., 2004). In either case, if unique to an experience, these synapses may bear a “synaptic engram” (Josselyn et al., 2015). On the other hand, sparsely distributed neurons that are selectively activated during formation or retrieval of a memory can bear a “neuronal engram” (Josselyn et al., 2015; Tonegawa et al., 2015). To capture engram neurons and synapses experimentally, many laboratories take advantage of activity-dependent biomarkers, with the assumption that the engram substrates would exhibit increased activities during memory formation and retrieval. Consequently, a relatively small number of engram neurons and synapses are tagged and defined among their numerous peers in response to a specific experience (Josselyn et al., 2015; Tonegawa et al., 2015). These synaptic and neuronal engrams are not mutually exclusive but are, at least in part, linked mechanistically. For example, some neurons can be “tuned” by remodeled synaptic inputs to selectively respond to a specific experience, thus becoming engram neurons. In this case, synaptic engrams may serve as the subcellular basis for neuronal engrams.

It has been demonstrated convincingly that selective inhibition of neurons in a neuronal engram (i.e., engram neurons) for a memory disrupts the behavioral expression of this memory (Tonegawa et al., 2015; Josselyn and Tonegawa, 2020). However, some neurons in one neuronal ensemble may also participate in another neuronal ensemble that encodes a different memory

(Cai et al., 2016; Rashid et al., 2016; Yokose et al., 2017; Abdou et al., 2018). Such an overlap provides a mechanism for memory linkage or generalization (Cai et al., 2016; Yokose et al., 2017), but it also indicates the likelihood that targeting individual engram neurons may simultaneously interfere with nontargeted memories. Indeed, it is the temporal activities, rather than their physical existence, of engram neurons that express the memory for a neuronal engram. Specifically, neurons can participate in multiple engrams, but at a given time (e.g., during memory recall), activity of neurons within a pretuned neuronal engram supports a unique network activity pattern. The uniquely patterned temporal circuit dynamics are thought to be a circuit representation for a specific memory (Sussillo, 2014; Yuste, 2015; Kyriazi et al., 2018; Ruff et al., 2018; Gonzalez et al., 2019; Ju and Bassett, 2020). As such, the synaptic engram, neuronal engram, and circuit dynamics can be regarded as neural manifestations of the same memory perceived from three different anatomic angles, with synapses as the key subcellular basis defining the circuit connectivity that orchestrates the temporal dynamics of ensemble neurons and circuit activities.

In theory, generation of new memory-encoding circuit-activity patterns can be achieved by modification of the weight of pre-existing synapses without changing the synaptic connectivity of the circuit. For example, experience-induced LTP and LTD may preferentially occur at different synapse populations to redefine the circuit activity dynamics. However, the fixed synaptic

connectivity also entails a “range constraint” on a circuit and limits the encoding capacity (Sadler et al., 2014; Oby et al., 2019). This constraint can be overcome at least in part by experience-dependent modification of the number of synapses. Indeed, experience-dependent synapse formation and elimination have been associated with the emergence of new circuit activity patterns (Peters et al., 2014) and formation of new memories (Yang et al., 2009; Fu et al., 2012; Lai et al., 2012; Parkhurst et al., 2013; Chen et al., 2015), including memories associated with SUD (Russo et al., 2010; Muñoz-Cuevas et al., 2013). Formation of neuronal engrams is also associated with synaptogenesis (Ryan et al., 2015; Choi et al., 2018), and reactivation of engram neurons during memory retrieval is dependent on these new synaptic connections (Ryan et al., 2015). Furthermore, synapses generated by different experiences may coexist on the same neurons but encode distinct memories, such that disrupting the function of one synapse population impairs only its associated memory (Yang et al., 2014; Cichon and Gan, 2015; Hayashi-Takagi et al., 2015). Such a compartmentalized arrangement allows different synaptic populations to embed different memory traces in the same neurons (Losonczy et al., 2008; Cichon and Gan, 2015), such that the same neurons may contribute to multiple memories (Abdou et al., 2018).

The above literature analysis supports the hypothesis that a memory can be selectively encoded in a specific synapse population, or a synaptic engram, that is generated *de novo* during learning. This hypothesis is similar to the previously proposed synaptic tagging hypothesis (Chklovskii et al., 2004; Rogerson et al., 2014; Holtmaat and Caroni, 2016) but conceptualizes new synapses generated by an experience as an independent synaptic engram that encodes nonoverlapping memory traces. Such synapses should have several key features: (1) they are generated by a learning experience, (2) they contribute to subsequent memory recall, and (3) changes in their functional state underlie the dynamics of a memory. Below, we review the features and mechanisms of cocaine-generated silent synapses, followed by our perspective of how these synapses may represent a synaptic engram encoding specific aspects of cocaine memories.

Mechanisms and functions of cocaine-generated silent synapses

Cocaine-induced generation of silent synapses

During brain development, immature glutamatergic synapses often contain only NMDARs without functionally stable AMPARs, and are thus called AMPAR-silent synapses (Kerchner and Nicoll, 2008). After development, synaptogenesis and silent synapses decline to low levels (Durand et al., 1996; Petralia et al., 1999), but the basic synaptogenic capacity remains throughout adulthood to contribute to experience-induced synaptic remodeling (Holtmaat and Svoboda, 2009). Under certain learning conditions, new glutamatergic synapses are generated in the adult brain, and these, like nascent synapses during development, often have thin or filopodia-like postsynaptic structures initially (Holtmaat et al., 2006; Knott et al., 2006), and then undergo gradual enlargement, a process corresponding to functional maturation via incorporation of AMPARs (Matsuzaki et al., 2004). Similar generation of silent synapses is observed during fear conditioning and likely other learning processes (Suvrathan et al., 2013; W. Ito et al., 2015; Y. Wang et al., 2018; W. Ito and Morozov, 2019). Thus, silent synapse-based synaptogenesis may serve as a general, basic strategy for certain learning processes to remodel related neural circuits during memory encoding.

Exposure to cocaine and other psychostimulants increases the density of dendritic spines in NAc MSNs (Robinson and Kolb, 1997, 1999; Robinson et al., 2001), suggesting that synaptogenesis occurs. Echoing these findings, cocaine experience induces generation of silent synapses in NAc MSNs (Y. H. Huang et al., 2009; Koya et al., 2012; Lee et al., 2013; Whitaker et al., 2016) (Fig. 1). Several features of these cocaine-generated silent synapses are consistent with their being nascent, immature synapses: (1) they are enriched in GluN2B-containing NMDARs (Y. H. Huang et al., 2009; Brown et al., 2011; Wright et al., 2020), a hallmark of immature glutamatergic synapses during development (Monyer et al., 1994; Kirson and Yaari, 1996; Tovar and Westbrook, 1999); (2) the majority of cocaine-generated silent synapses are not formed by AMPAR internalization at preexisting synapses (Graziane et al., 2016; Y. Q. Wang et al., 2021); (3) cocaine-induced generation of silent synapses is tightly correlated with a selective increase in thin and filopodia-like spines, which may represent immature glutamatergic synapses (Graziane et al., 2016; Wright et al., 2020); and (4) disrupting astrocytic thrombospondin- $\alpha 2\delta-1$ signaling, a signaling pathway that promotes synaptogenesis during brain development, prevents both the generation of silent synapses and the increase in spine density after cocaine experience (J. Wang et al., 2021a). These results argue that cocaine-generated silent synapses are a discrete set of new synapses related to cocaine experience.

The mechanisms governing experience-induced synaptogenesis in the adult brain are complex and incompletely understood (Waites et al., 2005; Südhof, 2018). To date, several mechanistic processes that have been characterized for generating silent synapse in the adult brain are similar to those underlying synaptogenesis during development (Dong and Nestler, 2014). A prominent molecular mechanism is upregulation of the activity of CREB and other transcriptional signaling pathways, which promotes transcription of synaptogenic proteins (McClung and Nestler, 2003) and drives generation of silent synapses (Marie et al., 2005; Brown et al., 2011; Grueter et al., 2013; for thorough reviews, see Dong and Nestler, 2014; Nestler and Lüscher, 2019). Another cellular step is synaptic insertion of GluN2B-containing NMDARs (Y. H. Huang et al., 2009; Y. Q. Wang et al., 2021), an NMDAR subtype critically involved in synaptogenesis and silent synapse generation during both development and adulthood (Tovar and Westbrook, 1999; Nakayama et al., 2005; Gambrill and Barria, 2011; Chung et al., 2017). Recent results suggest that GluA2-containing AMPARs are inserted simultaneously with GluN2B-NMDARs during initial synapse generation, but the AMPARs are quickly internalized, resulting in an AMPAR-silent state shortly after synaptogenesis (Y. Q. Wang et al., 2021). Such a two-step process is again reminiscent of synaptogenesis during development (Xiao et al., 2004; Groc et al., 2006). In addition to neuronal mechanisms, cocaine-induced generation of silent synapses requires an astrocyte-mediated synaptogenic signaling pathway. Specifically, cocaine experience induces astrocytic release of thrombospondin-2, which, in turn, activates its neuronal receptor $\alpha 2\delta-1$, promoting silent synapse generation in the NAc (J. Wang et al., 2021a). This and other astrocytic signaling pathways have been critically implicated in synapse generation, maintenance, and elimination during both development and adulthood (Eroglu and Barres, 2010; Allen and Eroglu, 2017; J. Wang et al., 2021b). Through localized activities, astrocyte processes can initiate and regulate synaptogenesis at specific dendritic segments (Eroglu and Barres, 2010; Martín et al., 2015; Allen and Eroglu, 2017; Martín-Fernández et al., 2017), and may even define the location within a circuit where new synapses are formed. These findings highlight that cocaine-induced generation of new

synapses involves synaptogenic mechanisms normally used by the developing brain.

While the majority of silent synapses generated after cocaine experience are new, immature synapses, a portion of them originate from preexisting synapses after AMPAR internalization (Y.D., unpublished data), and this synaptic weakening process may eventually lead to synapse elimination. As another effective means to restructure the circuit connectivity, it is not surprising that experience-induced synapse elimination is also important for memory formation (Yang et al., 2009; Chen et al., 2015; Li et al., 2017). Related to this, morphine experience induces synapse elimination in NAc D2-MSNs, whereas disrupting this elimination compromises the retention of morphine-conditioned place preference, a reward conditioned spatial memory (Graziane et al., 2016).

Maturation or elimination: “to be or not to be” of cocaine-generated synapses

After cocaine experience, silent synapses do not persist in the AMPAR-silent state indefinitely but “disappear” after several days of withdrawal (Y. H. Huang et al., 2009; Lee et al., 2013). In general, nascent synapses have two possible fates: (1) to mature and, thus, be unsilenced and incorporated into the circuit; or (2) to be eliminated (Yang et al., 2009; Dong and Nestler, 2014). The first scenario is supported by observations that the increase in spine density following cocaine experience persists through withdrawal periods; and importantly, it is accompanied by a conversion of thin/filopodia-like spines to mushroom-like spines (Graziane et al., 2016; Wright et al., 2020), suggesting synaptic maturation. Furthermore, the disappearance of silent synapses corresponds with an upregulation of AMPARs, specifically atypical, Ca^{2+} -permeable AMPARs (CP-AMPA). Blocking CP-AMPA restores high levels of silent synapses after drug withdrawal (Conrad et al., 2008; Lee et al., 2013; Wright et al., 2020).

Recent results have begun to reveal key mechanistic steps during maturation of cocaine-generated silent synapses. First, it appears that GluN2B-containing NMDARs regulate the pace of maturation. During development, GluN2B-NMDARs at nascent glutamatergic synapses inhibit AMPAR insertion, maintaining the synapses in the AMPAR-silent state (Adesnik et al., 2008; Gray et al., 2011). Subsequent synaptic maturation through AMPAR stabilization coincides with a switch of NMDARs from GluN2B- to GluN2A-containing subtypes (Monyer et al., 1994). Mirroring this developmental process, GluN2B NMDARs at cocaine-generated synapses are replaced with non-GluN2B, presumably GluN2A, NMDARs during early withdrawal from cocaine (Y. H. Huang et al., 2009; Wright et al., 2020). This replacement is required for the maturation of cocaine-generated synapses, such that disrupting this replacement keeps cocaine-generated synapses in the immature, silent state (Y. Q. Wang et al., 2021). Second, recruitment and stabilization of AMPARs at newly matured silent synapses during cocaine withdrawal may require coordinative interactions between postsynaptic density (PSD) scaffold proteins. Scaffold proteins, particularly PSD-95, stabilize synaptic AMPARs within the PSD, whereas genetic deletion of PSD-95 results in high basal levels of silent synapses in the adult brain (El-Husseini et al., 2000; Béique et al., 2006; Ehrlich et al., 2007; Cane et al., 2014; Meyer et al., 2014; X. Huang et al., 2015; Favaro et al., 2018). Upon PSD-95 knockout or knockdown, cocaine experience still generates silent synapses in NAc MSNs, suggesting intact generation processes. However, these synapses do not mature after prolonged drug withdrawal,

indicating failed insertion and/or stabilization of AMPARs (Shukla et al., 2017).

While a large portion of cocaine-generated silent synapses mature after drug withdrawal, it is speculated that some of them do not (Lee et al., 2013). This is not surprising because a similar phenomenon has been documented in the cortex, in which a portion of experience-generated synapses do not mature but are eliminated eventually (Yang et al., 2009; Li et al., 2017). Thus, selection mechanisms must exist for preservation versus elimination of these synapses. One potential mechanism involves activity-dependent synaptic maturation. It is believed that new synapse generation tends to be excessive, and only those that are frequently used eventually mature and are integrated into the circuit (Katz and Shatz, 1996; Cohen-Cory, 2002; Waites et al., 2005; Stephan et al., 2012; Holtmaat and Caroni, 2016). In this scenario, synapse generation has a degree of randomness, allowing sufficient connection opportunities, while maturation refines and specifies the circuit connectivity that supports consolidated/persistent memories.

However, memories are typically consolidated in the absence of direct exposure to related experience (Dudai et al., 2015; Klinzing et al., 2019). What activities drive synaptic maturation during such periods of quiescence? One possibility is the replay of patterned circuit activity that represents an experience. In the hippocampus, neuronal ensembles that are activated during learning are often reactivated to replay their temporal dynamics at later times in the absence of new learning (Ólafsdóttir et al., 2018), and this replay contributes to memory consolidation (Girardeau et al., 2009; Jadhav et al., 2012). Such replay may provide essential activity signals for selecting relevant synapses for maturation. In line with this viewpoint, it has been shown in the cortex that reactivation of a dendrite promotes the stabilization of nascent synapses on that dendrite (Cichon and Gan, 2015), and reactivation of a neuronal ensemble strengthens the functional connectivity between ensemble neurons to improve the circuit representation of the memory (Sugden et al., 2020). In the NAc, the neuronal ensembles related to rewarding experiences display reactivation dynamics during periods of quiescence (Pennartz et al., 2004; Lansink et al., 2008), suggesting the existence of similar replay mechanisms, which may contribute to the maturation of silent synapses and consolidation of drug-associated memories during withdrawal periods after cocaine self-administration training.

In addition to experience-dependent activity, homeostatic plasticity may contribute to synaptic maturation and circuit remodeling (Turrigiano and Nelson, 2004). By mobilizing a complex set of mechanisms, homeostatic plasticity regulates synaptic strength and intrinsic membrane excitability of neurons to maintain an output near a set point (Turrigiano, 2008; Davis, 2013). A form of homeostatic plasticity, termed synapse-membrane homeostatic crosstalk, has been characterized in NAc MSNs, through which the excitatory synaptic input and membrane excitability of MSNs are homeostatically adjusted to functionally compensate for changes in one or the other (Ishikawa et al., 2009). After short-term withdrawal from cocaine, upregulation of GluN2B NMDARs at cocaine-generated silent synapses produces a false signal of increased synaptic input that induces synapse-to-membrane homeostatic plasticity, resulting in decreased membrane excitability of NAc MSNs (J. Wang et al., 2018). This membrane change subsequently induces another round of membrane-to-synapse homeostatic plasticity, resulting in strengthening of excitatory synapses through insertion of CP-AMPA (J. Wang et al., 2018). Such a large-scale upregulation of CP-AMPA

suggests a rather nonselective maturation of cocaine-generated synapses. This potential feature may contribute to the substantial and persistent increase in spine density after drug exposure, which is not typical for other memories but potentially critical for the robustness of cocaine-associated memories.

Dynamics of cocaine-generated synapses in cocaine memories

Cocaine-associated memories are multifaceted, containing information related to unconditioned responses, contextual and discrete cues, value, and actions. Cocaine-generated silent synapses and the resulting circuit remodeling contribute to several, but not all, aspects of cocaine-associated memories. Preventing cocaine-induced generation of silent synapses in the NAc does not prevent the acquisition of cocaine self-administration, suggesting that NAc silent synapses are not involved in unconditioned stimulus-driven instrumental learning (J. Wang et al., 2021a). This is not surprising since NAc lesions do not prevent the acquisition of cocaine self-administration (R. Ito et al., 2004), nor instrumental learning during other goal-directed behaviors (Corbit et al., 2001; Jonkman and Everitt, 2011). Instead, cocaine-generated NAc synapses may contribute to conditioned associations, particularly associations between conditioned stimuli and behavioral outcomes (Goldstein et al., 2012; West and Carelli, 2016; Gmaz et al., 2018). Supporting this viewpoint, animals trained in cue-conditioned self-administration exhibit robust cue-induced cocaine seeking after prolonged drug withdrawal, and preventing NAc silent synapse generation during self-administration or weakening these synapses after their maturation during withdrawal impairs cue-induced cocaine seeking (Lee et al., 2013; Ma et al., 2014; Wright et al., 2020; J. Wang et al., 2021a). Furthermore, these manipulations do not affect the learning that extinguishes cocaine seeking in the absence of conditioned cues, nor the general motivation to obtain cocaine (J. Wang et al., 2021a), further narrowing in on cue-conditioned associations as the key aspect of cocaine memories that cocaine-generated NAc synapses may encode.

NAc MSNs receive convergent glutamatergic projections from many limbic and paralimbic areas (Sesack and Grace, 2010; Xia et al., 2020). Cocaine-generated silent synapses have been detected in projections from all the brain regions examined thus far, including the BLA, prelimbic and infralimbic PFC, and paraventricular nucleus of the thalamus (Lee et al., 2013; Ma et al., 2014; Neumann et al., 2016), and may be present in projections from other brain regions, such as the hippocampus, which have not yet been investigated. Accumulating evidence suggests that cocaine-generated synapses in projections from different brain regions contribute differentially to cocaine-related behaviors. For example, BLA and prelimbic silent synapses promote cue-induced cocaine seeking, whereas infralimbic silent synapses suppress this behavior (Lee et al., 2013; Ma et al., 2014). These results raise the possibility that cocaine-generated synapses within NAc afferents from different brain regions transmit different information, such as discrete or spatial cues, to the same NAc MSNs to differentially regulate behavior. Thus, cocaine-generated synapses dispersed in different afferents may collectively form a synaptic engram that encodes a broader, multifaceted representation of the cocaine experience.

Memories are not static, but highly dynamic. For example, consolidated memories become destabilized and susceptible to modification on reactivation, and then restabilize through a reconsolidation process (Tronson and Taylor, 2007; Torregrossa and Taylor, 2013). If memories are indeed encoded in synaptic and neuronal engrams as theorized, the engram neurons and

synapses would be expected to exhibit dynamic changes corresponding to these memory dynamics. Such functional dynamics of cocaine-generated NAc synapses are observed. Specifically, after formation and consolidation of cue-associated cocaine memories following withdrawal from cocaine self-administration, the already mature synapses are transiently resiled on reactivation of cocaine memories, via internalization of CP-AMPA; the synapses then mature again during the period of memory reconsolidation (Wright et al., 2020). This rematuration is essential for the reconsolidation of cue-associated cocaine memories, as preventing rematuration during the destabilization period impairs subsequent cue-induced cocaine seeking (Wright et al., 2020). Thus, the functional states of cocaine-generated synapses contribute to the dynamics of cocaine memories. Similar dynamics of AMPAR trafficking are observed during destabilization and reconsolidation of other types of memories (Rao-Ruiz et al., 2011), suggesting that the synaptic dynamics are a common mechanism underlying memory dynamics. Theoretically, resiliencing of memory-encoding synapses provides an opportunity for certain synapses to be selected for rematuration and others to be pruned away, with the redefined circuit connectivity updating the memory content.

Another important phenomenon revealed by this line of studies is the dissociation between the functional state of experience-generated synapses and the behavioral expression of memory. During the 1 h testing of cue-induced cocaine seeking, which measures the behavioral expression of cue-associated cocaine memories, cue reexposure resiliences cocaine-generated NAc synapses instantly (<10 min), but high levels of cocaine seeking persist beyond this initial 10 min and last throughout the testing (Wright et al., 2020). A similar dissociation is observed in the behavioral expression of fear memories, in which cue-conditioned freezing persists throughout the memory destabilization window, despite the AMPAR internalization-mediated synaptic weakening (Monfils et al., 2009; Rao-Ruiz et al., 2011). However, if cocaine-generated synapses are resiled before memory reactivation, subsequent cue-induced cocaine seeking is impaired (Wright et al., 2020). These results suggest that cocaine-generated synapses are key substrates for the storage and/or reactivation of cocaine memories, but once the memories are reactivated, behavioral expression is maintained by an independent set of mechanisms. It remains unknown what these mechanisms are, but one possibility is that, once the memory-encoding neural substrates are activated, the resulting circuit dynamics are self-sustaining, supporting the ongoing behavior. Alternatively, other transient adaptations may compensate for the weakening of encoding neural substrates to support the ongoing behavior. Such “compensatory” adaptations are detected in the NAc as transient potentiation of potentially non-cocaine-generated glutamatergic synapses during cue-induced cocaine seeking (Gipson et al., 2013), as well as in the hippocampus as a transient increase in the membrane excitability of engram neurons following memory retrieval (Pignatelli et al., 2018). Regardless of the mechanisms, this division of labor allows memory encoding substrates to be modified without sacrificing the ongoing behavior, and thus has adaptive advantages.

A synaptic substrate for cocaine memories

The concept that memories are encoded by a select population of synapses, or a synaptic engram, has been elaborated in various forms (Chklovskii et al., 2004; Rogerson et al., 2014; Holtmaat and Caroni, 2016). However, it is technically challenging to identify and manipulate such a select set of synapses among large

synapse populations. By taking advantage of their unique cellular features, targetable generation mechanisms, and robust behavioral correlates, investigating cocaine-generated synapses provides a compelling case that cocaine-generated synapses form a synaptic engram to encode aspects of cocaine memories. However, there still remains much to consider regarding how such synaptic engrams contribute to the circuit dynamics that support long-lasting memories.

Theoretical considerations of synaptic engrams

A key form of experience-dependent synaptic plasticity is achieved by modification of the weight at preexisting synapses. Through LTP, LTD, or other forms of weight changes at synapses, select routes of informational flow within the preexisting circuit can be strengthened or weakened, redefining the circuit activity dynamics. However, studies of artificial neural networks predict that such weight changes alone do not provide sufficient capacity to encode multiple memories over extended periods of time (French, 1999; Fusi and Abbott, 2007). This is partially because of spontaneous fluctuation in synaptic strength driven by spurious neural activity within the circuit. While the potential transition of synapses from a relatively plastic state to a rigid state promotes memory stability (Fusi et al., 2005; Benna and Fusi, 2016; Kirkpatrick et al., 2017; Masse et al., 2018), such spontaneous fluctuations, which are commonly detected in animal experiments, can still lead to degradation of precise synaptic weights that encode a given memory, resulting in catastrophic forgetting (Fusi and Abbott, 2007; Ziv and Brenner, 2018). These limitations restrain the capacity of preexisting synapses for forming new, functionally stable synaptic engrams.

On the other hand, experience-induced synapse formation or elimination may substantially rewire the circuit connectivity and provide improved capacity for memory encoding. Counterintuitively, the actual rate at which dendrites make synapses with bypassing axons is extremely low, such that neurons are connected in a sparse wiring framework within a circuit (Markram et al., 1997; Holmgren et al., 2003). This is exemplified in individual NAc MSNs, in which the bypassing axons from the PFC, amygdala, or thalamus exhibit low rates (i.e., 1%–2%) in forming synapse-like connections on a given dendritic segment (Xia et al., 2020). Within such a sparse wiring setup, even moderate levels of synapse formation or elimination can effectively redefine the circuit matrix for new, distinct connectivity patterns with a much higher predicted capacity for memory encoding than the modification of synaptic weight alone (Chklovskii et al., 2004; Knoblauch et al., 2014; Knoblauch and Sommer, 2016). In animal experiments, nascent and immature synapses are shown to be generated by new experience (Arendt et al., 2013; Suvrathan et al., 2013; Chung et al., 2017), and high levels of synaptogenesis are detected during periods of novel learning (Holtmaat and Svoboda, 2009; Peters et al., 2014; Chen et al., 2015; Holtmaat and Caroni, 2016). We argue that these experience-generated synapses are key substrates in creating new connectivity patterns underlying unique circuit activity dynamics; they stand as a distinct synaptic population, a synaptic engram, that supports memory encoding (Fig. 2).

While generation and elimination of synapses are likely more metabolically costly than modifying synaptic weight, this cost is predicted by modeling work to favor circuit modularity, which can facilitate sparse encoding to reduce interference between memories and ultimately increase memory capacity (Ellefsen et al., 2015). Such connectivity-oriented models also emphasize the contribution of the modification of preexisting synapses. For example, formation of new connections may encode core and,

particularly, novel information to form the skeleton of a memory, while modification of preexisting synapses may adjust and rearrange the already encoded information to flesh out the skeleton with details and richness. This is particularly important when considering that few experiences during adulthood are completely novel and new memories often incorporate previously learned information.

An unsolved issue for the connectivity model of memory is spontaneous synaptic turnover (Yang et al., 2009; Attardo et al., 2015). While some synapses persist for the lifetime of a memory, many others undergo continuous turnover (Xu et al., 2009; Yang et al., 2009; Attardo et al., 2015). If every individual synapse is essential for a complete memory, synaptic turnover would lead to progressive memory fragmentation. In the mammalian brain, two neurons are often connected through a compound connection comprising multiple synapses (Stepanyants et al., 2002). For NAc MSNs, compound connections can be achieved by short terminal branches (also called terminal tufts) from a single axonal projection, which forms multiple synapses onto the same dendritic segment (Tripathi et al., 2010; Aransay et al., 2015). Within a relatively homogeneous microenvironment, synapses in a compound connection may share similar spatial and temporal activation profiles. In this case, although activation of individual synapses is stochastic, the integrated activity of these synapses, referred to as the collective dynamics, is predicted to be strikingly stable, resilient to the probabilistic failure or reasonably paced synaptic turnover (Fauth et al., 2015). As a theoretical extrapolation, compound connections may serve as the basic functional modules for a synaptic engram to support stable circuit dynamics for a memory. Apparently, the compound connection is not the only anatomic organization for synapses. Mechanisms that determine how the activity of individual synapses is integrated to generate stable circuit dynamics remain a hot topic for current computational and experimental neuroscience.

The role of engram synapses in neuronal engram and circuit dynamics

Neuronal engrams are formed during learning when individual neurons are recruited into a functionally correlated population to encode a memory (Josselyn and Tonegawa, 2020). Several studies detect increased numbers and/or efficacy of synapses in engram neurons (Ryan et al., 2015; Kitamura et al., 2017; Roy et al., 2017; Choi et al., 2018; Zhou et al., 2019). Furthermore, experience-dependent synaptogenesis or synaptic strengthening appears to occur preferentially between engram neurons across different brain regions in support of the same memory (Kitamura et al., 2017; Choi et al., 2018; Zhou et al., 2019). On the other hand, disrupting experience-dependent synaptogenesis or synaptic potentiation prevents the effective reactivation of neuronal engrams by natural recall cues, resulting in memory failure (Ryan et al., 2015; Kitamura et al., 2017; Roy et al., 2017). While this memory failure can be rescued by strong optogenetic stimulation of engram neurons (Ryan et al., 2015; Roy et al., 2017), the rescue requires the preserved synaptic connections between engram neurons (Roy et al., 2017). These findings suggest that formation and potentiation of synaptic connections are key mechanistic steps in forming and maintaining neuronal engrams and related circuits for memory encoding and recall.

In the context of SUD-related memories, it is tempting to hypothesize that cocaine-generated synapses contribute to the formation of neuronal engrams that encode specific aspects of cocaine memory. As alluded to above, cocaine-generated silent synapses detected in randomly sampled NAc MSNs mature after

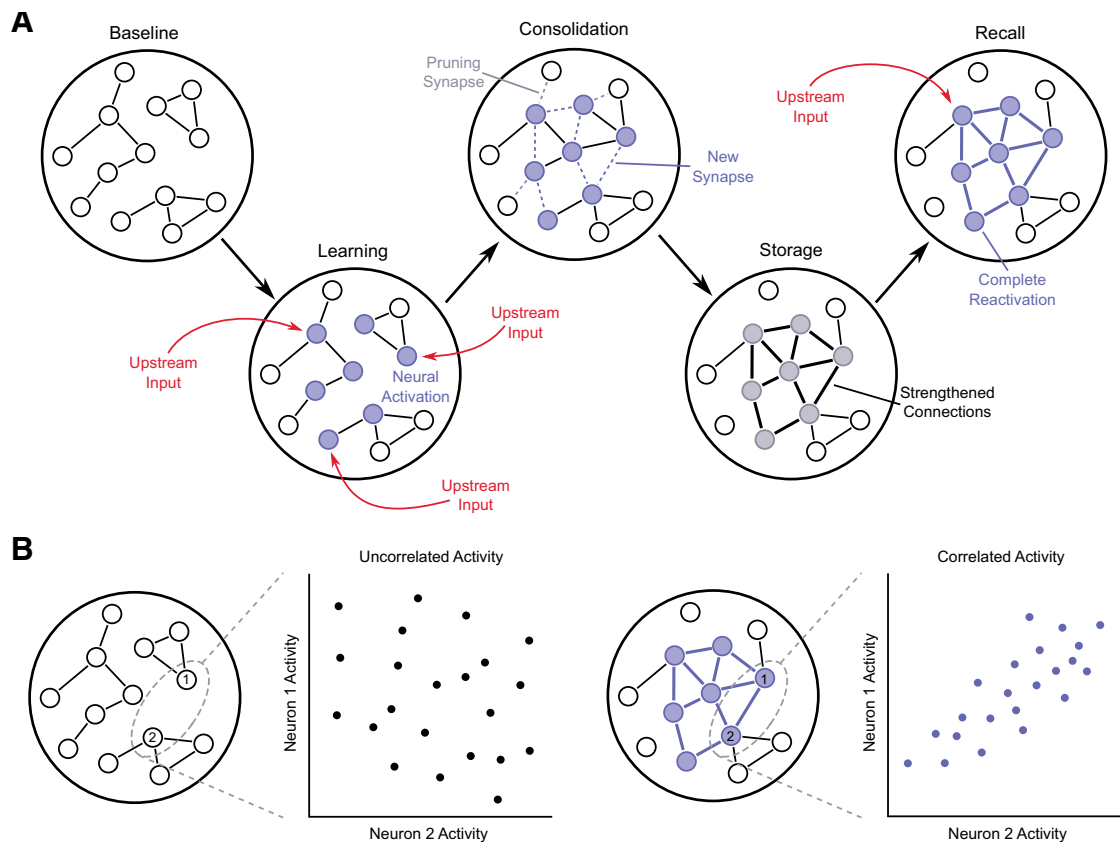


Figure 2. Potential role of synaptogenesis in circuit formation and dynamics. **A**, Schematic depiction of how synaptic remodeling may lead to the formation of a neuronal engram. During learning, strong inputs to the circuit from various upstream areas leads to the activation of select neurons. Activity triggers synaptic remodeling, with formation of new connections between neurons activated together while also potentially pruning away connections between neurons not activated together. This may connect neurons that previously did not interact with one another. Consolidation then proceeds to strengthen the new and preexisting connections between engram neurons while eliminating some connections with other neurons. The strong interconnectivity between engram neurons subsequently allows weaker and incomplete inputs to trigger complete reactivation of the neural engram and allow for memory recall. **B**, Example illustrating one way that synaptogenesis may reshape coactivity dynamics between neurons within a circuit. Before learning, neurons within a circuit may not be connected or share the same inputs. This results in their activity being largely independent of each other and uncorrelated. Following learning-induced formation of new synaptic connections between them, the activity of one of the neurons becomes dependent on the other, resulting in correlated dynamics. Such changes throughout an entire circuit may lead to dramatic changes in the neuronal coactivity patterns.

withdrawal from cocaine self-administration through two cellular steps, switching GluN2B NMDARs to nonGluN2B NMDARs and recruiting AMPARs (Y. Q. Wang et al., 2021). After maturation, these synapses become resilenced on cue reexposure-induced retrieval of cocaine memories (Wright et al., 2020) (Fig. 1). However, another population of silent synapses is detected in a potential cocaine engram/ensemble in the NAc. After withdrawal from repeated intraperitoneal injections of cocaine, silent synapses are preferentially detected in NAc MSNs that express high levels of cFos within 2 h after a locomotor sensitization test, in which animals are reexposed to cocaine injection and/or cocaine-associated cues (Koya et al., 2012; Whitaker et al., 2016). These synapses do not express high levels of GluN2B NMDARs, but they do contribute to the decreased AMPAR-mediated responses and decreased frequencies of AMPAR-mediated EPSCs observed in cFos-positive neurons (Koya et al., 2012; Whitaker et al., 2016). Thus, rather than being formed by synaptogenesis, these synapses are generated through internalization of AMPARs from synapses that have already existed before the final sensitization test. In theory, these silent synapses in cFos-enriched neurons may not belong to the same population of synapses that are generated by the initial cocaine experience, but instead represent a new synapse population specific for the cFos-defined neuronal engram/ensemble. Alternatively, they may be

part of the silent synapse population generated by the initial cocaine experience, mature after drug withdrawal (Fig. 1), but exhibit substantially robust resilencing dynamics within the cFos-defined engram neurons on reexposure to cocaine or cocaine-associated cues after drug withdrawal. In either case, silent synapse-mediated circuit remodeling is correlated with cocaine-induced formation of neuronal engram/ensembles in the NAc.

As discussed above, temporal circuit dynamics are possibly the circuit-level functional manifestation of synaptic and neuronal engrams. Recent results suggest that a unique set of temporal circuit dynamics is often formed gradually through learning processes, relying on synapse-mediated remodeling of circuit connectivity. For example, movement-related circuit activities in the motor cortex become progressively correlated, exhibiting consistent spatiotemporal sequences over repeated motor learning (Komiya et al., 2010; Peters et al., 2014; Adler et al., 2019). In conditioned fear learning, coactivity patterns of the amygdala neuronal population that encode conditioned stimuli become progressively similar to those that encode unconditioned stimuli (Grewe et al., 2017). The range of coactivity patterns of a neural circuit is constrained by the synaptic connectivity, which can, theoretically, be expanded by experience-generated synapses. Consistent with this, disrupting synapse formation or elimination during motor learning prevents the emergence of

stereotypical and spatiotemporal sequences in the motor cortex and impairs behavioral performance (Chen et al., 2015; Adler et al., 2019). These findings suggest a compelling possibility that experience-induced synapse formation and elimination provide key mechanisms for generation of new patterns of circuit activity dynamics that encode new memories.

In associative learning, the emergence of new circuit dynamics is, in part, driven by neurons acquiring stimulus- or behavior-specific responses over time (Vega-Villar et al., 2019; Ahmed et al., 2020). For cue-associated drug memories, following cocaine self-administration training and withdrawal, some prior cue-insensitive NAc neurons become cue-responsive, with the relative number of new responsive neurons predicting the magnitude of cue-induced cocaine seeking (Hollander and Carelli, 2005, 2007; Guillem et al., 2014). These newly recruited neurons may be particularly important in conditioning cues with cocaine to form a cue-cocaine association. To form such an association, neural substrates encoding cues versus cocaine must interact to establish a link between neurons of different engrams. Interestingly, the emergence of new, cue-responsive NAc neurons after cocaine experience follows a similar time course to the generation and maturation of silent synapses (Hollander and Carelli, 2007; Lee et al., 2013; Guillem et al., 2014), leading us to speculate that such an associative link is mediated by new synaptic connections.

Conclusions and future directions

In the context of a circuit- and engram-based understanding of memory, we have attempted to provide a conceptual perspective that silent synapses generated by cocaine experience stand as a distinct set of synapses, or a synaptic engram, that encodes key aspects of cocaine memories. Either by remodeling an established circuit or expanding the circuit through recruiting additional neurons, these synapses may create new connectivity patterns and, thus, new circuit activity dynamics that represent newly acquired cocaine-associated memories.

Our literature analyses reveal some key questions for future studies. A clearly important one is how synaptic remodeling creates new, memory-encoding circuit activity dynamics. Based on limited evidence, we speculate that new synaptic connections contribute to the formation of both neuronal engrams and new circuit activity patterns, yet direct evidence linking the synaptic modification to circuit dynamics remains lacking. This missing link is particularly prominent in the context of SUD-associated memories, in which the circuit activity patterns that are unique to drug experience have not been identified. Another question regards the relative contributions of experience-generated synapses versus experience-modified synapses to memory encoding. We speculate that modifications of preexisting synapses preferentially retune the established circuit connectivity for incorporating previously learned information while synaptogenesis preferentially creates new circuit connectivity patterns for novel information/experiences. This speculation needs to be tested and refined with empirical data. While exceedingly challenging, addressing these and other related questions will open the possibility to precisely target and manipulate memory traces uniquely associated with drug memories and treat addictive disorder.

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