REVIEW ARTICLE

Preventing incubation of drug craving to treat drug relapse: from bench to bedside

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In 1986, Gawin and Kleber reported a progressive increase in cue-induced drug craving in individuals with cocaine use disorders during prolonged abstinence. After years of controversy, as of 2001, this phenomenon was confirmed in rodent studies using self-administration model, and defined as the incubation of drug craving. The intensification of cue-induced drug craving after withdrawal exposes abstinent individuals to a high risk of relapse, which urged us to develop effective interventions to prevent incubated craving. Substantial achievements have been made in deciphering the neural mechanisms, with potential implications for reducing drug craving and preventing the relapse. The present review discusses promising drug targets that have been well investigated in animal studies, including some neurotransmitters, neuropeptides, neurotrophic factors, and epigenetic markers. We also discuss translational exploitation and challenges in the field of the incubation of drug craving, providing insights into future investigations and highlighting the potential of pharmacological interventions, environment-based interventions, and neuromodulation techniques.

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INTRODUCTION

Substance use disorder is a chronic brain disorder that is associated with high relapse rates (Box 1) [1, 2]. Some information or cues related with these substances can promote the relapse problem, such as life stress or returning the context where drugs were taken [3]. Either stress [4] or related contexts [5] can retrieve intense desires to reexperience the effects of addictive drugs and substances among individuals who are abstinent. This intense desire is referred to as *drug craving*, which is a critical factor for inducing drug-taking behaviors and causing relapse [6, 7]. Therefore, how to reduce drug craving attracts much attention for treatment of drug relapse.

Notably, drug craving does not seem to decrease with chronic abstinence. In 1986, Gawin and Kleber [8] reported that drug craving in individuals with cocaine use disorders increased after 1–10 weeks of abstinence. About 15 years later, this phenomenon was confirmed by drug *self-administration* models in rats and defined as the *incubation of drug craving*, in which cue-induced drug craving progressively intensifies during the first several months of abstinence [9–11]. The incubation of drug craving is widely observed with many drugs, including cocaine [12–15], nicotine [16], alcohol [17–19], heroin [20, 21], morphine [22], methamphetamine [23, 24], oxycodone [25], and fentanyl [26]. Many studies have investigated the mechanisms, and several excellent reviews [27–30] have summarized critical factors that modulate incubated craving. The present review mainly focuses on translational opportunities in the field of the incubation of drug craving, highlighting the value of several drug targets that have been well studied in animal studies and addressing the possibility and importance of pharmacological therapies, environment-based interventions, and neuromodulation in reducing the incubation of drug craving. We also propose translational obstacles, such as the scarcity of clinical research and different indicators of incubation in animals and humans, to provide insights into future investigations.

POTENTIAL DRUG TARGETS

Most studies have focused on the molecular mechanisms that underlie the incubation of drug craving, and identified various potential drug targets covering kinds of neurotransmitters, neuropeptides, neurotrophic factors, and epigenetics (Fig. 1; Table 1) [30]. In the following parts, we mainly introduce several well-studied molecules in the field of incubation of drug craving.

Neurotransmitters

Glutamate system. Glutamate is the primary excitatory neurotransmitter in the central nervous system and plays a critical role in addiction and relapse [31]. Consistent with an increase in glutamate release in the ventromedial prefrontal cortex (vmPFC) after abstinence [32], a microinjection of mGlu2/3 agonist LY379268 in the prelimbic cortex (PrL) markedly reduced glutamates levels and prevented cue-induced cocaine craving during late abstinence [33]. However, the function of glutamate in the infralimbic cortex (IL) on the incubation of cocaine craving

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Box 1. Glossary

- Drug craving: an affective state in humans that can be induced by exposure to stress, drug-related cues, environments, or partners who used the drug together. In rodents, craving is reflected by drug-seeking behaviors, such as nosepoke an lever presses.
- Incubation of drug craving: craving that is induced by drug-related cues that time-dependently increases over the first several weeks of abstinence.
- Self-administration: an experimental procedure in which rodents are trained to receive an intravenous drug infusion through an operant response (such as a lever presses or nosepokes) that is often paired with cues (such as a tone or light) and contexts.
- Forced abstinence: a procedure in which animals are kept in their homecage after training so that they do not have access to addictive drugs.
- Relapse: the resumption of drug taking in individuals with substance use disorder during abstinence or treatment. In rodents, relapse is indicated by the reinstatement of drug-seeking behavior after a period of abstinence, which can be induced by drug priming, drug-related cues, or stress.
- Social-choice voluntary abstinence: a procedure in which animals can choose between a drug or socially interacting with partners during abstinence.

shows heterogeneity. Cue-induced cocaine craving during late abstinence was attenuated by increasing glutamate levels in the IL with the excitatory amino acid transporter inhibitor threo-b-benzyloxyaspartate (TBOA) [33], suggesting an association between the role of glutamate in incubation of cocaine craving and brain regions where it is released. Despite the brain region-specific role of glutamate, research has shown that systemic injections of LY379268 reduce cue-induced cocaine craving after 21 days of withdrawal [34], indicating that the targeted inhibition of glutamate by systemic administration can effectively prevent the incubation of cocaine craving.

More attention has been paid on the glutamate receptors in the field of incubated drug craving. Given that many excellent reviews have described detailed mechanisms [35, 36], here we provide only a brief introduction of how these findings would be translated into possible treatments. Glutamate receptors are divided into two types: ionotropic receptors and metabotropic ones [37]. Ionotropic glutamate receptors mainly consist of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and *N*-methyl-D-aspartate receptors (NMDARs). Of these, calcium-permeable AMPARs (CP-AMPARs) are critical for mediating incubation of drug craving. CP-AMPARs are mainly composed of GluR1, with high conductance and fast kinetics, contributing to synapses strengthening [35]. During long-term abstinence, the insertion of CP-AMPARs occurs in medium spiny neurons (MSNs) in the nucleus accumbens (NAc), accompanied by an increase in GluR1 levels but no changes in GluR2 [38, 39]. The blockade of CP-AMPARs by 1-naphthylacetylsperimine (Naspm) decreased excitatory postsynaptic currents (EPSCs) in MSNs in the NAc shell (NAcsh), which receives inputs from the basolateral amygdala (BLA) [40] and IL [41]. However, incubated cocaine craving was attenuated only when inputs from the BLA were inhibited [40], suggesting that the role of CP-AMPARs in the incubation of cocaine craving is influenced by upstream projections. CP-AMPARs insertion was also found in the NAc core (NAcc) in "incubated rats" after methamphetamine self-administration training. An intra-NAcc injection of Naspm significantly reduced incubated methamphetamine craving after 40 days of withdrawal [42]. These findings reveal the necessity of CP-AMPARs for the incubated craving of psychostimulant drugs, including cocaine and methamphetamine, but remaining unclear is whether CP-AMPARs play a similar role for other addictive drugs. Direct evidence is lacking, but research has found that an intra-BLA injection of Naspm dose-dependently decreased the rate of alcohol self-administration [43], raising the possibility that CP-AMPARs may also mediate the incubation of other addictive substances.

In addition to directly blocking the insertion of CP-AMPARs via Naspm, indirectly targeting metabotropic glutamate receptors (mGluRs) is more effective and has more translational values, due to the development of agents with fewer side effects. Given that the inhibitory effect of mGluRs on the accumulation of CP-AMPARs during abstinence [36], intraperitoneal and intracranial stimulation of mGluR2/3 in the central nucleus of the amygdala (CeA) significantly reduced cue-induced cocaine craving after prolonged withdrawal [34]. However, the side effects of these traditional mGluRs agonists, such as low tolerance [44], and unclear physiological functions [45], hamper their further clinical use. In recent years, development of novel mGluRs-targeting agonists has become a hotspot in the field of incubation of drug craving, among which positive allosteric modulators (PAMs) have received the most attention [46]. Different from orthostatic agonists that bind to extracellular N-terminal sites of mGluRs, PAMs bind the heptahelical transmembrane domain, allowing it to have higher selectivity for mGluR subtypes, fewer side effects, and better pharmacokinetic characteristics [47, 48]. PAMs also show a role in suppressing incubation of drug craving. After 35 days of withdrawal, cue-induced cocaine craving was attenuated by systemic injection of the mGluR1 PAM SYN119 before a relapse test or every other day from abstinence day 15 to day 33 [49]. Similarly, systemic administration of SYN119 also decreased incubated methamphetamine craving after 40 days of withdrawal [42], demonstrating the inhibitory effect of SYN119 on incubation of craving for psychostimulant drugs. Notably, the function of SYN119 in incubated craving is specific to cocaine and methamphetamine, in which no change in incubated cue-induced sucrose craving was found after a SYN119 injection [49]. These results indicate that SYN199 has a therapeutic effect on incubated craving for addictive drugs rather than natural rewards. In addition to SYN119, AZD8529, a mGluR2 PAM, has also been applied in reducing incubation of methamphetamine craving [50]. Despite the effectiveness of PAMs in preventing incubated craving, previous studies mainly confirmed the influence of mGluR PAMs on the incubation of craving for cocaine and methamphetamine. Remaining unclear is whether the administration of SYN119 or AZD8529 effectively reduces the incubated craving of other classes of addictive drugs, such as opioids, nicotine, and alcohol. Additional preclinical and clinical studies are needed.

Dopamine. Dopamine plays a key role in drug addiction [51], and its role in incubated drug craving has been gradually concerned in recent years. In the vmPFC, dopamine release time-dependently waned during abstinence in cocaine-trained rats [32], along with an increase in the levels of dopamine transporter (DAT) both at early and late abstinence time [52]. Consistently, maximal dopamine uptake rate in the NAc also increased after a prolonged abstinence of cocaine [53]. These results suggest an inverse relationship between enhanced cocaine craving during withdrawal and dopamine levels. When dopamine receptors are targeted, the inhibitory effect on incubation will occur. Microinjections of dopamine D_1 or D_2 receptor antagonists (1.0 μg SCH39166 per side or 1.0 μg raclopride per side) in the NAcc but not NAcsh markedly reduced cue-induced methamphetamine craving after 15 days of voluntary abstinence, suggesting the necessity of D_1 and D_2 receptors in the NAcc for the incubation of methamphetamine craving [54]. Similar results were also found that microinjection of SB-277011A (the selective D_3 receptor antagonist) into the NAc or CeA decreased incubated cocaine craving [55], demonstrating the importance of mesolimbic dopamine system for incubation of craving for psychostimulant drugs. Unexpectedly, the systemic administration of SB-277011A inhibited cue-induced cocaine craving after 2, 10, and 30 days of abstinence [55], and sucrose seeking after 30 days of withdrawal was also attenuated by a

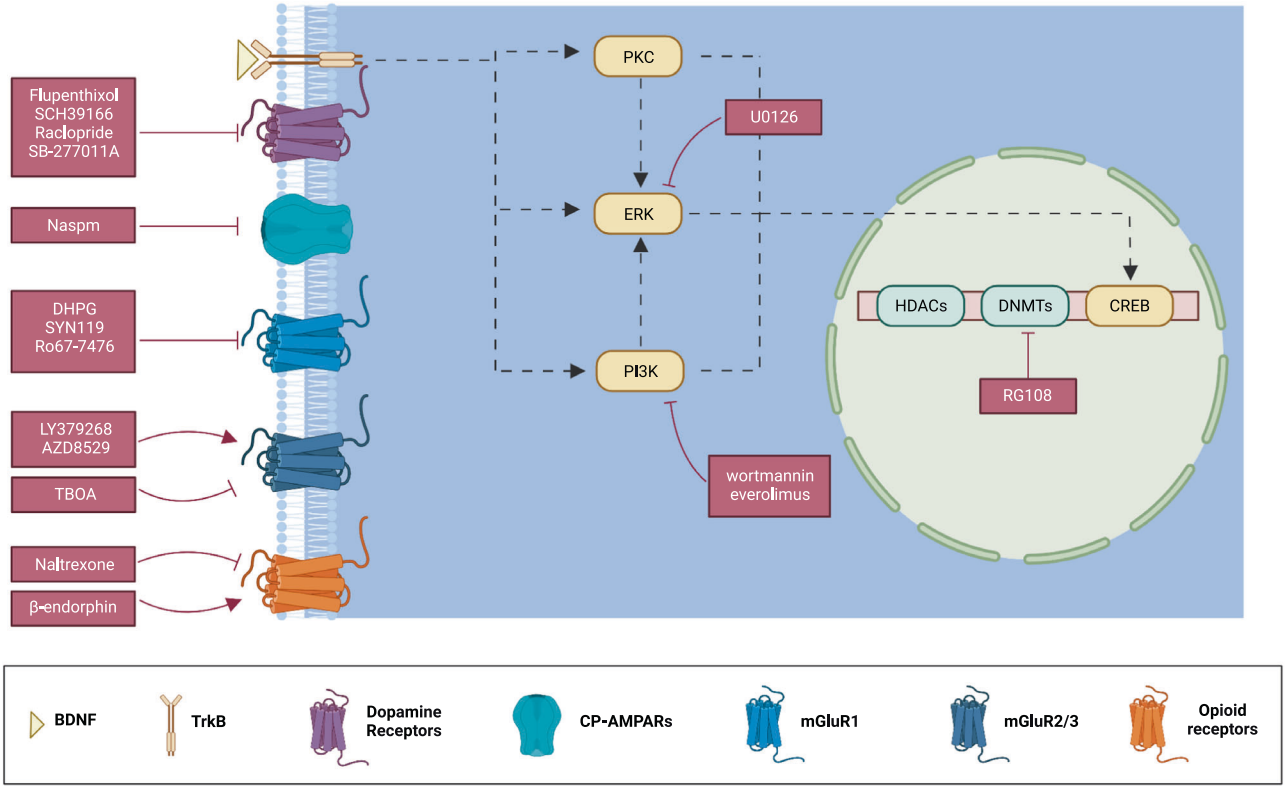


Fig. 1 Promising drug agents for preventing the incubation of drug craving. Some agents that target neurotransmitter receptors exert inhibitory effects on incubated craving, including Flupenthixol (D1R and D2R antagonist), SCH39166 (D1R antagonist), raclopride (D2R antagonist), SB-277011A (D3R antagonist), LY376298 (mGluR2/3 agonist), AZD8529 (mGluR2 PAM), TBOA (excitatory amino acid transporter inhibitor), DHPG (mGlu1 agonist), Ro67–7476 (mGluR1 PAM), SYN119 (mGluR1 PAM), Naspm (CP-AMPAR blocker), and naltrexone (opioid receptor antagonist). Additionally, BDNF binds to TrkB and induces the activation of downstream signaling (including ERK, PI3K, and PKC) and phosphorylation of CREB, thereby mediating the incubation of craving during late abstinence. U0126 (ERK phosphorylation inhibitor), wortmannin (PI3K inhibitor), and everolimus (PI3K inhibitor) exert therapeutic effects on the incubation of drug craving. The inhibition of DNMTs or HDACs also prevents the incubation of drug craving. For example, microinjections of RG108 (DNA methyltransferase inhibitor) markedly reduces cue-induced cocaine craving during late abstinence.

systemic injection of the D_1 receptor antagonist SCH 23390 (25 μg/kg) [56]. These results raise the possibility that the role of the dopamine system is not specific to incubated craving during abstinence, but it is necessary for the motivational response to drug- and non-drug-related cues during abstinence.

Neuropeptides

Endogenous opioid system. The endogenous opioid system includes opioid peptides and receptors, contributing to drug craving and relapse [57]. Among various opioid peptides, β-endorphin shows a role in decreasing incubated cocaine craving. In 2013, Dikshtein et al. [58] used an enzyme-linked immunosorbent assay, and found that β-endorphin level in the NAc was higher after exposure to cocaine-related cues on abstinence day 1 than that on day 30. Microinjections of a synthetic β-endorphin peptide in the NAc decreased cue-induced cocaine craving on abstinence day 30, which can be reversed by co-injection of the δ-opioid receptor antagonist naltrindole. This finding not only suggests that higher level of β-endorphin in the NAc can inhibit incubated cocaine craving through binding to the δ-opioid receptor, but also provide a new drug target for treatment, that is the opioid receptors.

The opioid receptors consist of three kinds, μ-, δ-, and κ-receptor [57], and opioid receptors antagonists have been examined potential for treatment of substance use disorder for many years [59], among which naltrexone is a promising agent for reducing incubated craving. Naltrexone is an opioid receptor antagonist, and chronic injections of naltrexone (7.5, 15, and 30 mg/kg/day) via minipumps from abstinence day 1 to day 13 significantly suppressed incubated heroin craving [60]. Additionally, subcutaneous injections of naltrexone at a dose of 1 mg/kg also reduced incubation of heroin craving. However, subcutaneous injection at doses of 15 and 30 mg/kg failed to decrease incubation [61], not only suggesting a dose-dependent effect of naltrexone on incubation of heroin craving, but also indicating an association between the naltrexone effect and the delivery methods. Notably, the therapeutic effect of naltrexone does not apply to all drugs of abuse. Research has showed that incubation of methamphetamine craving did not change by naltrexone injection either immediately before the test or chronically during abstinence [60]. These findings suggest that naltrexone may only be suitable for treatment of incubated opioids craving. But clinical research has found more possibilities of naltrexone in incubation treatment, and we will talk about it in the later section (see "Opportunities with translational values" section below).

Hypocretin/orexin. Hypocretin (Hcrt; also called orexin) plays an important role in reward-driven motivation, and there are some researches reporting its role in drug relapse [62, 63]. During abstinence from cocaine, activated Hcrt-positive neurons significantly increased in the lateral hypothalamic subregion, and this activated state persisted during withdrawal for at least 150 days [64]. In Hcrt knockout mice, although cue-induced cocaine craving remained elevated during the first week of abstinence, craving failed to incubate beginning in the second week [65], indicating

Table 1. Effect of various agents at different drug targets on the incubation of drug craving.

Drug target	Modulator (Dose)	Mechanism	Treatment regimen	Brain region	Model	Species/Sex	Training drug	Abstinence time (days)	Incubation of drug craving	FDA approval	Reference
CP-AMPARs	Naspm (40 μg/side)	CP-AMPAR blocker	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Methamphetamine	45	↓	No	Scheyer et al., 2016 [42]
mGluR2/3	LY379268 (20 mM)	mGlu2/3 agonist	Single injection (i.c.)	PrL	Self-administration	Rats/male	Cocaine	30	↓	No	Shin et al., 2018 [33]
	TBOA (300 μM)	Excitatory amino acid transporter inhibitor	Single injection (i.c.)	IL	Self-administration	Rats/male	Cocaine	30	↓	No	Shin et al., 2018 [33]
	LY379268 (0.5 or 1.0 μg/side)	mGluR2/3 agonist	Single injection (i.c.)	CeA	Self-administration	Rats/male	Cocaine	21	↓	No	Lu et al., 2007 [34]
	LY379268 (1.5 or 3 mg/kg)	mGluR2/3 agonist	Single injection (i.p.)		Self-administration	Rats/male	Cocaine	21	↓	No	Lu et al., 2007 [34]
	AZD8529 (20 and 40 mg/kg)	mGluR2 PAM	Single injection (s.c.)		Self-administration	Rats/male	Methamphetamine	21	↓	No	Caprioli et al., 2015 [50]
mGluR1	DHPG (500 μM)	mGluR1 agonist	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Cocaine	45	↓	No	Loweth et al., 2014 [49]
	Ro67–7476 (10 μM)	mGluR1 PAM	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Cocaine	45	↓	No	Loweth et al., 2014 [49]
	SYN119 (10 μM)	mGluR1 PAM	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Cocaine	45	↓	No	Loweth et al., 2014 [49]
	SYN119 (10 mg/kg)	mGluR1 PAM	Single injection (i.p.)		Self-administration	Rats/male	Cocaine	45	↓	No	Loweth et al., 2014 [49]
	SYN119 (10 mg/kg)	mGluR1 PAM	Chronic (i.p., every other day, Ad15–33)		Self-administration	Rats/male	Cocaine	35	↓	No	Loweth et al., 2014 [49]
	SYN119 (10 mg/kg)	mGluR1 PAM	Single injection (i.p.)		Self-administration	Rats/male	Methamphetamine	> 40	↓	No	Scheyer et al., 2016 [42]
D_3 receptor	SB-277011A (1.5 or 3 μg/μl/side)	D_3 receptor antagonist	Single injection (i.c.)	NAc/CeA	Self-administration	Rats/male	Cocaine	30	↓	No	Xi et al., 2013 [55]
	SB-277011A (24 mg/kg)	D_3 receptor antagonist	Single injection (i.p.)		Self-administration	Rats/male	Cocaine	30	↓	No	Xi et al., 2013 [55]
D_1 and D_2 receptors	Flupenthixol (10 μg/side)	D_1 and D_2 receptor antagonist	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Methamphetamine	15	↓	No	Rossi et al., 2020 [54]
D_1 receptor	SCH39166 (1.0 μg/side)	D_1 receptor antagonist	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Methamphetamine	15	↓	No	Rossi et al., 2020 [54]
D_2 receptor	Raclopride (1.0 μg/side)	D_2 receptor antagonist	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Methamphetamine	15	↓	No	Rossi et al., 2020 [54]
β-endorphin	Synthetic β-endorphin peptide (100 ng)		Single injection (i.c.)	NAc	Self-administration	Rats/male	Cocaine	30	↓	No	Dikshtein et al., 2013 [58]
μ-opioid receptor	Naltrexone (1.0 mg/kg)	μ-opioid receptor antagonist	Single injection (s.c.)		Self-administration	Rats/male	Heroin	15	↓	Yes	Theberge et al., 2012 [61]
μ-opioid receptor	Naltrexone (7.5, 15, 30 mg/kg/day)	μ-opioid receptor antagonist	Chronic (s.c., abstinence day 1–13)		Self-administration	Rats/male	Heroin	13	↓	Yes	Theberge et al., 2013 [60]
	Naltrexone (15, 30 mg/kg)	μ-opioid receptor antagonist	Single injection (s.c.)		Self-administration	Rats/male	Heroin	13	↔	Yes	Theberge et al., 2013 [60]

Table 1. continued

Drug target	Modulator (Dose)	Mechanism	Treatment regimen	Brain region	Model	Species/Sex	Training drug	Abstinence time (days)	Incubation of drug craving	FDA approval	Reference
	Naltrexone (15, 30 mg/kg/day)	μ-opioid receptor antagonist	Chronic (s.c., abstinence day 1–13)		Self-administration	Rats/male	Methamphetamine	13	↔	Yes	Theberge et al., 2013 [60]
	Naltrexone (15/30 mg/kg)	μ-opioid receptor antagonist	Single injection (s.c.)		Self-administration	Rats/male	Methamphetamine	13	↔	Yes	Theberge et al., 2013 [60]
Hypocretin					Self-administration	Hypocretin knockout mice/ female, male	Cocaine	14	↓	No	Steiner et al., 2018 [65]
TrkB	AAV-TrkB.T1 ($3–3.6 \times 10^9$ TU/ml, 0.5 μl/side)	Reduction of BDNF-induced TrkB phosphorylation	Single injection (i.c.)	NAcc	Self-administration	Rats/male	Cocaine	30	↔	No	Li et al., 2013 [70]
								90	↑	No	Li et al., 2013 [70]
	AAV-TrkB.T1 ($3–3.6 \times 10^9$ TUml, 0.5 μl/side)	Reduction of BDNF-induced TrkB phosphorylation	Single injection (i.c.)	NAcsh	Self-administration	Rats/male	Cocaine	45	↔	No	Li et al., 2013 [70]
								90	↓	No	Li et al., 2013 [70]
ERK	U0126 (100 ng/side)	ERK phosphorylation inhibitor	Single injection (i.c.)	CeA	Self-administration	Rats/male	Cocaine	30	↓	No	Lu et al., 2005 [72]
	U0126 (100 ng/side)	ERK phosphorylation inhibitor	Single injection (i.c.)	CeA	CPP	Rats/male	Morphine	14	↓	No	Li et al., 2008 [22]
PI3K	Wortmannin (50 μM, 100 ng/ side)	PI3K inhibitor	Single injection (i.c.)	vmPFC	Self-administration	Rats/male, C57BL/ 6 J (B6) mice/male	Cocaine	30	↓	No	Szumlinski et al., 2019 [74]
PI3K	Everolimus (1.0 mg/ kg)	PI3K/Akt effector mTOR inhibitor	Single injection (oral)		Self-administration	Rats/male	Cocaine	30–46	↓	Yes	Chiu et al., 2021 [75]
Epigenetics	RG108 (100 μM)	DNA methyltransferase inhibitor	Single injection (i.c.)	NAc	Self-administration	Rats/male	Cocaine	30–60	↓	No	Massart et al., 2015 [78]
	AAV-short hairpin RNA against HDAC5		Single injection (i.c.)	Dorsal striatum	Self-administration	Rats/male	Methamphetamine	30	↓	No	Li et al., 2018 [80]

i.c. intracranial, *i.p.* intraperitoneal, *s.c.* subcutaneous, *CeA* central nucleus of the amygdala, *DHPG* 3,5-dihydroxyphenylglycine, *IL* infralimbic cortex, *NAc* nucleus accumbens, *NAcc* NAc core, *NAcsh* NAc shell, *PAM* positive allosteric modulator, *PrL* prelimbic cortex, *TU* transducing units, *VTA* ventral tegmental area, *vmPFC* ventral medial prefrontal cortex; ↓, decrease; ↔, no effect; ↑, increase.

that Hcrt mainly mediates cue-induced cocaine craving at late abstinence time points.

Neurotrophic factors

Neurotrophic factors play an important role in cell growth and synaptic plasticity [66], such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and fibroblast growth factors (FGFs). As secreted proteins, these neurotrophic factors transmit signals by binding to transmembrane receptors and triggering activation of various downstream signaling pathways (e.g., extracellular-signal-regulated kinase [ERK]/mitogen-activated protein kinase [MAPK] pathway, protein kinase C [PKC] pathway, and phosphatidylinositol 3-kinase [PI3K] pathway), thereby activating secondary transmitter systems and inducing the transcription of related genes to mediate neuroplasticity [67].

BDNF. Studies of incubation of drug craving have reported a critical role of the BDNF signaling pathway. After extended cocaine self-administration, the protein and mRNA levels of BDNF in the ventral tegmental area (VTA) increased from the abstinence day 7 [68], and can persist until day 90 [69]. Along with that, the surface expression of tyrosine kinase receptor B (TrkB), the specific receptor of BDNF, also increased [70]. Similar increases in BDNF levels were also found in other brain regions, such as the NAc and amygdala in "cocaine-incubated rats" [69, 70] and mPFC in "heroin-incubated rats" [71]. However, the role of BDNF in incubation of drug craving is inconsistent in different brain region. For example, heterogeneous effects of BDNF-TrkB pathway on incubation of cocaine craving occur in the NAcc and NAcsh. Li et al. [70] found that cue-induced cocaine craving after 90 days of abstinence was strengthened by TrkB knockdown in the NAcc, whereas opposite result was found after blocking BDNF-TrkB binding in the NAcsh on abstinence day 90.

Downstream targets of neurotrophic factors. In addition to directly targeting BDNF, indirect manipulations of its downstream factors also have potential to decrease incubation of drug craving. Various molecules are successively activated by BDNF, such as ERK, the active state of which relies on direct phosphorylation [66]. After 2 weeks of abstinence, incubated craving for morphine and cocaine strengthened the phosphorylation of ERK in the CeA, together with activation of its downstream transcription factor cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB) [22, 72]. Consistently, microinjections of the ERK phosphorylation inhibitor U0126 in the CeA significantly decreased incubation of morphine or cocaine craving [22, 72], suggesting the potentiality of U0126 in treatment of incubated craving for psychostimulant drugs and opioids. ERK activity in the VTA is also required for GDNF-induced potentiation of cue-induced cocaine seeking after withdrawal [73]. Besides, PI3K is an important downstream pathway triggered by BDNF-TrkB activation [67]. Either intracranial injections of the PI3K inhibitor wortmannin [74] or single oral dosing with the FDA-approved PI3K inhibitor everolimus [75], suppressed incubated cocaine craving in rats. Notably, the effect of everolimus on decreasing incubated cocaine craving lasted 24 h [75], indicating that everolimus has immediate and long-term therapeutic effects on incubation of cocaine craving.

Epigenetics

In recent years, the role of epigenetics in drug addiction and relapse has attracted much attention [76]. Several studies have investigated the mechanisms that underlie the incubation of drug craving from the perspective of epigenetics, identifying activated DNA methylation in the corpus callosum and the higher mRNA expression of some epigenetic enzymes in the dorsal striatum after prolonged abstinence from cocaine [77]. Consistently, intracranial injections of the DNA methyltransferase inhibitor RG108 in the NAc decreased cue-induced cocaine craving after 30 days of abstinence [78], indicating the potential of targeting DNA methylation for suppressing incubation of cocaine craving. Moreover, histone deacetylases (HDACs), which restrain gene transcription [79], have been reported to time-dependently increase during long-term abstinence from methamphetamine [80]. And incubated methamphetamine craving can be attenuated by inhibition of HDAC5 with short-hairpin RNA. These results reveal the importance of epigenetic marks for decreasing the incubation of psychostimulant drugs.

These findings identify some promising targets that may be exploited to explore the function of existing drugs and develop novel agents with fewer adverse effects. More importantly, several potential agents, such as SYN119, naltrexone, and everolimus, showed favorable effects in decreasing incubated craving for some addictive drugs through systemic administration. In the next section below, we describe evidence of the incubation of drug craving in humans and several obstacles that need to be overcome in translational research.

TRANSLATION OF INCUBATION OF DRUG CRAVING

Measures

To date, the dominant method for detecting drug craving in humans has involved subjective measures. Participants are exposed to neutral and drug-related cues, such as visual and olfactory stimuli, and subjective measures are collected before and after cue exposure. The incubation of drug craving in humans was first found in cigarette smokers [16]. Using a between-subjects design, three groups of participants were abstinent for 7, 14, and 35 days, and a single cue session was conducted on the final abstinence day. In the within-subjects design, one group of participants was abstinent for 35 days, and cues were presented on abstinence days 7, 14, and 35. Subjective measures were collected through questionnaires, such as the Tobacco Craving Questionnaire–Short Form (TCQ-SF) Total and the Brief Questionnaire of Smoking Urges (QSU-B) Factor 1 and 2 subscales. The results showed that craving in response to smoking-related cues on the TCQ-SF increased with abstinence in the between-group analysis, and the incubation of cue-induced craving was weaker in the within-subjects assessment, thus identifying the incubation of nicotine craving in humans. Additional clinical evidence has subsequently emerged, demonstrating an increase in cue-induced craving for methamphetamine [23] and alcohol [17, 19] with abstinence. However, some surprising results on subjective self-reported cue-induced craving have been found in heroin and cocaine, which have already been confirmed in animal studies. Measured using a Visual Analog Scale (VAS), craving for heroin-related cues was not different after 1, 3, 12, and 24 months of abstinence [81]. This is likely attributable to the prolonged interval between abstinence time points, which likely missed the time at which cue-induced craving may be elevated. Cue-induced cocaine craving, which was measured by answering the question, "Rate how much you want (or do not want) cocaine in response to this picture," exhibited a linear decline from 2 days to 1 year of abstinence [12]. This inconsistency may have resulted from the different methods that were used to quantify craving [12]. The above study defined "cue-induced craving" as the rating when participants viewed the drug cues, whereas other previous studies usually quantified "cue-induced craving" as differences between craving before and after cue sessions. Although these findings demonstrate the availability and convenience of subjective measures for detecting incubated craving in humans, self-reports are limited by some factors, such as compromised self-awareness [82] and social desirability bias [83].

To provide more objective evidence of the incubation of drug craving in humans, electroencephalography (EEG) was applied to

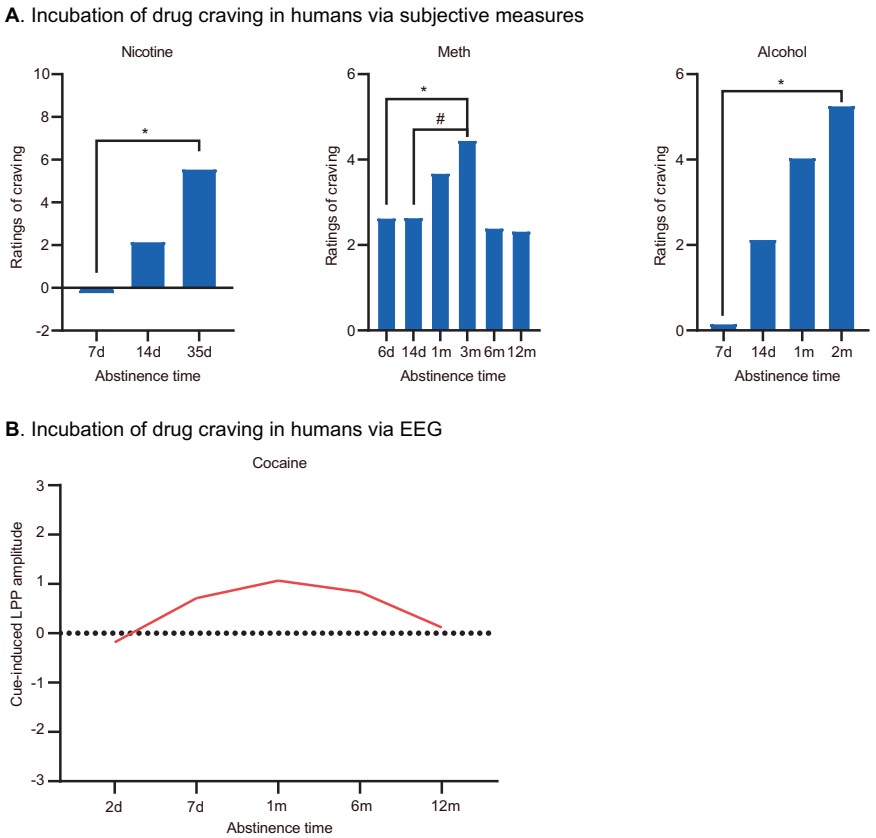


Fig. 2 Incubation of drug craving in humans. A Incubation of drug craving in humans detected by subjective measures, showing ratings of craving in response to drug-related cues. The data are expressed as the mean number of rating scores at different abstinence time points for nicotine [16], methamphetamine [23], and alcohol [17]. **B** Incubation of cocaine craving in humans detected by electroencephalography (EEG) [12]. The data are shown as cue-induced late positive potential (LPP) amplitude at different days of abstinence. *Different from abstinence day 6 or 7. #Different from abstinence day 14. The data were extracted by the WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) from the above cited references, and redrawn by the Graphprism9.4.1.

detect late positive potential (LPP) to track the incubation of cocaine craving [12]. The LPP is electrocortical positivity that can be elicited by emotional and neutral stimuli, with higher levels in response to emotional cues than in response to neutral cues [84, 85]. In cocaine-dependent patients, the LPP became larger in response to cocaine-related cues and exhibited a robust association with cue-induced cocaine craving that was measured by self-reports [86]. Parvaz et al. [12] used the LPP as an objective marker of cue-induced craving. The study recruited 76 participants with cocaine use disorder and divided them into five groups according to self-reported abstinence length (approximate average abstinence time: 2 days, 1 week, 1 month, 6 months, and 1 year). Age, sex, lifetime duration of cocaine use, and dependence severity were matched between groups. Participants viewed four types of pictures, including pleasant, unpleasant, neutral, and cocaine-related pictures, during which the LPP was detected by EEG. Following EEG recordings, the subjects' levels of self-reported cue-induced craving were also recorded. The results showed that the mean LPP-indexed drug-cue reactivity was higher at 1 and 6 months of abstinence and lower at 2 days, 1 week, and 1 year of abstinence, exhibiting an inverted-U trajectory during abstinence. This finding is consistent with the incubation of cocaine craving in rodents, suggesting that the LPP is a potential objective marker of incubated cocaine craving. Notably, however, self-reported cocaine craving in response to related cues declined with abstinence in this study. The decline in cue-induced craving may stem from the different statistical methods that were applied. The opposite findings between objective recordings and subjective measurements also raise a question. Does the increase in LPP amplitude during abstinence reflect changes in cue-induced craving? Future investigations should study associations between the LPP and cue-induced craving in abstinent individuals by utilizing more spatiotemporal-specific techniques, such as EEG-informed functional magnetic resonance imaging (fMRI).

Obstacles

Translational research on the incubation of drug craving is already underway, but some important issues need to be considered. The largest problem is the lack of adequate clinical research. It has been 21 years since the term "incubation of drug craving" was first proposed and about 10 years since the first clinical study that verified "incubated cue-induced craving" in humans. However, related clinical research is still rare, which may be attributable to inconsistent and inaccurate incubation time windows for different types of drugs, thereby making it difficult to detect this phenomenon clinically. For the incubation of methamphetamine craving, the highest cue-induced craving occurs at 3 months of abstinence (Fig. 2). For the incubation of cocaine craving, in contrast, cue-induced craving is highest after 2 months of abstinence. Although cue-induced alcohol and nicotine craving was higher at abstinence days 35 and 60, respectively, the exact time of intensification cannot be precisely defined because of the lack of data on longer abstinence periods. This uncertainty makes clinical measurement difficult. Unknown is whether abstinent individuals are at the peak of cue-induced craving for predicting relapse risk. A critical question is which abstinence times are the best phase for drug administration. Rodent studies mostly chose a late abstinence time point as the timing for intervention. The

translational value of this single dosing timeframe in animal studies is quite small, because it is still difficult to pinpoint the exact time point of incubation in abstinent individuals. More attention should be paid to results of interventions that are implemented at early abstinence time points. For example, naltrexone exerts a therapeutic effect on incubated craving after chronic administration during early abstinence periods. Future studies should more clearly define the time window of incubation to determine the best time window of treatment.

Another obstacle to translational research is that the definition of "cue-induced craving" in animals is not exactly the same as the clinical definition of "drug craving." In humans, self-reports are widely used to measure the degree to which individuals want drugs when they are exposed to drug-related cues. In animal studies, because it is impossible to measure their level of "desire", behavioral indicators are used to measure craving, such as nosepoke or lever-press responding. Thus, what we detect in rodents is actually the behavioral response to drug-related cues that is driven by desire or motivation, rather than the desire itself. Moreover, detailed neural mechanisms are heterogeneous between animals and humans [87], raising questions of whether findings from animals can be translated to humans and whether interventions that work in animals will also work in clinical trials. In addition to advancing clinical research, we also need to explore whether there are more objective metrics of incubated drug craving that can be detected in both animals and abstinent humans.

Although investigations focusing on incubation of drug craving in humans, especially these about the treatment, is still scarce, some promising therapies merit more attention because they have a good translational potential. In the next section, we describe more possible translational paths for moving forward.

OPPORTUNITIES WITH TRANSLATIONAL VALUES

In addition to well-studied drug targets in the field of incubation of drug craving, other kinds of interventions have also been emerging, including sleep manipulation [88], environmental improvement procedures [89], exercise [90, 91], voluntary abstinence [20, 92–94], and extinction sessions (Table 2) [95]. Of these, environment-based interventions gets more attention in recent years, due to its consideration of social factors which are critical for addiction and relapse [96]. Meanwhile, with the research about neurophysiological signatures of incubated craving in individuals with methamphetamine use disorder [24], neuromodulation also becomes a promising method for reducing incubation of drug craving.

Pharmacological interventions

Although several promising drug targets have been emerging, related clinical research is scarce, but still give us partially preliminary support in screening effective medications. In 2020, Bach et al. [97] found that naltrexone exerted a promising therapeutic effect on incubation of alcohol craving. 55 male alcohol-dependent patients and 35 healthy controls were recruited. Following about 3 weeks of controlled abstinence, the participants received either 21 days of Intensive Withdrawal Treatment (IWT, including occupational therapy, physical activation, psychoeducation, psychological group therapy, and multiple medical rounds [98]) or IWT with adjunct oral naltrexone. Instead of using subjective measures to detect cue-induced craving, the author applied a fMRI task to measure the neural response to alcohol-related cues. In the control group, the mesolimbic alcohol cue reactivity was enhanced over the first weeks of abstinence, consistent with incubation of alcohol craving. Individual who received naltrexone exhibited lower cue reactivity in the left putamen, along with a decrease in relapse (relapse was defined as that the alcohol consumption exceeded 60 g/day for men) risk. Despite that this study only included male subjects and did not measure self-reported cue-induced craving, the results suggest the potentiality of naltrexone for reducing incubated alcohol craving. Naltrexone has been approved by the FDA for weight loss medications in obese people [99] and the treatment for smoking behavior and alcoholism [100, 101]. But some clinical studies have reported adverse effects of naltrexone, such as vomiting, nausea, and abnormal pain, and during the early administration, some patients have reported fatigue and headache [102, 103]. Various side effects of medications are an inescapable problem, limiting their clinical use. In spite of this problem, it is still worthy to develop novel agents with fewer adverse effects, and the occurrence of mGluRs PAMs is a good example.

Environment-based interventions

Environmental improvements show potential and many studies have found that favorable living conditions, such as environmental enrichment, can reduce cue- and stress-induced drug craving [104, 105]. Incubation of cocaine craving can also be attenuated by environmental improvement procedures [89]. However, this therapeutic effect is unstable and disappears if these enriched environments are removed [89], demonstrating the importance of continually improving living conditions to prevent incubation of drug craving. Moreover, several studies have explored the effects of a kind of environmental enrichment where rodents are allowed to exercise, and cue-induced cocaine or nicotine craving at late abstinence was significantly attenuated [90, 91], suggesting that exercise is helpful for reducing incubated drug craving.

Voluntary abstinence models are good examples of "reverse translation," such as punishment-induced voluntary abstinence, food-choice voluntary abstinence, and social-choice voluntary abstinence, considering that abstinent individuals do not go through withdrawal without access to anything. However, the effect of voluntary abstinence on incubated drug craving is questionable because after punishment-induced voluntary abstinence, methamphetamine- and oxycodone-trained rats still showed higher cue-induced craving at late abstinence time points [94, 106]. Food-choice voluntary abstinence, in which rats have access to food by pressing a lever, did not decrease incubated methamphetamine craving during late abstinence [50, 107]. Incubated heroin craving in both male and female rats was reduced by food-choice voluntary abstinence, consistent with clinical findings in humans with heroin use disorder [108–110], suggesting a role for food in combating heroin craving [92, 93].

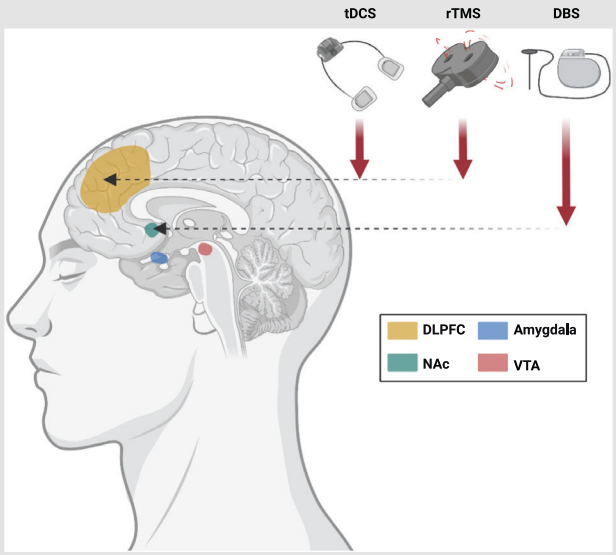
Of these, *social-choice voluntary abstinence* gets the most attention, which is a novel abstinence model with potential implications for preventing incubation of drug craving [111]. Compared with *forced abstinence*, social-choice voluntary abstinence allows drug-dependent rats to choose between a drug and contacting familiar peers by pressing levers during abstinence. This manipulation markedly reduced incubation of methamphetamine and heroin craving, and the therapeutic effect was independent of gender and abstinence duration [112, 113]. These studies suggest the critical role of social reward in decreasing incubated craving during abstinence, highlighting the importance of positive social interaction for preventing drug craving and treatment for relapse. Despite the lack of research on the influence of voluntary abstinence in abstinent humans, similar interventions for the treatment of individuals with substance use disorders already exist, possibly providing some important insights. For example, the community reinforcement approach (CRA), usually combined with contingency management, applies familial, social, recreational, and occupational reinforcers to help people with substance use disorder achieve full abstinence [114]. During the course of the CRA, there is a "relationship counseling" component, in which clients practice communication with their partner and rebuild a good relationship [115]. By reconstructing a healthy lifestyle, abstinent individuals may gradually rid themselves of

Table 2. Effect of various behavioral therapies on the incubation of drug craving.

Therapy	Model	Training drug	Species/sex	Treatment duration	Abstinence time (days)	Incubation of drug craving	Reference
Environment enrichment	Self-administration	Cocaine	Rats/male	Abstinence day 1–30	30	↓	Chauvet et al., 2012 [89]
				Abstinence day 1–30	60	↔	Chauvet et al., 2012 [89]
				Abstinence day 30–60	60	↓	Chauvet et al., 2012 [89]
				Abstinence day 1–60	30	↓	Chauvet et al., 2012 [89]
				Abstinence day 1–60	60	↓	Chauvet et al., 2012 [89]
Aerobic exercise	Self-administration	Cocaine	Rats/female	Abstinence day 1–30	30	↓	Zlebnik et al., 2015 [90]
Aerobic exercise	Self-administration	Nicotine	Rats/male	Abstinence day 1–10	10	↓	Sanchez et al., 2019 [91]
Social-choice voluntary abstinence	Self-administration	Methamphetamine	Rats/male, female	Abstinence day 1–14	15	↓	Venniro et al., 2020 [111], Venniro et al., 2018 [112]
Social-choice voluntary abstinence	Self-administration	Heroin	Rats/male, female	Abstinence day 1–14	15	↓	Venniro et al., 2019 [20]
Food-choice voluntary abstinence	Self-administration	Methamphetamine	Rats/male, female	Abstinence day 1–19	21	↔	Caprioli et al., 2015 [50]; Caprioli et al., 2017 [107]
Food-choice voluntary abstinence	Self-administration	Heroin	Rats/male, female	Abstinence day 1–19	21	↓	Venniro et al., 2017 [92]; D'Ottavio et al., 2022 [93]
Electric barrier-induced voluntary abstinence	Self-administration	Oxycodone	Rats/male, female	Abstinence day 1–13, Abstinence day 1–28	15; 30	↑	Fredriksson et al., 2020 [106]
Electric barrier-induced voluntary abstinence	Self-administration	Methamphetamine	Rats/male	Abstinence day 1–10	21	↔	Krasnova et al., 2014 [94]
Chronic sleep restriction	Self-administration	Cocaine	Rats/male	Abstinence day 22–42, Abstinence day 1–42	45	↓	Chen et al., 2015 [88]
Cue-extinction training	Self-administration	Cocaine	Rats/male	Abstinence day 1–7	30	↓	Madsen et al., 2017 [95]

↓, decrease; ↔, no effect; ↑, increase.

Box 2. Potential neuromodulation techniques to reduce cue-induced drug craving



Both noninvasive and invasive neuromodulation techniques have been applied for treating psychiatric disorders [136], such as depression, Alzheimer's disease, and substance use disorder. Milder side effects than pharmacological interventions and more direct modulation relative to behavioral therapies make neuromodulation methods promising for clinical application. Although evidence of the efficacy of rTMS, tDCS, and DBS in reducing incubated drug craving is still scarce, animal studies have found some critical brain regions that are involved in the incubation of drug craving that could be possible treatment targets, including the mPFC, NAc, CeA, and VTA [27]. Below we mainly introduce recent clinical studies that applied rTMS, tDCS, and DBS to reduce cue-induced drug craving with follow-up studies during abstinence, looking into the possibilities of using neuromodulations to treat incubated cue-induced craving.

Transcranial magnetic stimulation is a noninvasive treatment modality that delivers electric field pulses to the brain, and modulates specific brain regions via magnetic stimuli which are generated with a coil that is placed over the scalp [119]. A single TMS pulse lasts ~0.2–0.3 ms [119], and the most commonly used method for substance use disorder treatment is rTMS, which may produce long-term plasticity changes and confer an enduring therapeutic effect. Notably, the effects of rTMS on cortical excitability rely on the specific stimulation parameters. High-frequency (≥5 Hz) and low-frequency (~1 Hz) stimulation typically have higher and lower effects, respectively [122]. Both types of rTMS can exert a therapeutic effect on cue-induced drug craving. In 2020, Liu et al. [137] reported the results of a randomized, two-center clinical trial that recruited 112 male patients with heroin use disorder who were assigned to three groups: 10 Hz rTMS group, 1 Hz rTMS group, and waitlist control group. The left dorsolateral prefrontal cortex (DLPFC) was targeted in 20 consecutive daily sessions over 28 days. Cue-induced heroin craving was attenuated in both 10 and 1 Hz groups compared with the control waitlist group. This effect lasted for 1 month after treatment, suggesting that rTMS is a potential treatment that can decrease cue-induced heroin craving. Similar results were also reported by clinical research in alcohol [138], cigarettes [139], methamphetamine [140], and cocaine [141] dependent patients.

Compared with rTMS that requires customized pads to deliver stimulation, tDCS has the advantage of being relatively inexpensive. tDCS modulates brain sites by passing low-amplitude direct currents (0.5–2 mA) with at least two electrodes [122]. tDCS at ≥1 mA is usually used for the treatment of substance use disorder and preventing drug craving. A randomized controlled trial with 75 individuals with methamphetamine use disorder was performed with tDCS (1.5 mA) over prefrontal cortex plus computerized cognitive addiction therapy (CCAT) [142]. The CCAT + tDCS group exhibited lower cue-induced craving after treatment compared with the combined CCAT + sham group and control group, suggesting that tDCS may be useful for treating the incubation of methamphetamine craving. tDCS has less severe side effects than rTMS, but lower spatial and temporal resolution and higher sensitivity to anatomical differences [122], which might diminish the effectiveness of tDCS. Additionally, tDCS has not been approved by the FDA for any indication, thus hindering its further clinical use.

DBS is the most developed invasive neuromodulation method. It consists of an intracranial electrode, an extension wire, and a pulse generator, which allow it to directly interface with impaired brain regions and circuits [120]. Many studies have verified its effectiveness for treatment, such as Parkinson's disease (FDA approved) [143], tremor [144], dystonia [145], and psychiatric disorders [120]. Some studies have investigated the efficacy of DBS for the treatment of substance use disorder. In 2020, Zhu et al. [146] employed bilateral DBS (160 Hz) of the NAc combined with anterior capsulotomy in a 28-year-old man with polysubstance use disorders. After treatment, this patient reported a decrease in cue-induced craving for multiple drugs (bucinnazine, morphine, zopiclone, and alprazolam), measured by a VAS, at the 12-month follow-up. Symptoms of depression and anxiety, sleep, quality of life, cognitive function, and health status all progressively improved after treatment. Additionally, no significant side effects were noted, suggesting that DBS may be promising for treating the incubation of drug craving after abstinence.

addictive drugs. However, the social environment in humans is highly complex, and not everyone would rank social rewards first. Thus, medication combined with behavioral interventions may be more promising. Some indirect evidence shows the possibility of naltrexone combined with the CRA for decreasing drug craving. For example, 272 heroin-dependent patients were recruited and received 10 months of naltrexone combined with a CRA. The results showed that self-reported craving decreased during the 16-month follow-up period [116]. Although this study did not measure cue-induced heroin craving during abstinence, the findings raise the possibility that a modified CRA helps attenuate incubated craving in humans.

Neuromodulation

Neuromodulation methods have drawn much attention in recent years, which aim to probe, target, and remodel impaired brain circuits [117]. To date, the best developed devices for neuromodulation include transcranial direct current stimulation (tDCS) [118] and repetitive transcranial magnetic stimulation (rTMS) [119], which act on superficial brain regions through surface electrodes or stimulation coils that are affixed to the scalp. In contrast, deep brain stimulation (DBS) is an invasive therapy that implants microelectrodes into deeper brain regions [120]. Numerous studies have identified the efficiency of neuromodulation on suppressing drug craving (Box 2) [121, 122]. But unclear is remain whether these techniques can be applied for decreasing incubated cue-induced craving in abstinent individuals.

One advantage of neuromodulation techniques to treat the incubation of drug craving is that animal studies have identified potential targets of action, such as the mPFC, NAc, and amygdala [29]. For example, using the Daun02 chemogenetic inactivation procedure, the selective inhibition of neuronal ensembles in the

Box 3. Outstanding directions for future work

- Decrease incubated craving through the administration of FDA-approved agents that act on critical targets that mediate the incubation of drug craving.
- Develop novel agents with fewer and milder adverse effects.
- Decrease incubated drug craving during prolonged abstinence via rTMS, tDCS, transcranial ultrasound brain stimulation, or DBS.
- Prevent the incubation of drug craving using combined therapy with pharmacological interventions plus behavioral interventions or neuromodulation methods plus behavioral interventions.

dorsomedial striatum reduced incubated methamphetamine craving [107]. Additionally, the circuit from BLA-NAcsh [40], or circuit from PrL-NAcc [41], can be modulated by optogenetic procedures to suppress the incubation of cocaine craving. These results suggest the essential role of the NAc in the incubation of drug craving, which may be a promising target for neuromodulation via DBS.

Although direct investigations are still lacking, preliminary experiments [24] have provided some support for future studies that utilize neuromodulation to reduce incubated craving. For example, 156 male methamphetamine-dependent individuals were recruited and divided into five groups based on self-reported abstinence duration: <1 month, 1–3 months, 3–6 months, 6–12 months, and 12–24 months. After rating their drug craving in response to methamphetamine-related videos, resting-state 128-high-density channel EEG signals were recorded. Along with that cue-induced methamphetamine craving got higher at 1–3 months of abstinence, the power spectrum for θ (5.5–8 Hz) and α (8–13 Hz) declined, and β (16.5–26.5 Hz) increased. Notably, an association was found between the incubation of methamphetamine craving and the increase in β activity in the mPFC. Previous studies confirmed the potential role of targeting the mPFC via rTMS or tDCS to treat substance use disorder [123, 124]. Future studies should investigate whether targeting β activity in the mPFC via tDCS or rTMS is useful for decreasing cue-induced craving during abstinence.

Additionally, some indirect studies have shown that the application of neuromodulation techniques can reduce cue-induced drug craving during abstinence. In a multicenter double-blind randomized controlled trial, 262 chronic smokers with tobacco use disorder were recruited and randomly divided into two groups: active rTMS group (who received 3 weeks of daily bilateral active rTMS of the lateral prefrontal and insular cortices followed by once weekly rTMS for 3 weeks) and sham group [125]. Cue-induced craving, measured by a VAS, significantly decreased in the active rTMS group compared with the sham group, and the average weekly reduction of TCQ total score was also significantly greater in the active rTMS group. Although this study did not investigate whether cue-induced craving would be at a lower level at longer abstinence time points, the findings still suggest that rTMS may be applied from early abstinence time period to prevent the incubation of nicotine craving.

Other interventions

In addition to the above three kinds of interventions, other methods are also worthy further investigation. For example, Chen et al. [88] reported that incubated cocaine craving during late abstinence markedly decreased after chronic sleep restriction, supporting the effectiveness of sleep manipulation. And the other promising way is 7-day cue-extinction session performed during abstinence, which suppressed incubation of cocaine craving [95]. Although rare studies focus on the effectiveness of these interventions on decreasing incubation of drug craving, it possibly provides some insights for exploring the mechanism from new perspectives such as sleep and memory. And some potential treatments for preventing drug relapse which target the memory process have already been developed, such as the retrieval-extinction procedures [126, 127].

Compared with pharmacological interventions, behavioral therapies are characterized by safety and no adverse effects. As for neuromodulation, milder side effects than pharmacological interventions and more direct modulation relative to behavioral therapies make it more promising for clinical application. More advanced neuromodulation techniques should also be applied to treat substance use disorder and relapse in future studies, such as transcranial ultrasound brain stimulation [128], closed-loop systems [121], and adaptive DBS [129]. Combined therapies will also be a good research direction, such as neuromodulation plus behavioral therapies and pharmacological therapies plus behavioral interventions. Combinations of multiple treatment modalities might make up for shortcomings of one type of therapy and have superior efficacy.

CONCLUSIONS AND PERSPECTIVES

Drug addiction is a complex psychiatric disease that interrupts various systems [130], along with a high rate of relapse even after treatment [1]. The relapse rate for individuals with opioid use disorders is over 60% after 1 year of abstinence, and over 80% for cocaine users [131]. High risk of relapse has become the primary problem for treating substance use disorder. Among kinds of factors inducing relapse, cue-induced drug craving is a critical one, and exhibits an increasing trend during the first several months of abstinence, which is termed as incubation of drug craving. Using subjective measures and EEG, several clinical studies have verified this phenomenon in nicotine, methamphetamine, alcohol, and cocaine. Numerous excellent investigations have explored the mechanisms, not only providing potential pharmacological therapies but also proposing preliminary supports for social-choice voluntary abstinence and neuromodulation techniques.

The following two points should be noted in future research. First, gender differences have received more attention in recent years in the field of incubation of drug craving [132]. Animal studies have found that incubated cue-induced drug craving is also present in abstinent female rats, but evidence of sex differences is mixed in studies of the incubation of cocaine craving. In studies that found sex differences, female rats exhibited higher and longer-lasting incubation [13, 133–135]. Remaining unclear is whether possible sex differences exist between abstinent men and women. Most clinical research on the incubation of drug craving has recruited only male participants. Whether gender affects the efficacy of interventions in reducing the incubation of drug craving is also unknown.

Second, there is heterogeneity in the mechanisms that underlie incubated craving between different drugs of abuse. For example, incubated heroin craving in rats and incubated alcohol craving in humans can be attenuated by systemic naltrexone administration, whereas no effect on the incubation of methamphetamine craving was found. This difference may stem from the different mechanisms of action between psychostimulants, opioids, and alcohol. Notably, detailed mechanisms of incubated craving during abstinence may also be influenced by the quality or degree of prior drug exposure. Therefore, in addition to developing interventions, exploring whether there are common or distinct mechanisms that underlie the incubation of craving for different types of addictive drugs is also a future research direction.

In conclusion, the present review summarized promising drug targets that have been well studied in rodents, and highlight the potential of pharmacological interventions, social-choice voluntary abstinence and neuromodulations. We hope that the presented information will be useful for developing more effective treatments, investigating underlying mechanisms from new perspectives, and promoting translational research in the future (Box 3).

REFERENCES

1. Brandon TH, Vidrine JI, Litvin EB. Relapse and relapse prevention. Annu Rev Clin Psychol. 2007;3:257–84.
2. Volkow ND, Boyle M. Neuroscience of addiction: Relevance to prevention and treatment. Am J Psychiatry. 2018;175:729–40.
3. Ahmed SH, Badiani A, Miczek KA, Muller CP. Non-pharmacological factors that determine drug use and addiction. Neurosci Biobehav Rev. 2020;110:3–27.
4. Ruisoto P, Contador I. The role of stress in drug addiction. An integrative review. Physiol Behav. 2019;202:62–8.
5. O'Brien CP, Childress AR, McLellan AT, Ehrman R. Classical conditioning in drug-dependent humans. Ann N. Y Acad Sci. 1992;654:400–15.
6. George O, Koob GF. Control of craving by the prefrontal cortex. Proc Natl Acad Sci USA. 2013;110:4165–6.
7. Sayette MA. The role of craving in substance use disorders: theoretical and methodological issues. Annu Rev Clin Psychol. 2016;12:407–33.
8. Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Arch Gen Psychiatry. 1986;43:107–13.
9. Grimm JW, Hope BT, Wise RA, Shaham Y. Incubation of cocaine craving after withdrawal. Nature. 2001;412:141–2.
10. Shalev U, Morales M, Hope B, Yap J, Shaham Y. Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. Psychopharmacology. 2001;156:98–107.
11. Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF. Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. J Neurosci. 2000;20:798–805.
12. Parvaz MA, Moeller SJ, Goldstein RZ. Incubation of cue-induced craving in adults addicted to cocaine measured by electroencephalography. JAMA Psychiatry. 2016;73:1127–34.
13. Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You ZB, et al. Incubation of cocaine craving after intermittent-access self-administration: sex differences and estrous cycle. Biol Psychiatry. 2019;85:915–24.
14. Nugent AL, Anderson EM, Larson EB, Self DW. Incubation of cue-induced reinstatement of cocaine, but not sucrose, seeking in C57BL/6J mice. Pharm Biochem Behav. 2017;159:12–7.
15. Lubbers BR, Matos MR, Horn A, Visser E, Van der Loo RC, Gouwenberg Y, et al. The extracellular matrix protein brevican limits time-dependent enhancement of cocaine conditioned place preference. Neuropsychopharmacology. 2016;41:1907–16.
16. Bedi G, Preston KL, Epstein DH, Heishman SJ, Marrone GF, Shaham Y, et al. Incubation of cue-induced cigarette craving during abstinence in human smokers. Biol Psychiatry. 2011;69:708–11.
17. Li P, Wu P, Xin X, Fan YL, Wang GB, Wang F, et al. Incubation of alcohol craving during abstinence in patients with alcohol dependence. Addict Biol. 2015;20:513–22.
18. Koskela M, Piepponen TP, Andressoo JO, Võikar V, Airavaara M. Towards developing a model to study alcohol drinking and craving in female mice housed in automated cages. Behav Brain Res. 2018;352:116–24.
19. Treloar Padovano H, Miranda R Jr. Incubation of alcohol craving as it naturally occurs in a developmentally diverse sample of dependent and nondependent drinkers. Addict Biol. 2021;26:e12934.
20. Venniro M, Russell TI, Zhang M, Shaham Y. Operant social reward decreases incubation of heroin craving in male and female rats. Biol Psychiatry. 2019;86:848–56.
21. Airavaara M, Pickens CL, Stern AL, Wihbey KA, Harvey BK, Bossert JM, et al. Endogenous GDNF in ventral tegmental area and nucleus accumbens does not play a role in the incubation of heroin craving. Addict Biol. 2011;16:261–72.
22. Li YQ, Li FQ, Wang XY, Wu P, Zhao M, Xu CM, et al. Central amygdala extracellular signal-regulated kinase signaling pathway is critical to incubation of opiate craving. J Neurosci. 2008;28:13248–57.
23. Wang G, Shi J, Chen N, Xu L, Li J, Li P, et al. Effects of length of abstinence on decision-making and craving in methamphetamine abusers. PLoS One. 2013;8:68791–6.
24. Zhao D, Zhang M, Tian W, Cao X, Yin L, Liu Y, et al. Neurophysiological correlate of incubation of craving in individuals with methamphetamine use disorder. Mol Psychiatry. 2021;26:6198–208.
25. Fredriksson I, Tsai PJ, Shekara A, Duan Y, Applebey SV, Lu H, et al. Orbitofrontal cortex and dorsal striatum functional connectivity predicts incubation of opioid craving after voluntary abstinence. Proc Natl Acad Sci USA. 2021;118:e2106624118.
26. Gyawali U, Martin DA, Sulima A, Rice KC, Calu DJ. Role of BNST CRFR1 receptors in incubation of fentanyl seeking. Front Behav Neurosci. 2020;14:153.
27. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. Trends Neurosci. 2011;34:411–20.
28. Li X, Caprioli D, Marchant NJ. Recent updates on incubation of drug craving: a mini-review. Addict Biol. 2015;20:872–6.
29. Altshuler RD, Lin H, Li X. Neural mechanisms underlying incubation of methamphetamine craving: a mini-review. Pharm Biochem Behav. 2020;199:173058–71.
30. Venniro M, Reverte I, Ramsey LA, Papastrat KM, D'Ottavio G, Milella MS, et al. Factors modulating the incubation of drug and non-drug craving and their clinical implications. Neurosci Biobehav Rev. 2021;131:847–64.
31. Kalivas PW. The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci. 2009;10:561–72.
32. Shin CB, Serchia MM, Shahin JR, Ruppert-Majer MA, Kippin TE, Szumlinski KK. Incubation of cocaine-craving relates to glutamate over-flow within ventromedial prefrontal cortex. Neuropharmacology. 2016;102:103–10.
33. Shin CB, Templeton TJ, Chiu AS, Kim J, Gable ES, Vieira PA, et al. Endogenous glutamate within the prelimbic and infralimbic cortices regulates the incubation of cocaine-seeking in rats. Neuropharmacology. 2018;128:293–300.
34. Lu L, Uejima JL, Gray SM, Bossert JM, Shaham Y. Systemic and central amygdala injections of the mGluR(2/3) agonist LY379268 attenuate the expression of incubation of cocaine craving. Biol Psychiatry. 2007;61:591–8.
35. Wolf ME, Tseng KY. Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: When, how, and why? Front Mol Neurosci. 2012;5:72–100.
36. Dong Y, Taylor JR, Wolf ME, Shaham Y. Circuit and synaptic plasticity mechanisms of drug relapse. J Neurosci. 2017;37:10867–76.
37. Pomierny-Chamiolo L, Rup K, Pomierny B, Niedzielska E, Kalivas PW, Filip M. Metabotropic glutamatergic receptors and their ligands in drug addiction. Pharm Ther. 2014;142:281–305.
38. Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, et al. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature. 2008;454:118–21.
39. Ferrario CR, Loweth JA, Milovanovic M, Ford KA, Galiñanes GL, Heng LJ, et al. Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca^{2+}-permeable AMPA receptors during the incubation of cocaine craving. Neuropharmacology. 2011;61:1141–51.
40. Lee BR, Ma YY, Huang YH, Wang X, Otaka M, Ishikawa M, et al. Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. Nat Neurosci. 2013;16:1644–51.
41. Ma YY, Lee BR, Wang X, Guo C, Liu L, Cui R, et al. Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections. Neuron. 2014;83:1453–67.
42. Scheyer AF, Loweth JA, Christian DT, Uejima J, Rabei R, Le T, et al. AMPA receptor plasticity in accumbens core contributes to incubation of methamphetamine craving. Biol Psychiatry. 2016;80:661–70.
43. Faccidomo S, Cogan ES, Hon OJ, Hoffman JL, Saunders BL, Eastman VR, et al. Calcium-permeable AMPA receptor activity and GluA1 trafficking in the basolateral amygdala regulate operant alcohol self-administration. Addict Biol. 2021;26:e13049.
44. Liechti ME, Lhuillier L, Kaupmann K, Markou A. Metabotropic glutamate 2/3 receptors in the ventral tegmental area and the nucleus accumbens shell are involved in behaviors relating to nicotine dependence. J Neurosci. 2007;27:9077–85.
45. Schoepp DD. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J Pharm Exp Ther. 2001;299:12–20.
46. Caprioli D, Justinova Z, Venniro M, Shaham Y. Effect of novel allosteric modulators of metabotropic glutamate receptors on drug self-administration and relapse: a review of preclinical studies and their clinical implications. Biol Psychiatry. 2018;84:180–92.
47. Conn PJ, Christopoulos A, Lindsley CW. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nat Rev Drug Disco. 2009;8:41–54.
48. Conn PJ, Lindsley CW, Meiler J, Niswender CM. Opportunities and challenges in the discovery of allosteric modulators of GPCRs for treating CNS disorders. Nat Rev Drug Disco. 2014;13:692–708.
49. Loweth JA, Scheyer AF, Milovanovic M, LaCrosse AL, Flores-Barrera E, Werner CT, et al. Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving. Nat Neurosci. 2014;17:73–80.
50. Caprioli D, Venniro M, Zeric T, Li X, Adhikary S, Madangopal R, et al. Effect of the novel positive allosteric modulator of metabotropic glutamate receptor 2 AZD8529 on incubation of methamphetamine craving after prolonged voluntary abstinence in a rat model. Biol Psychiatry. 2015;78:463–73.
51. Volkow ND, Wise RA, Baler R. The dopamine motive system: Implications for drug and food addiction. Nat Rev Neurosci. 2017;18:741–52.

52. Grimm JW, Shaham Y, Hope BT. Effect of cocaine and sucrose withdrawal period on extinction behavior, cue-induced reinstatement, and protein levels of the dopamine transporter and tyrosine hydroxylase in limbic and cortical areas in rats. Behav Pharm. 2002;13:379–88.
53. Alonso IP, O'Connor BM, Bryant KG, Mandalaywala RK, Espana RA. Incubation of cocaine craving coincides with changes in dopamine terminal neurotransmission. Addict Neurosci. 2022;3:100029.
54. Rossi LM, Reverte I, Ragozzino D, Badiani A, Venniro M, Caprioli D. Role of nucleus accumbens core but not shell in incubation of methamphetamine craving after voluntary abstinence. Neuropsychopharmacology. 2020;45: 256–65.
55. Xi ZX, Li X, Li J, Peng XQ, Song R, Gaál J, et al. Blockade of dopamine D3 receptors in the nucleus accumbens and central amygdala inhibits incubation of cocaine craving in rats. Addict Biol. 2013;18:665–77.
56. Grimm JW, Harkness JH, Ratliff C, Barnes J, North K, Collins S. Effects of systemic or nucleus accumbens-directed dopamine D1 receptor antagonism on sucrose seeking in rats. Psychopharmacol (Berl). 2011;216: 219–33.
57. Le Merrer J, Becker JA, Befort K, Kieffer BL. Reward processing by the opioid system in the brain. Physiol Rev. 2009;89:1379–412.
58. Dikshtein Y, Barnea R, Kronfeld N, Lax E, Roth-Deri I, Friedman A, et al. β-endorphin via the delta opioid receptor is a major factor in the incubation of cocaine craving. Neuropsychopharmacology. 2013;38:2508–14.
59. Bailey CP, Husbands SM. Novel approaches for the treatment of psychostimulant and opioid abuse - focus on opioid receptor-based therapies. Expert Opin Drug Disco. 2014;9:1333–44.
60. Theberge FR, Li X, Kambhampati S, Pickens CL, St Laurent R, Bossert JM, et al. Effect of chronic delivery of the Toll-like receptor 4 antagonist (+)-naltrexone on incubation of heroin craving. Biol Psychiatry. 2013;73:729–37.
61. Theberge FR, Pickens CL, Goldart E, Fanous S, Hope BT, Liu QR, et al. Association of time-dependent changes in mu opioid receptor mRNA, but not BDNF, TrkB, or MeCP2 mRNA and protein expression in the rat nucleus accumbens with incubation of heroin craving. Psychopharmacol (Berl). 2012;224:559–71.
62. Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. Nature. 2005;437:556–9.
63. DiLeone RJ, Georgescu D, Nestler EJ. Lateral hypothalamic neuropeptides in reward and drug addiction. Life Sci. 2003;73:759–68.
64. James MH, Stopper CM, Zimmer BA, Koll NE, Bowrey HE, Aston-Jones G. Increased number and activity of a lateral subpopulation of hypothalamic orexin/hypocretin neurons underlies the expression of an addicted state in rats. Biol Psychiatry. 2019;85:925–35.
65. Steiner N, Rossetti C, Sakurai T, Yanagisawa M, de Lecea L, Magistretti PJ, et al. Hypocretin/orexin deficiency decreases cocaine abuse liability. Neuropharmacology. 2018;133:395–403.
66. Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nat Rev Drug Disco. 2011;10:209–19.
67. Wang CS, Kavalali ET, Monteggia LM. BDNF signaling in context: From synaptic regulation to psychiatric disorders. Cell. 2022;185:62–76.
68. Schmidt HD, Sangrey GR, Darnell SB, Schassburger RL, Cha JH, Pierce RC, et al. Increased brain-derived neurotrophic factor (BDNF) expression in the ventral tegmental area during cocaine abstinence is associated with increased histone acetylation at BDNF exon I-containing promoters. J Neurochem. 2012;120:202–9.
69. Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci. 2003;23:742–7.
70. Li X, DeJoseph MR, Urban JH, Bahi A, Dreyer JL, Meredith GE, et al. Different roles of BDNF in nucleus accumbens core versus shell during the incubation of cue-induced cocaine craving and its long-term maintenance. J Neurosci. 2013;33:1130–42.
71. Kuntz-Melcavage KL, Brucklacher RM, Grigson PS, Freeman WM, Vrana KE. Gene expression changes following extinction testing in a heroin behavioral incubation model. BMC Neurosci. 2009;10:95.
72. Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y. Central amygdala ERK signaling pathway is critical to incubation of cocaine craving. Nat Neurosci. 2005;8:212–9.
73. Lu L, Wang X, Wu P, Xu C, Zhao M, Morales M, et al. Role of ventral tegmental area glial cell line-derived neurotrophic factor in incubation of cocaine craving. Biol Psychiatry. 2009;66:137–45.
74. Szumlinski KK, Ary AW, Shin CB, Wroten MG, Courson J, Miller BW, et al. PI3K activation within ventromedial prefrontal cortex regulates the expression of drug-seeking in two rodent species. Addict Biol. 2019;24:1216–26.
75. Chiu AS, Kang MC, Huerta Sanchez LL, Fabella AM, Holder KN, Barger BD, et al. Preclinical evidence to support repurposing everolimus for craving reduction during protracted drug withdrawal. Neuropsychopharmacology. 2021;46:2090–100.
76. Robison AJ, Nestler EJ. Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci. 2011;12:623–37.
77. Nielsen DA, Huang W, Hamon SC, Maili L, Witkin BM, Fox RG, et al. Forced abstinence from cocaine self-administration is associated with DNA methylation changes in myelin genes in the corpus callosum: a preliminary study. Front Psychiatry. 2012;3:60–8.
78. Massart R, Barnea R, Dikshtein Y, Suderman M, Meir O, Hallett M, et al. Role of DNA methylation in the nucleus accumbens in incubation of cocaine craving. J Neurosci. 2015;35:8042–58.
79. Borrelli E, Nestler EJ, Allis CD, Sassone-Corsi P. Decoding the epigenetic language of neuronal plasticity. Neuron. 2008;60:961–74.
80. Li X, Carreria MB, Witonsky KR, Zeric T, Lofaro OM, Bossert JM, et al. Role of dorsal striatum histone deacetylase 5 in incubation of methamphetamine craving. Biol Psychiatry. 2018;84:213–22.
81. Wang GB, Zhang XL, Zhao LY, Sun LL, Wu P, Lu L, et al. Drug-related cues exacerbate decision making and increase craving in heroin addicts at different abstinence times. Psychopharmacol (Berl). 2012;221:701–8.
82. Moeller SJ, Goldstein RZ. Impaired self-awareness in human addiction: deficient attribution of personal relevance. Trends Cogn Sci. 2014;18:635–41.
83. Crowne DP, Marlowe D. A new scale of social desirability independent of psychopathology. J Consult Psychol. 1960;24:349–54.
84. Hajcak G, MacNamara A, Foti D, Ferri J, Keil A. The dynamic allocation of attention to emotion: Simultaneous and independent evidence from the late positive potential and steady state visual evoked potentials. Biol Psychol. 2013;92:447–55.
85. Hajcak G, Olvet DM. The persistence of attention to emotion: Brain potentials during and after picture presentation. Emotion 2008;8:250–5.
86. Franken IH, Dietvorst RC, Hesselmans M, Franzek EJ, van de Wetering BJ, Van Strien JW. Cocaine craving is associated with electrophysiological brain responses to cocaine-related stimuli. Addict Biol. 2008;13:386–92.
87. Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry. 2005;162:1403–13.
88. Chen B, Wang Y, Liu X, Liu Z, Dong Y, Huang YH. Sleep regulates incubation of cocaine craving. J Neurosci. 2015;35:13300–10.
89. Chauvet C, Goldberg SR, Jaber M, Solinas M. Effects of environmental enrichment on the incubation of cocaine craving. Neuropharmacology. 2012;63:635–41.
90. Zlebnik NE, Carroll ME. Prevention of the incubation of cocaine seeking by aerobic exercise in female rats. Psychopharmacol (Berl). 2015; 232:3507–13.
91. Sanchez V, Bakhti-Suroosh A, Chen A, Brunzell DH, Erisir A, Lynch WJ. Exercise during abstinence normalizes ultrastructural synaptic plasticity associated with nicotine-seeking following extended access self-administration. Eur J Neurosci. 2019;50:2707–21.
92. Venniro M, Zhang M, Shaham Y, Caprioli D. Incubation of methamphetamine but not heroin craving after voluntary abstinence in male and female rats. Neuropsychopharmacology. 2017;42:1126–35.
93. D'Ottavio G, Reverte I, Ragozzino D, Meringolo M, Milella MS, Boix F, et al. Increased heroin intake and relapse vulnerability in intermittent relative to continuous self-administration: Sex differences in rats. Br J Pharmacol. 2022; https://doi.org/10.1111/bph.15791.
94. Krasnova IN, Marchant NJ, Ladenheim B, McCoy MT, Panlilio LV, Bossert JM, et al. Incubation of methamphetamine and palatable food craving after punishment-induced abstinence. Neuropsychopharmacology. 2014;39: 2008–16.
95. Madsen HB, Zbukvic IC, Luikinga SJ, Lawrence AJ, Kim JH. Extinction of conditioned cues attenuates incubation of cocaine craving in adolescent and adult rats. Neurobiol Learn Mem. 2017;143:88–93.
96. Heilig M, Epstein DH, Nader MA, Shaham Y. Time to connect: Bringing social context into addiction neuroscience. Nat Rev Neurosci. 2016;17:592–9.
97. Bach P, Weil G, Pompili E, Hoffmann S, Hermann D, Vollstädt-Klein S, et al. Incubation of neural alcohol cue reactivity after withdrawal and its blockade by naltrexone. Addict Biol. 2020;25:12717–27.
98. Loeber S, Kiefer F, Wagner F, Mann K, Croissant B. Treatment outcome after inpatient alcohol withdrawal: Impact of motivational interventions: a comparative study. Nervenarzt. 2009;80:1085–92.
99. Narayanaswami V, Dwoskin LP. Obesity: current and potential pharmacotherapeutics and targets. Pharm Ther. 2017;170:116–47.
100. Epstein AM, King AC. Naltrexone attenuates acute cigarette smoking behavior. Pharm Biochem Behav. 2004;77:29–37.
101. Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP. Naltrexone in the treatment of alcohol dependence. Arch Gen Psychiatry. 1992;49:876–80.

102. Croop RS, Faulkner EB, Labriola DF. The safety profile of naltrexone in the treatment of alcoholism. Results from a multicenter usage study. The naltrexone usage study group. Arch Gen Psychiatry. 1997;54:1130–5.
103. Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, et al. Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. JAMA. 2006;295:2003–17.
104. Chauvet C, Lardeux V, Goldberg SR, Jaber M, Solinas M. Environmental enrichment reduces cocaine seeking and reinstatement induced by cues and stress but not by cocaine. Neuropsychopharmacology. 2009;34:2767–78.
105. Solinas M, Chauvet C, Thiriet N, El Rawas R, Jaber M. Reversal of cocaine addiction by environmental enrichment. Proc Natl Acad Sci USA. 2008;105:17145–50.
106. Fredriksson I, Applebey SV, Minier-Toribio A, Shekara A, Bossert JM, Shaham Y. Effect of the dopamine stabilizer (-)-OSU6162 on potentiated incubation of opioid craving after electric barrier-induced voluntary abstinence. Neuropsychopharmacology. 2020;45:770–9.
107. Caprioli D, Venniro M, Zhang M, Bossert JM, Warren BL, Hope BT, et al. Role of dorsomedial striatum neuronal wnsembles in incubation of methamphetamine craving after voluntary abstinence. J Neurosci. 2017;37:1014–27.
108. Weiss G. Food fantasies of incarcerated drug users. Int J Addict. 1982;17:905–12.
109. Zador D, Lyons Wall PM, Webster I. High sugar intake in a group of women on methadone maintenance in south western Sydney, Australia. Addiction. 1996;91:1053–61.
110. Neale J, Nettleton S, Pickering L, Fischer J. Eating patterns among heroin users: a qualitative study with implications for nutritional interventions. Addiction. 2012;107:635–41.
111. Venniro M, Shaham Y. An operant social self-administration and choice model in rats. Nat Protoc. 2020;15:1542–59.
112. Venniro M, Zhang M, Caprioli D, Hoots JK, Golden SA, Heins C, et al. Volitional social interaction prevents drug addiction in rat models. Nat Neurosci. 2018;21:1520–9.
113. Venniro M, Russell TI, Ramsey LA, Richie CT, Lesscher HMB, Giovanetti SM, et al. Abstinence-dependent dissociable central amygdala microcircuits control drug craving. Proc Natl Acad Sci USA. 2020;117:8126–34.
114. Smith JE, Meyers RJ, Miller WR. The community reinforcement approach to the treatment of substance use disorders. Am J Addictions. 2001;10:51–9.
115. Meyers RJ, Roozen HG, Smith JE. The community reinforcement approach: an update of the evidence. Alcohol Res Health. 2011;33:380–8.
116. De Jong CA, Roozen HG, van Rossum LG, Krabbe PF, Kerkhof AJ. High abstinence rates in heroin addicts by a new comprehensive treatment approach. Am J Addict. 2007;16:124–30.
117. Won SM, Song E, Reeder JT, Rogers JA. Emerging modalities and implantable technologies for neuromodulation. Cell. 2020;181:115–35.
118. Chase HW, Boudewyn MA, Carter CS, Phillips ML. Transcranial direct current stimulation: a roadmap for research, from mechanism of action to clinical implementation. Mol Psychiatry. 2020;25:397–407.
119. Diana M, Raij T, Melis M, Nummenmaa A, Leggio L, Bonci A. Rehabilitating the addicted brain with transcranial magnetic stimulation. Nat Rev Neurosci. 2017;18:685–93.
120. Krauss JK, Lipsman N, Aziz T, Boutet A, Brown P, Chang JW, et al. Technology of deep brain stimulation: current status and future directions. Nat Rev Neurol. 2021;17:75–87.
121. Habelt B, Arvaneh M, Bernhardt N, Minev I. Biomarkers and neuromodulation techniques in substance use disorders. Bioelectron Med. 2020;6:4–20.
122. Spagnolo PA, Goldman D. Neuromodulation interventions for addictive disorders: Challenges, promise, and roadmap for future research. Brain. 2017;140:1183–203.
123. Alizadehgoradel J, Nejati V, Sadeghi Movahed F, Imani S, Taherifard M, Mosayebi-Samani M, et al. Repeated stimulation of the dorsolateral-prefrontal cortex improves executive dysfunctions and craving in drug addiction: a randomized, double-blind, parallel-group study. Brain Stimul. 2020;13:582–93.
124. Su H, Zhong N, Gan H, Wang JJ, Han H, Chen TZ, et al. High frequency repetitive transcranial magnetic stimulation of the left dorsolateral prefrontal cortex for methamphetamine use disorders: a randomised clinical trial. Drug Alcohol Depend. 2017;175:84–91.
125. Zangen A, Moshe H, Martinez D, Barnea-Ygael N, Vapnik T, Bystritsky A, et al. Repetitive transcranial magnetic stimulation for smoking cessation: a pivotal multicenter double-blind randomized controlled trial. World Psychiatry. 2021;20:397–404.
126. Xue YX, Luo YX, Wu P, Shi HS, Xue LF, Chen C, et al. A memory retrieval-extinction procedure to prevent drug craving and relapse. Science. 2012;336:241–5.
127. Xue YX, Deng JH, Chen YY, Zhang LB, Wu P, Huang GD, et al. Effect of selective inhibition of reactivated nicotine-associated memories with propranolol on nicotine craving. JAMA Psychiatry. 2017;74:224–32.
128. Beisteiner R, Matt E, Fan C, Baldysiak H, Schonfeld M, Philippi Novak T, et al. Transcranial pulse stimulation with ultrasound in Alzheimer's disease-A new navigated focal brain therapy. Adv Sci (Weinh). 2020;7:1902583.
129. Provenza NR, Sheth SA, Dastin-van Rijn EM, Mathura RK, Ding Y, Vogt GS, et al. Long-term ecological assessment of intracranial electrophysiology synchronized to behavioral markers in obsessive-compulsive disorder. Nat Med. 2021;27:2154–64.
130. Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: The role of reward-related learning and memory. Annu Rev Neurosci. 2006;29:565–98.
131. Sinha R. New findings on biological factors predicting addiction relapse vulnerability. Curr Psychiatry Rep. 2011;13:398–405.
132. Nicolas C, Zlebnik NE, Farokhnia M, Leggio L, Ikemoto S, Shaham Y. Sex differences in opioid and psychostimulant craving and relapse: a critical review. Pharmacol Rev. 2022;74:119–40.
133. Kerstetter KA, Aguilar VR, Parrish AB, Kippin TE. Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. Psychopharmacol (Berl). 2008;198:63–75.
134. Corbett CM, Dunn E, Loweth JA. Effects of sex and estrous cycle on the time course of incubation of cue-induced craving following extended-access cocaine self-administration. eNeuro. 2021;8:ENEURO.0054–21.2021.
135. Johnson AR, Thibeault KC, Lopez AJ, Peck EG, Sands LP, Sanders CM, et al. Cues play a critical role in estrous cycle-dependent enhancement of cocaine reinforcement. Neuropsychopharmacology. 2019;44:1189–97.
136. Creed M. Current and emerging neuromodulation therapies for addiction: Insight from pre-clinical studies. Curr Opin Neurobiol. 2018;49:168–74.
137. Liu X, Zhao X, Liu T, Liu Q, Tang L, Zhang H, et al. The effects of repetitive transcranial magnetic stimulation on cue-induced craving in male patients with heroin use disorder. EBioMedicine. 2020;56:102809–15.
138. Ceccanti M, Inghilleri M, Attilia ML, Raccah R, Fiore M, Zangen A, et al. Deep TMS on alcoholics: effects on cortisolemia and dopamine pathway modulation. A pilot study. Can J Physiol Pharm. 2015;93:283–90.
139. Li X, Hartwell KJ, Henderson S, Badran BW, Brady KT, George MS. Two weeks of image-guided left dorsolateral prefrontal cortex repetitive transcranial magnetic stimulation improves smoking cessation: a double-blind, sham-controlled, randomized clinical trial. Brain Stimul. 2020;13:1271–9.
140. Yuan J, Liu W, Liang Q, Cao X, Lucas MV, Yuan TF. Effect of low-frequency repetitive transcranial magnetic stimulation on impulse inhibition in abstinent patients with methamphetamine addiction: a randomized clinical trial. JAMA Netw Open. 2020;3:e200910.
141. Garza-Villarreal EA, Alcala-Lozano R, Fernandez-Lozano S, Morelos-Santana E, Dávalos A, Villicaña V, et al. Clinical and functional connectivity outcomes of 5-Hz repetitive transcranial magnetic stimulation as an add-on treatment in cocaine use disorder: a double-blind randomized controlled trial. Biol Psychiatry Cogn Neurosci Neuroimaging. 2021;6:745–57.
142. Xu X, Ding X, Chen L, Chen T, Su H, Li X, et al. The transcranial direct current stimulation over prefrontal cortex combined with the cognitive training reduced the cue-induced craving in female individuals with methamphetamine use disorder: a randomized controlled trial. J Psychiatr Res. 2021;134:102–10.
143. Fasano A, Aquino CC, Krauss JK, Honey CR, Bloem BR. Axial disability and deep brain stimulation in patients with Parkinson disease. Nat Rev Neurol. 2015;11:98–110.
144. Oliveria SF, Rodriguez RL, Bowers D, Kantor D, Hilliard JD, Monari EH, et al. Safety and efficacy of dual-lead thalamic deep brain stimulation for patients with treatment-refractory multiple sclerosis tremor: a single-centre, randomised, single-blind, pilot trial. Lancet Neurol. 2017;16:691–700.
145. Vidailhet M, Yelnik J, Lagrange C, Fraix V, Grabli D, Thobois S, et al. Bilateral pallidal deep brain stimulation for the treatment of patients with dystonia-choreoathetosis cerebral palsy: A prospective pilot study. Lancet Neurol. 2009;8:709–17.
146. Zhu R, Zhang Y, Wang T, Wei H, Zhang C, Li D, et al. Deep brain stimulation of nucleus accumbens with anterior capsulotomy for drug addiction: a case report. Stereotact Funct Neurosurg. 2020;98:345–9.

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AUTHOR CONTRIBUTIONS

XXL wrote the draft manuscript. XXL, KY, and TSL constructed the figures and prepared the tables. XL, WZ, YXX, and JS revised the manuscript. LL and YH supervised this review and revised the manuscript. All authors contributed to the article and approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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