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Neural mechanisms underlying incubation of methamphetamine craving: A mini-review

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Abstract

Cue-induced drug craving and seeking progressively increases during abstinence. This "incubation of drug craving" phenomenon has been observed in both laboratory animals and humans. Preclinical studies identified several neural mechanisms underlying incubation of drug craving after forced abstinence, primarily focusing on cocaine. Recently, studies started focusing on another powerful psychostimulant, methamphetamine (Meth), and developed new incubation procedures (choice-induced and punishment-imposed abstinence). Here, we review mechanistic studies at the circuit, synaptic and molecular levels on incubation of Meth craving. First, we provide an overview of neural adaptations associated with prolonged forced abstinence after extended-access Meth self-administration. Next, we review studies examining the causal roles of discrete brain regions and associated circuits, glutamate transmission, histone deacetylase 5 and oxytocin in incubation of Meth craving after forced abstinence. Lastly, we review causal and correlational studies examining the mechanisms underlying incubation of Meth craving after choice-induced voluntary abstinence and punishment-induced abstinence, respectively. We conclude by discussing the translational potential of these mechanistic studies in Meth relapse prevention in human drug users.

Keywords

addiction; methamphetamine; incubation of craving; relapse; circuit; molecular

1. Introduction

Methamphetamine (Meth) is a powerful psychostimulant and currently no approved pharmacological treatments are available for Meth addiction[1]. A major obstacle for treating drug addiction, including Meth, is relapse during abstinence[2–4], often triggered by re-exposure to drug-associated cues[5]. To account for this persistent relapse, Gawin and Kleber[6] in 1986 proposed that cue-induced cocaine craving progressively increases during the early weeks of abstinence and remains high over prolonged abstinence. In the early 2000s, an

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analogous incubation phenomenon was identified in rats, termed incubation of drug craving, based on the findings that cocaine and heroin seeking progressively increases after forced abstinence from drug self-administration[7,8]. Subsequent studies demonstrated that incubation of drug craving also occurs in rats with a history of Meth[9,10], alcohol[11], nicotine[12] and sucrose[13] self-administration. Moreover, incubation of drug craving in rats is contributed by both discrete and contextual drug cues, with discrete cues potentiating the non-reinforced operant responding[14]. From a clinical perspective, this incubation phenomenon mimics relapse after forced abstinence, such as incarceration or hospitalization[15], and has been demonstrated in humans across multiple drug classes, including cocaine[16], alcohol[17,18], nicotine[19] and Meth[20].

Over the last two decades, several groups have identified neural mechanisms underlying incubation of drug craving after forced abstinence, primarily focusing on cocaine[21–24]. While early studies had investigated changes of dopaminergic functions in the mesolimbic system associated with prolonged abstinence after extended-access Meth self-administration[25,26], it was only five years ago when we and others began to examine the causal roles of distinct neural mechanisms underlying incubation of Meth craving[27–29]. Furthermore, modified incubation procedures were developed to investigate neural mechanisms underlying incubation of Meth craving after choice-induced (food or social interaction) voluntary abstinence [30,31] and after punishment-induced and forced abstinence [32] (hereafter shortened to punishment-induced abstinence). These procedures mimic relapse in humans after cessation of contingency management [33,34], which employs either non-drug rewards or negative consequences associated with drug use [35,36].

In this review, we discuss neural mechanistic studies at the circuit, synaptic and molecular levels on incubation of Meth craving after forced abstinence, choice-induced voluntary abstinence and punishment-induced abstinence in the past decade. In addition, we discuss similarities and differences in neural mechanisms between incubation of Meth and cocaine craving after forced abstinence. We also compare neural mechanisms underlying incubation of Meth craving after forced abstinence with after choice-induced voluntary abstinence. Note that unless specified, all studies discussed below used male rats. A glossary of terms used in this review (blue font) is in Box 1.

2. Neural mechanisms underlying incubation of Meth craving after forced abstinence

Most studies so far have focused on examining neural mechanisms underlying incubation of Meth craving after forced abstinence. Among these, several studies built on prior knowledge on incubation of craving to other drugs, primarily cocaine, and demonstrated either similar or distinct neural mechanisms. Below we first describe main findings from correlational studies that examined neural adaptations associated with prolonged withdrawal (beyond 24 h) from extended-access Meth self-administration (6 h or more/d), and refer readers for further details summarized in Table 1. Note that studies combining correlational and causal investigations in incubation of Meth craving are discussed in subsequent sections. Additionally, we focus on studies using extended-access self-administration because of the

early observations that incubation of craving is more robust after extended than short (e.g., 2 h/d) access self-administration training[37]. Next we describe studies that examined the causal roles of discrete brain regions and associated circuits, glutamate transmission, epigenetic mechanisms and oxytocin in incubation of Meth craving. We summarize these findings and list parallel cocaine studies in Table 2.

2.1 Neural adaptations associated with prolonged withdrawal from extended-access Meth self-administration

Early studies began focusing on dopaminergic pathways, but together with later findings, yielded conflicting results. Shepard et al.[25] combined *in situ* hybridization with Western blotting and reported that tyrosine hydroxylase (TH) mRNA and protein expressions in ventral tegmental area (VTA) and substantia nigra compacta (SNC) increase on withdrawal day 1 but not 30. On either withdrawal days, dopamine transporter (DAT) mRNA levels in VTA and SNC, or TH protein and DAT mRNA levels in nucleus accumbens (NAc) and dorsal striatum (DS) exhibit no change. A recent study also reported an increase of DAT, but not TH or dopamine 2 receptor (D2R) protein expression in striatum on withdrawal day 3[38].

In contrast, Krasnova et al.[26] used high performance liquid chromatography (HPLC) with electrochemical detection and reported persistent decrease of dopamine (DA) levels in rat striatum on withdrawal day 1, 7 and 14, as well as decreased DA levels in cortex on withdrawal day 14. Authors also observed decreased TH and DAT protein expressions in both rat striatum and cortex on withdrawal day 14. McFadden et al.[39] reported decreased dopamine uptake and DAT protein expression in striatal tissue on both withdrawal day 8 and 30, but with no changes of DA levels or TH protein expression. Krasnova et al.[40] later extended their previous findings and found sustained decrease in dorsal striatal DA after 1-month withdrawal. This is accompanied with decreased protein expressions of D2R [but not dopamine 1 receptor (D1R)], transcription factors [ΔFosB, cFos, cAMP response element protein (CREB) and phosphorylated CREB (pCREB)], brain-derived neurotrophic factor (BDNF) and tyrosine kinase B receptor (TrkB) in DS.

The main factor that contributes to the inconsistent results above is the daily Meth dose during self-administration. For example, the Meth intake in studies from Krasnova et al.[26,40] was about 15 mg/kg/d, more than doubled than those in other studies[25,38,39]. Therefore, neural adaptations observed by Krasnova et al.[26,40] are possibly due to neurotoxic effect of Meth. Indeed, authors reported increased protein expression of glial fibrillary acidic protein (GFAP, a cellular marker for astrocytes) in both striatum and cortex on withdrawal day 7[40]. In contrast, no changes of GFAP protein expressions across cortical and striatal areas are detected in rats with an average Meth intake about or below 6 mg/kg/d[39,41].

In the past 5 years, we also examined transcriptional regulations across multiple brain areas[42–44] during incubation of Meth craving. In the first study[42], we reported that mRNA expressions of *Bdnf* and *TrkB*, glutamate receptors, and epigenetic enzymes in DS homogenate exhibit minimal changes on either withdrawal day 2 and 35, compared with saline rats. In contrast, we found increased mRNA expressions of several genes in Fos [the neuronal activity marker[45]]-positive DS neurons [isolated by fluorescence activated cells sorting (FACS)] after the late withdrawal day-relapse tests (withdrawal day 30 to 50),

compared with non-activated DS neurons in Meth rats. These data suggest that unique molecular alterations associated with Meth seeking occur selectively in Fos-positive neurons during prolonged withdrawal from Meth self-administration. In a follow-up study[43], we examined the same genes in Fos-positive neurons in dorsomedial striatum (DMS) and anterior intralaminar nucleus of thalamus (AIT) after the day-30 relapse test in both saline and Meth rats. Unexpectedly, while we observed changes of mRNA expressions of several genes in Fos-positive neurons compared with Fos-negative neurons in both brain regions, these gene alterations are not drug-specific and also occur in rats that self-administered saline. In future studies, genome-wide analysis of these behaviorally-activated neurons may reveal drug-specific gene alterations.

Recently, we used RNA-sequencing and examined transcriptional regulations in the orbitofrontal cortex (OFC) and the central amygdala (CeA) during incubation of Meth craving[44]. We reported a 10-fold increase in differentially expressed genes (DEGs) in CeA on withdrawal day 35 compared to withdrawal day 2. Additionally, upregulated DEGs on withdrawal day 35 are enriched in various biological processes, such as histone modifications and protein ubiquitination. In contrast, much fewer DEGs are identified in OFC, with more DEGs on withdrawal day 2 than withdrawal day 35. These findings are consistent with the critical role of CeA, but not OFC, in incubated Meth seeking[27] (see more details in 2.2).

Additional focuses include neurogenesis in hippocampus and glutamate-associated synaptic plasticity across striatum, medial prefrontal cortex (mPFC) and perirhinal cortex (PRH). Recinto et al.[46] reported increased number of progenitor cells and expression of a biomarker (Ki-67) for cell proliferation in hippocampus on withdrawal day 28, implicating a potential role of neurogenesis in hippocampus in Meth relapse. Regarding glutamate-associated synaptic plasticity, Reichel et al.[47] and Schwendt et al.[48] reported on withdrawal day 14, metabotropic glutamate receptor 5 (mGlu5) expression in crude membrane fraction of PRH decreases, and surface and total protein expression of mGlu2/3 in both NAc and DS decrease, accompanied with decreased surface expression of mGlu2/3 and mGlu7 in mPFC. Furthermore, mGlu2 alone exhibits no changes in either mPFC or PRH on withdrawal day 7, although changes coupled with serotonin receptors were reported in both brain regions[49]. It is of note that Schwendt et al.[48] also demonstrated that extinction training after Meth self-administration reverses the decrease of mGlu2/3 in NAc and DS as described above, which support the notion that dissociable neural mechanisms underlie extinction training versus abstinence after drug self-administration[22,50–53]

In addition, Reichel et al.[54] and Scofield et al.[55] reported that PRH, on withdrawal day 7, exhibits decreased GluN2B expression in crude membrane fraction, decreased GluN2B surface expression and inability for LTD induction, an effect reversed by an NMDA receptor agonist. Furthermore, on withdrawal day 8, Mishra et al.[56] demonstrated decreased AMPA/NMDA ratio in mPFC, possibly contributed by increased NMDA-mediated currents and increased GluN2B surface expression. In contrast, in NAc, changes occur presynaptically, with decreased paired-pulse ratio and increased frequency of spontaneous excitatory postsynaptic potential (sEPSC)[56]. These observations above, especially in PRH, have been linked to novel object memory deficits after withdrawal from Meth self-administration, and

mGlu5 in PRH plays a critical role in shifting rats from seeking Meth-associated cues to novel cues during relapse tests[57].

Lastly, two studies examined sex differences after withdrawal from Meth self-administration. At the synaptic level, Pena-Bravo et al.[58] reported females exhibit decreased basal glutamate excitatory strength (e.g., lower amplitude of sEPSC) in prelimbic cortex (PL) between withdrawal day 9 and 14, compared with males. Furthermore, female Meth rats, but not male Meth rats, exhibit increased amplitude of evoked EPSC compared with their respective saline groups. At the transcriptional level, Daiwile et al.[59] reported that in NAc, females have higher baseline levels of prodynorphin (*Pdyn)* mRNA than males, but on withdrawal day 30, *Pdyn* mRNA expression selectively increases in males and hypocretin receptor 2 (*Hcrtr2)* mRNA expression selectively decreases in females. It is of note that while Pena-Bravo et al.[58] and Daiwile et al.[59] reported no sex differences in Meth seeking, Daiwile et al.[59] reported higher Meth intake in males than females during self-administration, which may be potentially due to different rat strains and contribute to the sex differences in transcriptional regulations.

2.2 Role of discrete brain regions and associated neural circuits

Central amygdala (CeA)—In a functional mapping study, we reported that CeA inactivation by a mixture of $GABA_A$ and $GABA_B$ agonists (muscimol + baclofen) decreases Meth seeking on withdrawal day 35, but not day 2, indicating a critical role of CeA in incubation of Meth craving[27]. This finding is in line with earlier studies showing that inactivation of CeA activity decreases incubation of cocaine[60,61], nicotine[62] and sucrose craving[63] assessed by the self-administration procedure, as well as incubation of morphine craving assessed by the conditioned place preference procedure[64]. Moreover, CeA has been identified as a common hub for incubation of cocaine, heroin and sucrose craving, based on the analyses of synaptic proteins, gene expressions and neurotransmitters related to glutamatergic, GABAergic and endocannabinoid systems across cortical, striatal and amygdalar regions[65]. Taken together, these findings implicated CeA as a common anatomical locus underlying incubation of craving to drug and non-drug reward.

Additionally, we reported that dorsomedial PFC (dmPFC), ventromedial PFC (vmPFC), OFC and basolateral amygdala (BLA) inactivation has no effect on Meth seeking on withdrawal day 35[27]. In contrast to the previous studies implicating these brain regions in incubation of cocaine[66–69] and opioid craving[70,71], the negative findings here suggest that the role of these brain regions in incubation of craving might be drug-specific or beyond acute neuronal activity assessed by the reversible inactivation approach.

Two studies further dissected the cell-type specific role of CeA subregions (lateral vs medial division, CeL vs CeM) in incubation of Meth craving after either forced abstinence or social choice-induced voluntary abstinence[31,72]. Here we briefly describe the main findings on the forced abstinence procedure (also see more discussion in Section 3). The authors reported that Meth seeking on withdrawal day 15 is associated with *Fos* induction in CeM output neurons and somatostatin (SOM)-expressing neurons in CeL[31]. Moreover, SOM knockdown in CeL, which selectively decreases *Fos* expression of SOM-expressing neurons, decreases Meth seeking on withdrawal day 15 (but not day 1), accompanied with decreased Fos

expression in CeM output neurons[72]. Overall, these data demonstrated a critical role of SOM-expressing neurons in CeL in incubation of Meth craving after forced abstinence.

Dorsal striatum (DS) and associated circuits—DS is another brain region implicated in incubation of Meth craving. First, we reported that Meth seeking between withdrawal day 30 and 50 is associated with increased *Fos* mRNA of both dopamine receptor 1 (*Drd1*)- and dopamine receptor 2(*Drd2*)-expressing neurons in DS[42]. DS Injections of SCH23390, a D1-family receptor antagonist that inhibits cue-induced Fos induction in striatum[73], decreases Meth seeking on withdrawal day 30, but not day 2[42], demonstrating a critical role of D1R-mediated signaling in DS in incubation of Meth craving. Furthermore, SCH23390 injections into either DS subregion (dorsomedial or dorsolateral striatum, DMS or DLS) alone also decreases Meth seeking on withdrawal day 30[42]. This finding is in contrast to the previous studies showing dissociable roles of DMS and DLS in cue-controlled cocaine seeking[74], and context-induced reinstatement of cocaine[75] and Meth seeking[76].

In a follow-up circuit mapping study[28], we reported that Meth seeking on withdrawal day 30 is associated with activation of AIT (assessed by Fos) and AIT→DMS projections (assessed by Fos and a retrograde tracer, cholera toxin B, CTb). Muscimol + baclofen injections into lateral AIT (AIT-L), but not medial AIT (AIT-M), decreases Meth seeking on withdrawal day 30. In addition, anatomical disconnections of AIT-L→DMS, which disrupted the local interaction between glutamatergic projections (muscimol + baclofen injections into AIT-L) and postsynaptic D1R signaling (SCH23390 injections into contralateral DMS), decrease Meth seeking on withdrawal day 30, but not day 1. Our recent circuit mapping study focusing on DLS also showed that incubated Meth seeking was associated with activation of AIT→DLS projections in female rats, suggesting that AIT→DLS may also play a role in incubation of Meth craving[77]. These results, together with recent findings showing the reinforcing effect of optogenetic activation of AIT→DS projections in mice[78], highlight the novel role of AIT→DS in reward seeking behavior.

2.3 Role of glutamate transmission

Caprioli et al.[30] assessed the role of mGlu2, primarily expressed on presynaptic glutamatergic neurons[79], on incubation of Meth craving after either forced abstinence or food choice-induced voluntary abstinence. Regarding the forced abstinence model, they found that systemic injections of the novel positive allosteric modulator (PAM) of mGlu2, AZD8529, which decreases evoked glutamate release[80,81], decreases Meth seeking on withdrawal day 21, but not day 1, indicating a critical role of mGlu2 in incubation of Meth craving. This finding is in agreement with the early observation that decreased mGlu2/3 surface expression in PFC, NAc, and DS, is associated with prolonged withdrawal from Meth self-administration[48], and that systemic injections of LY379268 (a mGlu2/3 agonist) also decreases incubation of cocaine craving after forced abstinence[61]. Moreover, systemic injections of AZD8529 decreases cue-induced reinstatement of nicotine and alcohol seeking in squirrel monkeys[82] and rats[83], suggesting that mGlu2 plays critical roles in relapse across drug classes.

Two studies from the Wolf group focused on the role of Ca^{2+}-permeable AMPA receptors (CP-AMPARs) and mGlu1-mediated synaptic depression in NAc core in incubation of Meth craving[29,84], which was built on their seminal findings with cocaine over the past 12 years[22,23,85–88]. First, Scheyer et al.[29] used whole-cell patch clamp electrophysiological recording and reported that bath-applied Naspm, a CP-AMPAR antagonist, produces a significantly greater reduction of EPSC held at −70 mv ($EPSC_{-70\ mv}$) in NAc core in Meth than saline rats on withdrawal day 7–8, an effect lasting beyond withdrawal day 40. Additionally, bath-applied SYN119, an mGlu1 PAM, decreases $EPSC_{-70\ mv}$ in NAc core of Meth rats after withdrawal day 40, with no further decrease observed after subsequent Naspm applications. Finally, authors reported that either a single intra-NAc injection of Naspm or systemic injection of SYN119 decreases incubated Meth seeking beyond withdrawal day 40. In a follow-up study, Murray et al.[84] used biochemical methods and reported that incubation of Meth craving is accompanied with increased GluA1 translation in NAc core, but no changes in GluA1–3, mGlu1 surface and total protein expression, or coupling between mGlu1 and its scaffolding protein Homer. Moreover, authors reported that repeated systemic injections of SYN119 during early withdrawal has no effect on delaying the elevation of Meth seeking on withdrawal day 9.

Taken together, two studies above demonstrated both similarities and differences in the role of glutamate adaptations in NAc core between incubation of Meth and cocaine craving. Similar to cocaine[85,88,89], CP-AMPARs and mGlu1-mediated synaptic depression emerge in NAc core during incubation of Meth craving and contribute to the long-term maintenance of incubated Meth seeking; another shared neural adaptation is increased GluA1 translation, suggesting that elevated CP-AMPARs in NAc core could be contributed by accumulation of homomeric GluA1 with both drugs. Regarding differences from cocaine, there are three major ones. First, incubation of Meth craving exhibits an accelerated time-course of elevating CP-AMPARs in NAc core, which occurs as early as 7-day withdrawal, compared with 1-month withdrawal from cocaine. In parallel, Meth seeking is maximal on withdrawal day 7[14,29], while cocaine seeking starts rising on withdrawal day 7 and reaches maximal 1–2 months later[21,37]. These results suggest that elevations of CP-AMPARs in NAc core might contribute the maximal expression of incubated cocaine or Meth seeking. However, no studies have directly examined the causal role of CP-AMPARs in NAc core in cocaine or Meth seeking on withdrawal day 7. Second, unlike cocaine[85,88], incubation of Meth craving is not accompanied with increased GluA1 or decreased mGlu1 surface expression that could underlie the electrophysiological manifestation of CP-AMPARs and mGlu1-mediated depression in NAc core. These results suggest that accumulation of CP-AMPARs in NAc core during incubation of Meth craving might be contributed by subtle biochemical changes that are below the sensitivity of detection. Finally, repeated systemic mGlu1 PAM blocks incubation of cocaine[85], but not Meth craving[84]. However, it is noted that the duration of mGlu1 PAM treatment for cocaine is twice as long as for Meth, which might explain the differences between two studies.

2.4 Other mechanisms

Two studies examined additional mechanisms underlying incubation of Meth craving after forced abstinence, including the epigenetic enzyme, histone deacetylase 5 (HDAC5) and the

neuropeptide, oxytocin. In the first study, we reported that viral-mediated overexpression of the nuclear-localized HDAC5 and knockdown of HDAC5 in DS increases and decreases Meth seeking on withdrawal day 30, respectively; neither manipulation affects Meth seeking on withdrawal day 2[90]. These findings together demonstrated a critical role of HDAC5 in DS in incubation of Meth craving, which are in contrast with the previous study showing that overexpression of the nuclear-localized HDAC5 in NAc decreases reinstatement of cocaine seeking after short-access cocaine self-administration training and extinction[91]. However, direct comparisons between cocaine and Meth study should be made with caution, because of different self-administration procedures (short-access vs long-access) and relapse models (reinstatement after extinction vs incubation of craving) used, and brain regions of interest (NAc and DS).

In the second study, Everett et al.[92] reported that daily systemic injections of oxytocin between withdrawal day 6 and 20 decrease incubated Meth seeking on withdrawal day 30 and subsequent Meth-primed reinstatement of Meth seeking in rats of both sexes, and yohimbine-induced reinstatement of Meth seeking in female rats. Interestingly, this effect of chronic oxytocin is selective in rats with a history of extended-access, but not short-access self-administration training, which may be attributed to the role of oxytocin in offsetting the increased anxiety observed in extended-access rats. Overall, these results extend previous findings on the role of oxytocin in reinstatement of Meth seeking[93–99], and from a clinical perspective, implicates oxytocin as a potential treatment strategy to prevent Meth relapse. Finally, these results are also consistent with cocaine studies in which systemic or brain-site specific injections of oxytocin reduce reinstatement of cocaine seeking[100–104], suggesting a shared role of oxytocin in cocaine and Meth relapse.

2.6 Summary

Several studies have demonstrated neural adaptations at transcription and synaptic levels across multiple brain regions during prolonged withdrawal from extended Meth self-administration, but direct comparison should be made with caution, because of different Meth self-administration procedures used across these studies. In contrast, only a handful of studies identified neural mechanisms that play causal roles in incubation of Meth craving. These studies not only uncovered similarities (e.g., critical roles of CeA and CP-AMPARs in NAc core) and differences (e.g., roles of vmPFC and BLA) between incubation of cocaine and Meth craving, but also demonstrated novel brain circuits underlying incubation of Meth craving (e.g., AIT→DMS projections). Finally, while all functional studies described above used male rats, it is possible that these findings do not apply to female rats, based on the observations that transcriptional and synaptic changes exhibit sex differences during withdrawal from Meth self-administration. Indeed, as described above, chronic oxytocin treatment led to selective decrease of yohimbine-induced reinstatement of Meth seeking in female rats[92].

3. Neural mechanisms underlying incubation of methamphetamine craving after choice-induced voluntary abstinence

Below we discuss five studies examining mechanisms underlying incubation of Meth craving after choice-induced voluntary abstinence, as well as similarities and differences compared with incubation of Meth craving after forced abstinence. It is important to note that while incubation of Meth craving occurs after food choice-induced voluntary abstinence[30], social choice-induced voluntary abstinence prevents incubation of Meth craving[31]. We summarized the causal findings from these studies in Table 2.

Food choice-induced voluntary abstinence

Caprioli et al.[30] reported that systemic injections of AZD8529, an mGlu2 PAM, decrease incubated Meth seeking after forced or food choice-induced voluntary abstinence on withdrawal day 21, but not day 1, indicating a generalized role of mGlu2 in incubation of Meth craving after forced (see more discussion in Section 2.2) and choice-induced voluntary abstinence. Two additional studies focused on the role of DS and NAc in incubation of Meth craving after food choice-induced voluntary abstinence. Caprioli et al.[105] reported that incubated Meth seeking on withdrawal day 21, but not day 1, is associated with *Fos* induction in *Drd1*- and *Drd2*-expressing cells in DMS, but not DLS. The absence of *Fos* induction in DLS, compared with the previous forced-abstinence study[42], provides the initial evidence for the dissociable mechanisms underlying incubation of Meth craving after forced versus choice-induced voluntary abstinence. Furthermore, DMS injections of SCH39166 or raclopride (D1R- and D2R-family antagonists, respectively) decreases Meth seeking on withdrawal day 21, but not day 1. Additionally, chemogenetic ablation of Fos-expressing DMS neurons activated by relapse tests in *Fos-LacZ* transgenic rats decreases Meth seeking on withdrawal day 21. Together with our previous study[42], these findings indicate that DMS plays a generalized role in incubation of Meth craving after forced abstinence and food choice-induced voluntary abstinence.

In a subsequent study, Rossi et al.[106] reported that incubated Meth seeking on withdrawal day 15, but not day 1, is associated with *Fos* induction in *Drd1*- and *Drd2*-expressing cells in NAc core, but not NAc shell. Furthermore, NAc core, but not shell, injections of muscimol +baclofen decreases Meth seeking on withdrawal day 15, but not day 1. NAc core injections of SCH39166 or raclopride also decrease Meth seeking on withdrawal day 15. Taken together with the previous study demonstrating a critical role of CP-AMPARs in NAc core in incubation of Meth craving after forced abstinence[29], these results suggest a generalized role of NAc core in incubation of Meth craving after either forced or choice-induced voluntary abstinence.

Social choice-induced voluntary abstinence

Two studies focused on CeA, previously implicated in both incubation of Meth craving after forced abstinence[27] and Meth relapse after food choice-induced voluntary abstinence[107], and explored neural mechanisms underlying the suppressing effect of social choice-induced voluntary abstinence on incubation of Meth craving. First, Venniro et al.[31] reported that on withdrawal day 15, while Meth seeking after forced abstinence is associated with *Fos*

induction in CeM and SOM-expressing neurons in CeL, Meth seeking after social choice-induced voluntary abstinence is only associated Fos induction in protein kinase C δ (PKCδ)-expressing neurons in CeL. In a follow-up study, Venniro et al.[72] demonstrated that SOM-expressing neurons in CeL plays a critical role in incubation of Meth craving after forced abstinence (see more details in Section 2.2). In contrast, PKCδ knockdown in CeL, which decreases neuronal activation of PKCδ-expressing neurons, increases Meth seeking on withdrawal day 15 (but not day 1) after social choice-induced voluntary abstinence, accompanied with increased Fos induction in CeM. These results indicated that PKCδ-expressing neurons in CeL, through inhibiting neuronal activation of CeM output neurons, play a critical role in the inhibitory effect of social choice-induced voluntary abstinence on incubation of Meth craving. Together, these findings provide the initial evidence at the function level for abstinence-dependent neural mechanisms underlying incubation of Meth craving.

4. Neural mechanisms underlying incubation of Meth craving after punishment-induced abstinence

So far only three correlations studies examined the molecular adaptions and circuit activities associated with incubation of Meth craving after punishment-induced abstinence. Below we discuss the main findings from these studies and further details are summarized in Table 1. At the molecular level, Krasnova et al.[108] reported mRNA levels of oxytocin in NAc and cocaine-and-amphetamine-regulated transcript (CART) prepropeptide (*CARTpt)* in DS selectively increase in shock-resistant rats 30 days after the last punishment session. Authors also observed changes of additional genes (e.g., oxytocin receptors, metabolic enzymes) in NAc or DS, but these changes are either similar or relative to yoked-shock control rats, indicating an effect of shock alone. Later, Torres et al.[109] focused on neurotrophins and associated mitogen-activated protein kinase (MAPK) signaling in DS and reported increased mRNA expression of several neurotrophins, including *Bdnf*, in shock-sensitive rats, compared with shock-resistant rats, 30 days after the last punishment session. In both shock-sensitive and shock-resistant rats, BDNF protein level increases in DS, while increases of tyrosine kinase A phosphorylation and several phosphorylated proteins associated with MAPK are only observed in short-sensitive rats. However, no shock control rats were used in this study and therefore it is unclear whether these effects are associated with shock or not.

At the circuity level, Hu et al.[110] used fMRI and identified neural circuits associated with Meth self-administration and punishment-induced abstinence. Authors reported an increase in circuit strength of OFC→DMS projections and a decrease in circuit strength of prelimbic cortex (PrL)→ventrolateral striatum(VLS) projections after 20-d Meth self-administration. Shock-resistant rats exhibit strengthening of the negative connectivity after 5-d punishment sessions, while circuit strength of PrL→VLS returns to baseline. Interestingly, circuitry strength in all rats return to baseline after 30-day withdrawal from the last punishment session, suggesting that these changes might not be implicated in incubation process after punishment-induced abstinence.

5. Conclusion and future directions

In the past five years, a growing body of studies started examining causal roles of neural mechanisms underlying incubation of Meth craving, which extended studies focusing on correlations between neural adaptation and prolonged withdrawal from extended-access Meth self-administration. The majority of these causal studies used forced abstinence procedures. Furthermore, these causal studies identified either parallel or distinct mechanisms between incubation of Meth and cocaine craving (see Table 2). Together with evidence demonstrating distinct neural mechanisms between incubation of craving to opioid and psychostimulants[27,70,71], these findings indicated that neural mechanisms underlying incubation of drug craving differ not only across drug classes, but also within drug classes.

A handful of studies examined causal neural mechanisms underlying incubation of Meth craving after choice-induced voluntary abstinence. Compared with studies using forced abstinence procedure, these studies also identified neural mechanisms that are either abstinence-independent or abstinence-dependent. Especially the new study from Venniro et al.[72] highlighted differences in cell-type specific microcircuitry within the CeA that plays critical roles in incubation of Meth craving after forced abstinence as opposed to social choice-induced abstinence. In contrast, studies focusing on punishment-induced abstinence have all been association studies. Furthermore, it is difficult to disentangle neuroadaptations using the punishment procedure as the shock alone leads to molecular changes (see details in Section 4).

Together, these causal studies provided potential therapeutic targets (e.g., CP-AMPARs, oxytocin) for future clinical studies to develop effective treatment for preventing Meth relapse and to further our understanding of the neurological process of Meth users who undergo voluntary abstinence. These studies also raised additional key questions for future preclinical studies: what contributes to the distinct neural mechanisms underlying incubation of cocaine versus Meth craving after forced abstinence? What neural mechanisms play a casual role in incubation of Meth craving after punishment-induced abstinence? Finally, correlational studies identified sex differences in neural substrates during incubation of Meth craving and it would be important to examine whether these substrates play causal roles at the behavioral level.

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Box 1.

Glossary of behavioral and biological terms

Behavioral terms	
Choice-induced (food or social interaction) voluntary abstinence	An abstinence procedure developed to model contingency management therapy[33]. Rats are first trained to self-administer alternative reward (food or social interaction), followed by Meth. The delivery of alternative reward and drug is paired with its respective discrete cues (e.g., tone and light). After training, rats undergo daily mutually exclusive choice procedures between alternative reward and Meth. Caprioli et al.[30] and Venniro et al.[31] demonstrated that during choice procedures, rats prefer alternative reward over drug, and therefore achieve choice-induced voluntary abstinence.
Conditioned-place preference	A Pavlovian conditioning procedure measuring the rewarding effects of drugs by determining preference for a drug-associated environment
Contingency management	A behavioral therapy that uses non-drug rewards (e.g., token) or punishments to reinforce abstinence in humans[33].
Forced abstinence	An abstinence procedure in which rats simply return to their home cages after self-administration training, with no access to the operant chamber.
Incubation of drug craving	Time-dependent increases in cue-induced drug seeking following withdrawal[7].
Punishment-induced and forced abstinence	An abstinence procedure to model voluntary abstinence imposed by adverse consequences, a phenomenon observed in humans[35,36,111,112]. Rats are first trained to self-administer Meth paired with a discrete cue. After training, rats undergo the daily punishment sessions, during which 50% of responses on the active lever and Meth infusions are paired with a foot shock[32]. Krasnova et al.[32,108] demonstrated that during the punishment phase, self-administration decreases in a subset of rats (shock-sensitive rats), achieving punishment-induced abstinence. In contrast, a subset of rats continues Meth self-administration despite of the shock (shock-resistant rats). After the punishment phase, incubation of drug craving occurs in both shock-resistant and shock-sensitive rats after a period of forced abstinence, with higher drug seeking behavior in shock-resistant than shock-sensitive rats during both early and prolonged withdrawal.
Reinstatement	A procedure modeling relapse behaviors testing for a reemergence of drug seeking behavior after the behavior has been previously extinguished. The reinstatement of drug seeking behavior can be elicited by a drug-associated cue, non-contingent drug exposure, or stress.
Biological terms	
Ca^{2+}-permeable AMPA receptor	A type of AMPA receptors that lack of GluA2 subunits. Unlike GluA2-containing AMPA receptors, GluA2-lacking AMPA receptors are calcium permeable, and exhibit greater conductance, inward rectifying currents and a unique pharmacological profile (e.g., blocked by polyamine drugs, such as Naspm).
Cocaine-and-amphetamine-regulated transcript prepropeptide	A neuropeptide that is differentially expressed in the striatum when a rat is injected with cocaine or amphetamine[113]. CARTpt modulates stimulant-induced behaviors such as locomotor activity[114].
Histone deacetylase 5	Class II histone deacetylase responsible for removing acetyl groups from the N-terminal tail of histones.
mGlu1-mediated synaptic depression	A type of synaptic depression expressed postsyanptically in response to activation of mGlu1, which leads to endocytosis CP-AMPA receptors.
Oxytocin	A neuropeptide hormone with anxiolytic effects
Positive allosteric modulator	Ligands that bind to an allosteric binding site on a receptor, resulting in an increased agonist affinity or efficacy at the receptor.

Highlights

- Neural adaptations occur during withdrawal from methamphetamine self-administration
- This review focuses on causal studies on incubation of methamphetamine craving
- Certain mechanisms are shared between cocaine and methamphetamine
- Dissociable mechanisms are also observed between cocaine and methamphetamine
- Neural mechanisms can differ between forced and voluntary abstinence

Table 1.

Neural adaptations beyond 24-h withdrawal from extended access Meth self-administration

Brain region	SA procedure (dose)	Withdrawal day	Technique	Specific molecular targets	Major findings	Citation
Forced abstinence						
SNC/VTA	9 h/d × 10 d (0.1 mg/kg/inf)	WD 30	ISH	*DAT*	—/—	Shepard et al., 2006
				TH	—/—	
			WB	TH	—/—	
CPu			WB	TH	—	
NAc					—	
SN					—	
Striatum	8 h/d × 7 d (0.06 mg/ml/inf)	WD 30	[^{3}H]DA uptake assay	DAT	▼	McFadden et al., 2012
				VMAT2	—	
	8 h/d × 7 d (0.12 mg/ml/inf)			DAT	▼	
	8 h/d × 7 d (0.24 mg/ml/inf)		WB	DAT	▼	
DS	1 h/d × 6 d, then 6 h/d × 22 d (0.05 mg/kg/0.1 ml inf)	WD 3	WB	DAT	▲	D'Arcy et al., 2016
				TH	—	
				D2R	—	
Striatum	15 h/d × 8 d (0.1 mg/kg/inf)	WD 7/14	HPLC analysis	DA	▼/▼	Krasnova et al., 2010
				DOPAC	▼/—	
				HVA	▼/—	
				NE	—/—	
				5-HT	▲/—	
				5-HIAA	—/—	
		WD 7	WB	GFAP	▲	
		WD 14	WB	TH	▼	
				DAT	▼	
				5-HTT	—	
Cortex		WD 7/14	HPLC analysis	DA	—/▼	
				DOPAC	—/—	
				NE	—/—	
				5-HT	—/—	
				5-HIAA	—/—	
		WD 7	WB	GFAP	▲	
		WD 14	WB	TH	▼	
				DAT	▼	
				5-HTT	—	
DS	15 h/d × 8 d (0.1 mg/kg/inf)	~WD 30	HPLC analysis	DA	▼	Krasnova et al., 2013
				DOPAC	—	
			qPCR/WB	*Drd1*/D1R	—/—	

Brain region	SA procedure (dose)	Withdrawal day	Technique	Specific molecular targets	Major findings	Citation
				Drd2/D2R	—/▼	
				cfos /cFos	—/▼	
				Fosb/FosB	—/—	
				ΔFosb/ ΔFosB	—/▼	
				Bdnf/BDNF	—/▼	
				Trkb/TrkB	—/▼	
			WB	CREB	▼	
				pCREB	▼	
			ChIP	H3K4me3	—	
DS	9 h/d × 10 d (0.1 mg/kg/ inf)	WD 2/35 (whole cell extracts) / WD30–50 (Fos-positive cells)	qPCR (whole cell extracts for WD2/35, compared with saline; Fos-positive cells for WD 30–50 relapse tests, compared with Fos-negative cells in Meth rats)	*Bdnf*	—/—/▲	Li et al., 2015d
				TrkB	—/—/▲	
				Gria1	—/—/▲	
				Gria2	—/—/▼	
				Gria3	—/—/▲	
				Grin1	—/—/—	
				Grin2a	—/—/▲	
				Grin2b	—/—/—	
				Grm1	—/—/▲	
				Grm5	—/—/—	
				Hdac1	—/—/—	
				Hdac2	—/—/—	
				Hdac3	—/—/▲	
				Hdac4	—/—/▲	
				Hdac5	—/—/▲	
				Sirt1	—/—/—	
				Sirt2	—/—/—	
				Crebbp	—/—/—	
				Suv39h1	—/—/—	
				G9a	—/—/—	
				GLP	—/—/▲	
				Kdm1a	—/—/▲	
				Mil1	—/▲/—	
				Dnmt3a	—/—/▲	
CeA	9 h/d × 10 d (0.1 mg/kg/ inf)	WD 2/35	RNA-seq	DEGs	— (215) / ▲ (2217)	Cates et al., 2018
OFC					▲ (118) / — (55)	
			qPCR	*Dcn*	▲/▼	
				Col3a1	▲/▼	
				Henmt1	—/—	
NAc	3 h/d × 20d (0.1 mg/kg/ inf)	WD 30	qPCR (male/female rats)	*Pdyn*	▲/—	Daiwile et al., 2019

Brain region	SA procedure (dose)	Withdrawal day	Technique	Specific molecular targets	Major findings	Citation
				Hcrtr1	—/—	
				Hcrtr2	▼/▼	
				Crh	▼/▼	
				Crhr1	—/—	
				Crhr2	▲/—	
				Avp	▼/—	
				Avpr1a	▼/▼	
				Avpr1b	▼/▼	
DMS/AIT	6 h/d × 10 d (0.1 mg/kg/inf)	WD 30 (after the 2-h relapse test)	qPCR (Fos-positive cells, different from Fos-negative cells)	*Bdnf*	—/—	Li et al., 2019
				TrkB	▲/▲	
				Gria1	—/—	
				Gria2	—/—	
				Gria3	▲/—	
				Grin1	▲/▲	
				Grin2a	—/—	
				Grin2b	▲/—	
				Grm1	▲/—	
				Grm5	—/—	
				Hdac1	—/—	
				Hdac2	—/—	
				Hdac3	▲/—	
				Hdac4	—/—	
				Hdac5	▲/▲	
				Sirt1	—/—	
				Sirt2	—/—	
				Crebbp	▲/—	
				Suv39h1	—/—	
				G9a	—/—	
				GLP	—/—	
				Kdm1a	—/—	
				Mll1	—/—	
				Dnmt3a	—/—	
PRh	1 h/d × 7 d, then 6 h/d × 14 d (20 μg/50 μl/inf)	WD 8/14	WB	mGlu2/3	—/—	Reichel et al., 2011
				mGlu5	—/▼	
HPC				mGlu2/3	—/—	
				mGlu5	—/—	
PFC		WD 8		mGlu2/3	—	
				mGlu5	—	
PFC	1 h/d × 7 d, then 6 h/d × 14 d (20 μg/50 μl/inf)	WD 14	WB (* surface, # total)	mGlu2/3	▼*	Schwendt, et al., 2012
				mGlu7	▼*	
NAc				mGlu2/3	▼*#	

Brain region	SA procedure (dose)	Withdrawal day	Technique	Specific molecular targets	Major findings	Citation
				mGlu7	—	
DS				mGlu2/3	▼*#	
				mGlu7	—	
PRh/HPC/PFC	1 h/d × 7 d, then 6 h/d × 14 d (20 μg/50 μl/inf)	WD 7	WB	GluN1	—/—/—	Reichel et al., 2014
				GluN2a	—/—/—	
				GluN2b	▼/—/—	
PRh	1 h/d × 7 d, then 6 h/d × 14 d (20 μg/50 μl/inf)	WD 7	Capillary electrophoresis	GluN1 (surface)	—	Scofield et al., 2015
				GluN1 (intracellular)	—	
				GluN2b (surface)	▼	
				GluN1 (intracellular)	—	
mPFC/HPC/PRh	1 h/d × 7 d, then 6 h/d × 14 d (20 μg/50 μl/inf)	WD 7	WB	5HT2A	▲/—/▲	Hamor et al., 2019
				mGlu2	—/—/—	
				Gq	▲/—/▲	
				Gi	—/—/—	
				Go	—/—/—	
NAc	6 h/d × 10 d (0.1 mg/kg/inf)	WD 3/21/48	WB	GluA1 (surface)	—/—/—	Murray et al., 2019
				GluA1 (total)	—/—/—	
				GluA2 (surface)	—/—/—	
				GluA2 (total)	—/—/—	
				GluA3 (surface)	—/—/—	
				GluA3 (total)	—/—/—	
				mGlu1 (surface)	—/—/—	
				mGlu1 (total)	—/—/—	
				Homer1b/c	—/—/—	
				Homer2	—/—/—	
		WD 30	Puromycin IP	GluA1	▲	
				GluA2	—	
mPFC	1 h/d × 7 d, then 6 h/d × 14 d (20 μg/50 μl/inf)	WD 8	Electrophysiology (sEPSCs)	AMPA/NMDA	▼	Mishra et al., 2017
				PPR	—	
				Avg. frequency	—	
				Avg. amplitude	—	

Brain region	SA procedure (dose)	Withdrawal day	Technique	Specific molecular targets	Major findings	Citation
				NMDA (current amplitude)	▲	
				AMPA (current amplitude)	—	
			Capillary electrophoresis	GluN2b	▲	
NAc			Electroph7ysiology (sEPSCs)	PPR	▼	
				Avg. frequency	▲	
				Avg. amplitude	—	
PL	6 h/d × 14 d (0.05 mg/kg/inf)	WD 9–14	Electrophysiology (sEPSCs, males/females)	Amplitude	—/—	Pena-Bravo et al., 2019
				Frequency	—/—	
				Rise	—/—	
				Decay	—/—	
			Electrophysiology (eEPSCs, males/females)	Amplitude	—/▲	
				Decay	▼/—	
				NMDA current	—/▲	
HPC	1 h/d × 8–10 d, then 6 h/d × 22d (0.05 mg/kg/inf)	WD 29	IHC	BrdU	—	Recinto et al., 2012
				Ki-67	▲	
				Fos	—	
mPFC/NAc				Fos	—/—	
Punishment-induced abstinence						
NAc/ Striatum	9 h/d × 20 d (0.1 mg/kg/inf)	WD 35 (5 d punishment, 30 d forced abstinence)	qPCR (SR) (*relative to yoked-SR shock saline control, # relative to no-shock saline control)	*Oxt*	▲*#/—	Krasnova et al., 2017
				OxtR	▲#/—	
				CARTpt	—/▲*#	
				FMO2	—/▼*	
				PDK4	▼*▲#/▼*#	
				PTPRO	▼*/▼*	
			qPCR (SS) (*relative to yoked-SS shock saline control, # relative to no-shock saline control)	*Oxt*	—/—	
				OxtR	▲#/—	
				CARTpt	—/—	
				FMO2	—/—	
				PDK4	▲#/—	
				PTPRO	▲#/—	
DS	9 h/d × 22 d (0.1 mg/kg/inf)	WD 43 (13 d punishment, 30 d forced abstinence)	PCR array (SS relative to SR)	*Bdnf*	▲	Torres et al., 2018
				Gdnf	—	
				Ngf	▲	
				Vgf	▲	
				Ntf3	▲	

Brain region	SA procedure (dose)	Withdrawal day	Technique	Specific molecular targets	Major findings	Citation
				Trka	—	
				Trkb	—	
				Gfra1	▲	
				Gfra2	▲	
				Ngfr	—	
				Crh	▲	
				Crhr1	▲	
				Crhr2	—	
				Crhpb	▲	
				Ucn	—	
			qPCR (SR/SS) (relative to saline control)	*Bdnf*	—/▲	
				Ngf	—/—	
				TrkA	—/▼	
				TrkB	—/—	
				Gfra2	—/▲	
				Crh	—/—	
				Crhr1	—/▲	
				Crhr2	—/—	
				Crhbp	—/▲	
				Ucn2	—/▲	
				Cfos	▲/▲	
				Fosb	—/—	
				Egr1	▲/—	
				Egr2	▲/—	
				Egr3	—/—	
			WB (SR/SS) (relative to saline control)	proBDNF	▼/▼	
				mature BDNF	▲/▲	
				TrkB	—/—	
				pTrkB	—/—	
				proNGF	—/—	
				mature NGF	▲/▲	
				TrkA	—/—	
				pTrkA	—/▲	
				p75NTR	—/▲	
				Sortilin	—/—	
				pc-Raf	—/—	
				pMek1/2	▲/▲	
				pErk1/2	—/—	
				pMSK1	—/▲	
				pCREB	—/▲	

Brain region	SA procedure (dose)	Withdrawal day	Technique	Specific molecular targets	Major findings	Citation
				pmTOR	—/▲	
				H3ac	—/▲	
				pMeCP2	—/▲	
OFC→DMS	9 h/d × 20 d (0.1 mg/kg/inf)	WD 35 (5 d punishment, 30 d forced abstinence)	fMRI (SR/SS) (compared to saline control)	Resting state functional connectivity	—/—	Hu et al., 2019
PL→VLS					—/—	

Comparisons are made in reference to saline controls unless otherwise stated in the table.

Abbreviations: 5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: 5-hydroxytryptamine; CeA: central amygdala; CPu: caudate putamen; DA: dopamine; DAT: dopamine transporter; DMS: dorsomedial striatum; DOPAC: 3,4-dihydroxyphenylacetic acid; DS: dorsal striatum; HPC: hippocampus; HVA: homovanillic acid; GFAP: glial fibrillary acidic protein; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; NE: norepinephrine; OFC: orbitofrontal cortex; PFC: prefrontal cortex; PL: prelimbic cortex; PRh: perirhinal cortex; SN: substantia nigra; SNC: substantia nigra pars compacta; SR: shock resistant; SS: shock sensitive; TH: tyrosine hydroxylase; VLS: ventrolateral striatum; VTA: ventral tegmental area; IHC: immunohistochemistry; DEG: differentially expressed genes; ISH: *in situ* hybridization; ChIP: chromatin immunoprecipitation; HPLC: high performance liquid chromatography; EPSC: excitatory postsynaptic currents; eEPSC: evoked EPSC; sEPSC; spontaneous EPSC; IP; immunoprecipitation; PPR: paired-pulse ratio; WB: western blot; WD: withdrawal day. Symbols: (▲): increase; (▼): decrease; (—): no change

Table 2.

Neural mechanisms underlying incubation of methamphetamine craving and parallel studies on incubation of cocaine craving

Site of manipulation	Manipulations	SA procedure (dose)	Withdrawal day	Behavioral outcomes	Citation	Parallel incubation studies with cocaine
Forced abstinence						
dmPFC	Mus + Bac (0.03 + 0.3 nmol/0.5 μl/side); 15 min before relapse tests	9 h/d × 10 d (0.1 mg/kg/inf)	WD 30	—	Li et al., 2015c	Koya et al., 2009; Ma et al., 2014
vmPFC				—		Koya et al., 2009; Ma et al., 2014; Miller et al., 2017
OFC				—		NA
NAc core	Naspm (40 μg/0.5 μl/side); 15 min before relapse tests	6 h/d × 10 d (0.1 mg/kg/inf)	WD 45	▼	Scheyer et al., 2016	Loweth et al., 2014b; ,Conrad et al., 2008
DS	SCH23390 (0.75 μg/1 μl/side);15 min before relapse tests	9 h/d × 10 d (0.1 mg/kg/inf)	WD 2 WD 30	— ▼	Li et al., 2015d	NA
	HDAC5 overexpression; AAV-mHDAC5 injections before SA training		WD 2 WD 30	— ▲	Li et al., 2018b	
	HDAC5 knockdown; AAV-shHDAC5 injections before SA training		WD 2 WD 30	— ▼	Li et al., 2018b	
DMS/DLS	SCH23390 (0.75 μg/1 μl/side); 15 min before relapse tests	9 h/d × 10 d (0.1 mg/kg/inf)	WD 30	▼	Li et al., 2015d	NA
	HDAC5 knockdown; AAV-shHDAC5 injections before SA training		WD 2 WD 30	— —	Li et al., 2018b	
BLA	Mus + Bac (0.03 + 0.3 nmol/0.5 μl/side); 15 min before relapse tests	9 h/d × 10 d (0.1 mg/kg/inf)	WD 2 WD 30	— —	Li et al., 2015c	Lee et al., 2013
CeA	Mus + Bac (0.03 + 0.3 nmol/0.5 μl/side); 15 min before relapse tests	9 h/d × 10 d (0.1 mg/kg/inf)	WD 2 WD 30	— ▼	Li et al., 2015c	Lu et al., 2005; Lu et al., 2007
	CeL SOM knockdown; AAV-shSOM injections before SA training	6 h/d × 12 d (0.1 mg/kg/inf)	WD 1 WD 15	— ▼	Venniro et al., 2020	
AIT-L	Mus + Bac (3 + 15 ng/0.3 μl/side); 15 min before relapse tests	6 h/d × 10 d (0.1 mg/kg/inf)	WD 30	▼	Li et al., 2018a	NA
AIT-M				—		
AIT→DMS	Mus + Bac (AIT-L; 3 + 15 ng/0.3 μl/one side) and SCH23390 (DMS; 0.75 μg/0.5 μl/ contralateral side); 15 min before relapse tests	6 h/d × 10 d (0.1 mg/kg/inf)	WD 1 WD 30	— ▼	Li et al., 2018a	NA

Site of manipulation	Manipulations	SA procedure (dose)	Withdrawal day	Behavioral outcomes	Citation	Parallel incubation studies with cocaine
	Mus + Bac (AIT-L; 3 + 15 ng/0.3 μl/one side) and SCH23390 (DMS; 0.75 μg/0.5 μl/ipsilateral side); 15 min before relapse tests		WD 30	▼		
Systemic	AZD8529 (20 mg/kg, s.c.); 3 h before relapse tests	9 h/d × 10 d (0.1 mg/kg/inf)	WD 1 WD 21	— —	Caprioli et al., 2015	Lu et al., 2007
	AZD8529 (40 mg/kg, s.c.); 3 h before relapse tests		WD 1 WD 21	— ▼		
	SYN119 (10 mg/kg, i.p.); 20 min before relapse tests	6 h/d × 10 d (0.1 mg/kg/inf)	WD1 WD 44 or 52 (counterbalanced with vehicle)	— ▼	Scheyer et al., 2016	Loweth et al., 2014b
	SYN119 (10 mg/kg, i.p.); WD 1 (after day 1 test), 3, 5, 7	6 h/d × 10 d (0.1 mg/kg/inf)	WD 9	—	Murray et al., 2019	
	Oxytocin (1 mg/kg, i.p.); WD 6–20	6 h/d × 10 d (0.1 mg/kg/50 μl inf)	WD 30	▼	Everett et al., 2020	NA
Food choice-induced voluntary abstinence						
NAc core	Mus+Bac (50 + 50 ng/0.5 μl/side); 15 min before relapse tests	Food: 6 h/d × 6 d, 1% sucrose + maltodextrin/ delivery Meth: 6 h/d × 12 d (0.1 mg/kg/inf)	WD 1 WD 15	— ▼	Rossi et al., 2020	NA
	SCH39166 (1 μg/0.5 μl/ side); 15 min before relapse tests		WD 15	▼		
	Raclopride (1 μg/0.5 μl/ side); 15 min before relapse tests		WD 15	▼		
	Flupenthixol (10 μg/0.5 μl/side); 15 min before relapse tests		WD 15	▼		
NAc shell	Mus+Bac (50 + 50 ng/0.5 μl/side); 15 min before relapse tests	Food: 6 h/d × 6 d, 1% sucrose + maltodextrin/ delivery Meth: 6 h/d × 12 d (0.1 mg/kg/inf)	WD 1 WD 15	— —	Rossi et al., 2020	NA
DMS	SCH39166 (1 μg/0.5 μl/ side) ; 15 min before relapse tests	Food: 6 h/d × 6 d (5 pellets/delivery) Meth: 6 h/d × 12 d (0.1 mg/kg/inf)	WD 1 WD 21	— ▼	Caprioli et al., 2017	NA
	Raclopride (1 μg/0.5 μl/ side); 15 min before relapse tests		WD 1 WD 21	— ▼		
	Daun02 injections (4 μg/1 μl/side); 75 min after 15-min induction sessions, 3 d before relapse tests		WD 21	▼		

Site of manipulation	Manipulations	SA procedure (dose)	Withdrawal day	Behavioral outcomes	Citation	Parallel incubation studies with cocaine
Systemic	AZD8529 (20 mg/kg, s.c.); 3 h before relapse tests	Food: 9 h/d × 6 d (5 pellets/delivery) Meth: 9 h/d × 10 d (0.1 mg/kg/inf)	WD 1 WD 21	— —	Caprioli et al., 2015	NA
	AZD8529 (40 mg/kg, s.c.); 3 h before relapse tests		WD 1 WD 21	— ▼		
Social choice-induced voluntary abstinence						
CeA	CeL PKCδ knockdown; AAV-shPKCδ injections before SA training	Social: 15 trials/d × 6 d Meth: 6 h/d × 12 d (0.1 mg/kg/inf)	WD 1 WD 15	— ▲	Venniro et al., 2020	NA

Abbreviations: Mus: muscimol; Bac: baclofen; dmPFC: dorsomedial prefrontal cortex; vmPFC: ventromedial prefrontal cortex; OFC: orbitofrontal cortex; NAc: nucleus accumbens; DS: dorsal striatum; DMS: dorsomedial striatum; DLS: dorsolateral striatum; BLA: basolateral amygdala; CeA: central nucleus of amygdala; CeM: medial CeA; CeL: lateral CeA; AIT: anterior intralaminar nucleus of thalamus; AIT-L: lateral AIT; AIT-M: medial AIT; SA: self-administration; AAV: adeno-associated virus; SOM: somatostatin; PKC: protein kinase C; i.p.: intraperitoneal injections; s.c.: subcutaneous injections; WD: withdrawal day; inf: infusion; NA: not available. Symbols: (▲) increased Meth seeking; (▼) decreased Meth seeking; (—) no effect