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Animal Models of Drug Relapse and Craving after Voluntary Abstinence: A Review

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***Abstract*——Relapse to drug use during abstinence is a defining feature of addiction. During the last several decades, this clinical scenario has been studied at the preclinical level using classic relapse/reinstatement models in which drug seeking is assessed after experimenter-imposed home-cage forced abstinence or extinction of the drug-reinforced responding in the self-administration chambers. To date, however, results from studies using rat relapse/reinstatement models have yet to result in Food and Drug Administration–approved medications for relapse prevention. The reasons for this state of affairs are complex and multifaceted, but one potential reason is that, in humans, abstinence is often self-imposed or voluntary and occurs either because the negative consequences of drug use outweigh the drug's rewarding effects or because of the availability of nondrug alternative rewards that are chosen over the drug. Based on these considerations, we and others have recently developed rat models of relapse after voluntary abstinence, achieved either by introducing adverse consequences to drug taking (punishment) or seeking (electric barrier) or by providing mutually exclusive choices between the self-administered drug and nondrug rewards (palatable food or social interaction). In this review, we provide an overview of these translationally relevant relapse models and discuss recent neuropharmacological findings from studies using these models. We also discuss sex as a biological variable, future directions, and clinical implications of results from relapse studies using voluntary abstinence models. Our main conclusion is that the neuropharmacological mechanisms controlling relapse to drug seeking after voluntary abstinence are often different from the mechanisms controlling relapse after home-cage forced abstinence or reinstatement after extinction.**

***Significance Statement*——This review describes recently developed rat models of relapse after voluntary abstinence, achieved either by introducing adverse consequences to drug taking or seeking or by providing mutually exclusive choices between the self-administered drug and nondrug rewards. This review discusses recent neuropharmacological findings from studies using these models and discusses future directions and clinical implications.**

I. Introduction

A main problem for treatment of drug addiction is *relapse* (see Table 1 for glossary of terms) to drug use after periods of abstinence (Wikler, 1973; O'Brien et al., 1992; Sinha, 2011). In humans, relapse and craving are commonly triggered by re-exposure to the drug itself, re-exposure to cues and contexts previously associated with drug use, or exposure to stressors (Wikler, 1973; O'Brien et al., 1992; Sinha, 2011). Over the last several decades, drug relapse and *craving* have typically been studied at the preclinical level using animal models in which laboratory mice, rats, or monkeys are trained to self-administer a drug and resumption of drug seeking (relapse) is assessed after experimenter-imposed *extinction* of the drug-reinforced responding in the self-administration chambers or *forced abstinence* in the home cage (Shaham et al., 2003; Bossert et al., 2013; Venniro et al., 2016).

Studies using the *reinstatement model* have shown that, after extinction of the drug-reinforced responding, drug seeking is reinstated after exposure to drug-priming injections (Stewart and de Wit, 1987), drug-associated *discrete cues* (See, 2002), contexts (Crombag et al., 2008), and different stressors (Mantsch et al., 2016). Studies using the home-cage forced-abstinence procedure have shown that across several drug classes, relapse to drug seeking in the presence of drug-associated cues and contexts progressively increases over time (Pickens et al., 2011; Wolf, 2016). This behavioral phenomenon is termed *incubation of drug craving* (Grimm et al., 2001) (Table 2). Recent translational studies reported that incubation of drug craving also

ABBREVIATIONS: AI, anterior insula; AIV, AI cortex ventral; BLA, basolateral amygdala; BNST, bed nucleus of stria terminalis; CART, cocaine and amphetamine-regulated transcript; CeA, central amygdala nucleus; CeL, lateral division of CeA; CeM, medial division of CeA; CTb, cholera toxin B; DLS, dorsolateral striatum; DMS, dorsomedial striatum; DREADD, designer receptor exclusively activated by designer drug; Drd1, dopamine D1 receptor; Drd2, dopamine D2 receptor; DSM-IV, Diagnostic and Statistical Manual IV; F1, filial 1; FDA, Food and Drug Administration; fMRI, functional magnetic resonance imaging; LH, lateral hypothalamus; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; OFC, orbitofrontal cortex; Pir, piriform cortex; PKCδ, protein kinase-Cδ; PVT, paraventricular thalamus; SOM, somatostatin; VP, ventral pallidum; vSub, ventral subiculum; VTA, ventral tegmental area.

occurs in humans (Bedi et al., 2011; Wang et al., 2013; Li et al., 2015a; Parvaz et al., 2016).

Over the years, many studies have shown good *postdictive validity* of the classic reinstatement and forced-abstinence models: Food and Drug Administration (FDA)-approved medications for opioid, nicotine, and alcohol addiction decrease relapse or reinstatement in these models (Epstein et al., 2006; Sinha et al., 2011; Heilig et al., 2016). However, results from studies using these models have yet to result in FDA-approved medications for relapse prevention or demonstrate true *predictive validity* (Reiner et al., 2019; Venniro et al., 2020a). Specifically, many translational clinical studies on the efficacy of potential pharmacological treatments identified in the reinstatement model were mostly negative [e.g., corticotropin-releasing factor receptor antagonists (Kwako et al., 2015; Schwandt et al., 2016), 5-HT2c (the 2c receptor subtype of 5-hydroxytryptamine) receptor agonists (Brandt et al., 2020), *n*-acetylcysteine (LaRowe et al., 2013), buspirone (Winhusen et al., 2014), α1 receptor antagonists (Simpson et al., 2018)] or showed moderate effects [e.g., α2 adrenoceptor agonists (Kowalczyk et al., 2015), see Venniro et al. (2020a)].

The reasons for this state of affairs are complex and multifaceted, but one possibility is potential lack of homology between the classic relapse/reinstatement models and the conditions that often lead to drug abstinence in humans. Specifically, in humans, abstinence is often self-imposed or voluntary despite drug availability and occurs either because the negative or adverse consequences (e.g., fear of incarceration, losing family and friends, securing money to obtain drugs) of drug use outweigh the drug's rewarding effect or because of the availability of nondrug alternative rewards (e.g., family, friends, employment) that are chosen over the addictive drug (Marlatt, 1996; Epstein and Preston, 2003). Therefore, one potential limitation of the classic relapse/reinstatement models is that the focus has been exclusively on forced abstinence (i.e., experimenter-imposed), which presumably models instances such as inpatient care or a drug-free prison environment. These procedures, however, do not model voluntary abstinence, which is presumably very common in humans who use drugs (Heyman, 2013).

To more closely mimic human relapse that occurs after self-imposed or voluntary abstinence, we and others have recently developed animal models of drug relapse and craving after *voluntary abstinence*, achieved either by introducing negative consequences to ongoing drug self-administration or by introducing alternative nondrug rewards using discrete-choice procedures (Fig. 1). These relapse models are different from the classic models in which drug is not available and laboratory animals are forced to abstain from the drug prior to the reinstatement/relapse tests (Shaham et al., 2003). These newer models include punishment- or electric barrier–induced voluntary abstinence relapse models (Panlilio et al., 2003; Cooper et al., 2007; Marchant et al., 2013a; Fredriksson et al., 2020) and palatable food– or social choice–induced voluntary abstinence relapse models (Caprioli et al., 2015a; Venniro et al., 2018). The goal of these new models is to increase the translation potential (or the predictive validity) of animal relapse models and to advance our understanding of behavioral and neurobiological factors involved in drug relapse.

In this review, we first describe the experimental procedures used in the voluntary abstinence–based relapse models. We then discuss behavioral and neuropharmacological findings from studies using these models. We conclude by discussing future directions, clinical implications, and the role of sex in relapse after voluntary abstinence. In Table 1 we provide a glossary of terms *(italic font* in the text).

II. Relapse after Abstinence Induced by Adverse Consequences of Drug Taking and Seeking

A. Punishment-Induced Voluntary Abstinence Model

1. Overview. The procedure includes three phases: drug self-administration training, punishment-induced voluntary abstinence, and relapse tests. [Some of the studies described below compared the effect of drug priming or context exposure on resumption of operant responding after punishment vs. extinction. To differentiate between the two operations, we use the term *relapse* to describe resumption of responding after punishment (or choice) and the term *reinstatement* to describe resumption of responding after extinction.] During the training phase, laboratory animals are trained to self-administer a drug, and each drug delivery is temporally paired with a discrete cue. Next, during the punishment-induced abstinence phase, drug-taking behavior is suppressed by response-contingent foot shock in either the self-administration context or in a different context (Marchant et al., 2013b). During testing, which is typically performed 1 day after termination of punishment, relapse is assessed without shock. Relapse to drug seeking after punishment can also be tested after different abstinence periods in a manner analogous to *spontaneous recovery* after extinction (Shaham et al., 1997). In the punishment-induced abstinence model, the negative consequences are associated with *drug taking* (Krasnova et al., 2014; Marchant et al., 2019), and the model attempts to mimic abstinence in humans due to negative consequences of drug taking (e.g., losing family, friends, and employment). Relapse after punishment-induced abstinence has been studied in rats trained

TABLE 1
Glossary of terms

Behavior Term	Definition
ABA context-induced reinstatement	A behavior procedure in which laboratory animals are first trained to self-administer a drug in an environment (termed context A) associated with a specific set of background stimuli (e.g., operant chamber fan, time of day, visual cues, tactile stimuli, olfactory stimuli). Lever pressing is then extinguished in a different environment (termed context B) with a different set of background stimuli. During reinstatement testing under extinction conditions, exposure to context A previously paired with the drug reinstates operant responding. The procedure is based on the ABA renewal procedure that has been used to assess the role of contexts in resumption of conditioned responses to aversive and appetitive cues after extinction.
Community reinforcement approach	A learning-based treatment developed for alcohol addiction in the 1970s and combined with contingency management for other addictions in the 1990s. Its goal is to substitute drug use with nondrug social rewards (family support, employment) contingent on decrease or cessation of drug use.
Contingency management	A learning-based treatment in which abstinence is maintained by providing nondrug rewards (monetary vouchers, prizes, or other incentives) given promptly and predictably in exchange for negative drug tests.
Daun02 chemogenetic inactivation procedure	A method to selectively disrupt the function of behaviorally activated neurons. This method enables investigation of whether "neuronal ensembles" (subsets of activated neurons) are involved learned behaviors. Selective inactivation is performed by injecting a prodrug, Daun02, into the brains of Fos-lacZ transgenic rats that express β-galactosidase in strongly activated neurons. β-Galactosidase converts Daun02 into daunorubicin, which reduces neuronal excitability.
Discrete cues	Neutral stimuli (e.g., light, tone) that become conditioned reinforcers after repeated temporal pairing with drug infusions and effects during self-administration training. In studies on discrete cue–induced reinstatement, rats are trained to self-administer a drug or food; each reward delivery is temporally paired with the discrete cue. Lever pressing is then extinguished in the absence of the discrete cue. During reinstatement testing, exposure to the discrete cue, which is earned contingently by responding on the drug-associated lever, reinstates drug or food seeking.
Drug craving	A subjective state that refers to a strong desire to consume an addictive drug. In animal models, craving is often used to describe the motivation state associated with drug taking and seeking in a manner analogous to the use of hunger as the motivational state associated with taking and seeking food rewards or fear as the motivation state associated with freezing induced by aversive stimuli such as high-intensity foot shock or predator odor.
Escalation model	An animal model of escalation of drug intake in which rats are given continuous extended access to drug (6–12 h per day). Under these experimental conditions, most rats increase their drug intake over time. In the escalation model, drug intake and drug brain levels are relatively constant during the daily sessions.
Extinction	A decrease in the frequency or intensity of learned responses after the removal of the reinforcer (e.g., food, drug) that has reinforced the learning.
Fixed-ratio reinforcement schedule	A schedule of reinforcement in which a reinforcer is presented upon the completion of a fixed number of responses.
Forced abstinence	A term refers to experimental conditions in which abstinence after drug self-administration is experimenter-imposed. In animal models, forced abstinence can be achieved by 1) extinction training in the drug self-administration context or a nondrug context or (2) keeping the subjects in their home cage during the abstinence period.
Incubation of drug craving	A hypothetical motivational process inferred from the findings of time-dependent increases in nonreinforced drug seeking during abstinence from drug self-administration in rats.
Intermittent-access drug self-administration model	An animal model of intermittent access of drug intake in which cycles of drug availability (typically 5 min ON, 25 min OFF for 6–8 h per day) are presented within a daily session. In the intermittent-access model, drug intake and drug brain levels fluctuate between peaks and troughs during the daily sessions.
Neuronal ensembles	Specific patterns of synchronously activated neurons that are hypothesized to encode highly specific and complex information underlying learning, memory, motivation, and other psychologic processes. Neuronal ensembles have been traditionally studied with in vivo electrophysiology using multielectrode recordings, which provide temporal information on neuronal activity patterns (i.e., when the neurons are activated in the brain during behavior). The immunohistochemical detection of IEGs such as Fos or Arc can also be used to study neuronal ensembles by providing information on the spatial expression patterns of behaviorally relevant activated neurons (i.e., where the neuronal ensemble neurons are in the brain).
Postdictive validity	The ability of a laboratory model to retrospectively demonstrate an established human phenomenon. This typically refers to the demonstration that a medication previously shown to be effective in the treatment of a human disease is also effective in the animal model of the disease.
Predictive validity	The extent to which laboratory-animal behavior induced by an experimental manipulation predicts human behavior induced by a similar event in the modeled condition. The concept often refers to a model's ability to identify new treatments that are effective in humans.
Progressive-ratio reinforcement schedule	A schedule of reinforcement in which a reinforcer is only presented upon the completion of a set number of responses. The number of required responses progressively increases after each presented reinforcement.
Reacquisition	The resumption of the original learned response when the reinforcer (operant or classic) is reintroduced after extinction.

(*continued*)

TABLE 1—*Continued*

Behavior Term	Definition
Reinstatement model	The most commonly used animal model of drug relapse. In the context of addiction research, reinstatement refers to the resumption of drug seeking after extinction of the drug-reinforced responding. The resumption is typically induced by exposure to priming drug injections, drug-associated cues, drug-associated contexts, or stressors.
Relapse	Resumption of drug-taking behavior during self-imposed (voluntary) or forced abstinence in humans and laboratory animals.
Renewal	The recovery of extinguished conditioned behavior, which can occur when the context is changed after extinction; renewal often occurs when the subject returns to the learning (training) environment (context) after extinction of the conditioned response in a different context.
Retro-DREADD dual-virus approach	A double-virus method that allows for selective inhibition or activation of defined neuronal projections. The method comprises the combined use of a CRE-recombinase–expressing CAV2 or AAV injected into a terminal region of interest and a second AAV virus that contains a DIO version of an inhibitory or an excitatory DREADD injected into the cell body region. CAV2 or the AAV retrogradely infects projection neurons, resulting in projection-specific expression of the DREADD receptor. During behavioral testing, injections of the otherwise inactive drugs like CNO or Sal B result in selective activation or inhibition (in the case of CNO) or selective inhibition (in case of Sal B) of the neuronal projection of interest.
Second-order reinforcement schedule	A reinforcement schedule in which completion of the response requirements of one schedule (the unit schedule) is treated as a unitary response that is reinforced according to another schedule.
Spontaneous recovery	The resumption of the extinguished conditioned response that occurs after time has passed after the conclusion of extinction.
Three-criteria DSM-IV model	An animal model of drug intake that is based on three DSM-IV criteria used in humans to identify addicted rats (Deroche-Gamonet et al., 2004). After self-administration training, the model evaluates three behaviors based on DSM-IV criteria: persistent drug seeking during periods when drug is not available, high motivation to self-administer the drug (progressive-ratio responding), and willingness to take drug despite adverse consequences (foot-shock punishment). Next, an addiction score (scale 0–3) based on the subjects' percentile on each measure's distribution is calculated. Approximately 20% of rats meet all three addiction criteria.
Voluntary abstinence	A term used to refer to experimental conditions in which the self-administered drug is available in the self-administration chamber but the laboratory animal either stops or significantly decreases the drug self-administration behavior. In animal models, voluntary abstinence can be achieved by introducing 1) mild foot-shock punishment after the drug-reinforced operant response, 2) an electric barrier that delivers mild shock near the drug-paired lever, 3) mutually exclusive alternative palatable food reward, and 4) mutually exclusive alternative social reward (see text).

AAV, adeno-associated virus; CAV2, canine adenovirus-2; CNO, Clozapine N Oxide; Cre recombinase, a tyrosine recombinase enzyme derived from the P1 bacteriophage; DIO, double floxed inverse open reading frame; IEG, immediate early gene; Sal B, Salvinorin B.

to self-administer remifentanil (a potent short-acting opioid agonist), cocaine, and methamphetamine (intravenously) and alcohol (orally delivered) (Table 3).

2. Review of Studies. a. Drug-priming–induced relapse. Panlilio et al. (2003) reported that priming injections of remifentanil accelerated *reacquisition* of remifentanil self-administration after punishment-induced abstinence in male rats. In another study, the same authors compared the effect of drug priming on relapse after punishment versus extinction in male rats. They reported that the effect of priming injections of the benzodiazepine lorazepam, but not heroin, is dependent on the abstinence condition. Specifically, priming injections of heroin or the benzodiazepine lorazepam induced relapse to remifentanil seeking after punishment (Panlilio et al., 2005). In contrast, priming injections of heroin but not lorazepam induced reinstatement of remifentanil seeking after extinction. We discuss the clinical implications of these findings in section *IV. Conclusions and Clinical Implications* below.

b. Context-induced relapse. i. Alcohol Studies. Marchant et al. (2013a) modified the *ABA context-induced reinstatement* [*renewal* (Bouton and Bolles, 1979)] procedure (Crombag and Shaham, 2002) to study context-induced relapse after punishment. In this procedure, rats are trained to self-administer a drug in context A. Next, abstinence is achieved in context B by probabilistic response-contingent foot-shock punishment. Context B is distinct from context A in its tactile, visual, auditory, and circadian (time of day) features. The rats are then tested for relapse in context A and B in the absence of foot shock or drug.

In the first study, Marchant et al. (2013a) used male alcohol-preferring rats (Penn et al., 1978) and compared context-induced relapse after punishment versus extinction in rats trained to self-administer oral alcohol in context A. Rats were first exposed to an intermittent-access alcohol-intake procedure (free choice between alcohol and water) in the home cage three to four times per week (Wise, 1973). The rats were then trained to self-administer alcohol in context A. Next, all rats continued to self-administer alcohol in a different context (context B). For one group, 50% of alcohol-reinforced responses were punished by mild foot shock; two other groups either received noncontingent shocks or no shock. A fourth group was given extinction training in context B.

TABLE 2
Incubation of drug craving: behavioral measures and learning processes

Initial incubation procedure	Time-dependent increases in drug seeking after cessation of drug self-administration were initially observed in studies that used the so-called between-within reinstatement procedure (Shalev et al., 2002). In this procedure, the extinction and reinstatement test phases are performed during a single session on different days after drug self-administration training (Tran-Nguyen et al., 1998; Neisewander et al., 2000; Grimm et al., 2001; Shalev et al., 2001). In the first study, in which this time-dependent drug-seeking phenomenon was termed "incubation of craving," Grimm et al. (2001) assessed cocaine seeking at different time points after forced abstinence (1 day to 60 days) in two ways. They first exposed rats to six to eight 1-h extinction sessions in the absence of a discrete tone-light cue previously paired with cocaine infusions during training. The authors then tested the rats immediately after the last extinction session for cue-induced reinstatement in a single 1-h session in which lever presses resulted in contingent presentations of the discrete cue. They found that lever presses in the extinction and cue-induced reinstatement tests follow a similar time course.
Current incubation procedure	Over the years, several studies used the between-within reinstatement procedure and showed robust incubation of extinction responding (without the discrete cue) and subsequent cue-induced reinstatement (Grimm et al., 2003; Lu et al., 2004; Kerstetter et al., 2008). However, most subsequent mechanistic studies on incubation of drug craving after forced or voluntary abstinence have primarily used a simplified procedure in which rats were tested at different time points after cessation of drug self-administration in a single extinction session in the presence of contextual cues previously paired with drug effects (the self-administration chambers) and lever presses or nose pokes result in contingent presentations of the discrete cue (Pickens et al., 2011; Wolf, 2016; Dong et al., 2017; Szumlinski and Shin, 2018). In the studies described in the current review, investigators have used the simplified procedure to study mechanisms of incubation after voluntary abstinence.
Learning processes of incubation	An unresolved question is the learning processes involved in the observed incubation of drug seeking in the simplified single-session procedure. This question was addressed by Adhikary et al. (2017) who used a variation of the between-within procedure and two different contexts to determine the unique contribution of the discrete cues, the contextual cues, and the learned operant response to the incubated methamphetamine seeking response, as typically assessed in the single-session procedure. They trained rats to self-administer methamphetamine in a distinct context (context A) for 14 days; lever presses were paired with a discrete light cue. Next, they tested groups of rats in context A or a different nondrug context (context B) after 1 day, 1 week, or 1 month for extinction responding with or without the discrete cue. The authors found that operant responding in the extinction sessions in contexts A or B was higher after 1 week and 1 month than after 1 day; this effect was context-independent. Independent of the forced-abstinence period, operant responding in the extinction sessions was somewhat higher when responding led to contingent delivery of the discrete cue. After extinction in context B in the absence of the discrete cue, cue-induced reinstatement in context B was modestly higher after 1 month than after 1 day or 1 week. After extinction in context B in the presence of the discrete cue, context-induced reinstatement in context A was similar after 1 day, 1 week, and 1 month. These results demonstrate that the incubation of drug craving phenomenon is primarily mediated by time-dependent increases in context-independent nonreinforced operant responding, and this incubation effect is modestly increased by exposure to discrete cues previously paired with drug infusions.

Rats were then tested for relapse to alcohol seeking under extinction conditions in contexts A and B. Marchant et al. (2013a) reported reliable context A–induced relapse to alcohol seeking after punishment that was similar in magnitude to context A–induced reinstatement after extinction. This renewal effect was specific to the punishment (contingent shock) manipulation and did not occur after unpaired (noncontingent) shock exposure in context B, which did not suppress responding. The context-specific ABA renewal effect of punishment was also observed with cocaine and food rewards (Bouton and Schepers, 2015; Pelloux et al., 2018a).

In an initial mechanistic study, Marchant et al. (2014) used alcohol-preferring male rats to investigate the role of lateral hypothalamus (LH) because of its role in context-induced reinstatement after extinction. The authors found that context-induced relapse after punishment is associated with increased activity of LH [assessed by the activity marker Fos (Morgan and Curran, 1991)] and that reversible inactivation of LH using the $GABA_{A+B}$ receptor agonists muscimol + baclofen (McFarland and Kalivas, 2001) decreases relapse (Marchant et al., 2014). In this study, Marchant et al. (2014) also used Fos labeling and the retrograde tracer cholera toxin B (CTb) to determine projection-specific activation of projections to LH during the relapse tests. They found that context-induced relapse is associated with selective activation of nucleus accumbens (NAc) shell and ventral bed nucleus of stria terminalis (BNST) neurons projecting to LH but not projections from ventral and dorsal medial prefrontal cortex (mPFC), lateral septum, and dorsal BNST (Marchant et al., 2014). The causal role of these projections in context-induced relapse after punishment is unknown.

In a follow-up study, the same authors used similar experimental methods to investigate the role of ventral subiculum (vSub) and its glutamatergic projection to NAc shell because of their roles in context-induced reinstatement after extinction (Bossert and Stern, 2014; Bossert et al., 2016). They found that in alcohol-preferring male rats, context-induced relapse after punishment is associated with increased vSub activity (assessed by Fos) and that muscimol + baclofen reversible inactivation of vSub decreases relapse (Marchant et al., 2016). Additionally, context-induced relapse was associated with selective activation of vSub projections

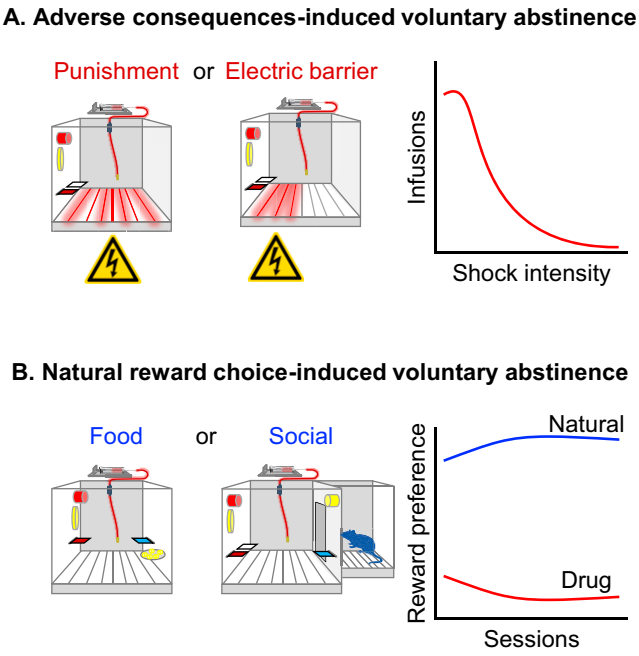

Fig. 1. Rodent models of voluntary abstinence. (A) Models of voluntary abstinence induced by adverse consequences. Schematic representation of voluntary abstinence achieved by either punishment [left panel—lever presses result in contingent drug delivery plus contingent foot shock (typically for 0.5 seconds) delivered throughout the entire metal grid] or electric barrier (middle panel—noncontingent shock is delivered throughout the session to the grid floor area near the drug-associated lever). Under these conditions, drug taking and seeking (right panel—red line) decreases by increasing the intensity of the foot shock. (B) Voluntary abstinence induced by providing alternative nondrug rewards. Schematic representation of voluntary abstinence achieved via mutually exclusive choice between natural rewards (palatable food—left panel; social interaction—middle panel) and drugs. Under these conditions, rats prefer the natural reward (right panel—blue line) over drugs (right panel—red line).

to NAc shell but not projections from ventral mPFC, paraventricular thalamus (PVT), and basolateral amygdala (BLA). Finally, Marchant et al. (2016) used a chemogenetic *retro-DREADD dual-virus approach* (Rothermel et al., 2013) and reported that inhibition of the vSub to NAc shell projection decreases context-induced relapse. In a follow-up study on the role of NAc shell in context-induced relapse after punishment in alcohol-preferring male rats, Marchant and Kaganovsky (2015) reported that intracranial injections of the dopamine D1 receptor (Drd1) antagonist SCH 23390 into NAc shell or core decrease relapse. NAc shell activity is dependent on glutamate and dopamine Drd1-mediated neurotransmission (O'Donnell, 2003). Thus, a potential account of the results of the two studies is that context-induced relapse after punishment is dependent on synergetic activation of glutamatergic projections from vSub (Groenewegen et al., 1987) and dopaminergic projections from ventral tegmental area (VTA) (Nauta et al., 1978).

Finally, Campbell et al. (2018) reported that in alcohol-preferring male rats, paternal alcohol exposure led to decreased resistance to punishment in context B and decreased context-induced relapse after punishment in context A in male F1 (filial 1) offspring. The reasons for this unexpected "protective" effect of paternal alcohol exposure on alcohol seeking in male F1 offspring are unknown.

ii. Cocaine Studies. In two studies, Pelloux and colleagues investigated mechanisms of context-induced relapse to cocaine seeking after punishment-induced abstinence in male rats. In the first study, Pelloux et al. (2018a) used Fos to investigate brain regions activated during context-induced relapse. The authors found that relapse was associated with selective activation of dorsal and ventral mPFC, BLA, vSub, PVT, LH, anterior insula (AI), dorsolateral striatum (DLS) and dorsomedial striatum (DMS), lateral habenula, substantia nigra, and dorsal raphe but not NAc core and shell, central amygdala nucleus (CeA), LH, VTA, orbitofrontal cortex (OFC), lateral and medial septum, ventral pallidum (VP), ventral BNST, medial habenula, and median raphe (Pelloux et al., 2018a). However, the results of this correlational study should be interpreted with caution because it is unknown whether Fos expression is the cause or the consequence of drug relapse (Bossert et al., 2011).

In a second study, Pelloux et al. (2018b) used muscimol + baclofen inactivation to investigate the causal role of BLA and CeA in context-induced relapse after punishment in male rats. The authors compared the role of the amygdala subregions in context-induced relapse after punishment versus context-induced reinstatement after extinction. The authors found that inactivation of BLA, but not CeA, *increased* context-induced relapse in context A after punishment in context B and that inactivation of either BLA or CeA induced relapse in context B after punishment. In contrast, inactivation of either BLA or CeA decreased context-induced reinstatement of cocaine seeking in context A after extinction in context B; the BLA results replicate results from a previous study (Fuchs et al., 2005). A surprising conclusion from this study is that, depending on the historical conditions that induce abstinence, amygdala activity can either promote or inhibit relapse.

Farrell et al. (2019) investigated the role of VP in relapse after punishment using Fos immunohistochemistry and DREADD inhibition in male and female rats. They first trained rats to self-administer cocaine in context A with a discrete cue paired with cocaine infusions and then exposed the rats to punishment-induced abstinence in context B in the presence of the discrete cue. Next, they tested the rats for context-induced relapse in context A in the presence or absence of the discrete cue and tested for relapse induced by cocaine priming in context A. Subsequently, they exposed different groups of rats to context A with or without the discrete cue, to context B without the cue, or did not expose to either context or cue (home cage) and determined Fos

TABLE 3
Relapse after punishment-induced voluntary abstinence: summary of main findings

No.	Reference	General Training Procedures	Test	Major Findings
1	Panlilio et al. (2003)	Rat strain and sex Long-Evans males. Drug training Completion of 100 infusions for 4 μg/kg/infusion remifentanil (FR1) for 3 or 27 sessions. Punishment Responses for remifentanil paired with a 0.5-s, 1.5-mA foot shock for three sessions.	Reinstatement Five priming intravenous infusions of remifentanil at the start of the session with additional priming injections at 1, 2, and 3 h if responding did not commence. Sessions lasted for 5 h or when 100 remifentanil trials were completed (n = 4 to 5 per priming condition).	Priming infusions of remifentanil increase reacquisition of remifentanil self-administration after punishment.
2	Panlilio et al. (2005)	Rat strain and sex Sprague-Dawley males. Drug training Completion of 100 infusions for 4 μg/kg/infusion remifentanil (FI5 for three sessions followed by VR4 for two sessions) within 5 h. Punishment Responses for remifentanil paired with foot shock (increasing in duration by 0.01 s per trial within session), which increased in intensity over training sessions (0.25 mA to 0.51 mA). Extinction After drug self-administration training and 5 additional days of FI5 training, a separate group of rats were placed on extinction training in which drug was no longer available for 16 sessions.	Reinstatement After 1 h without responding (foot shock set to 1 mA for 0.5 s), test injection of vehicle or lorazepam was administered (0.08, 0.16, 0.31, 0.62, 1.25, 2.5, 5, and 10 mg/kg, i.p.). After injection, the remainder of test sessions continued with foot shock off but remifentanil available. Sessions lasted for 4 h after injection or when 100 remifentanil trials were completed (n = 4). Reinstatement (with extinction training) Same as above, but remifentanil was not available. Additional double injections of lorazepam and heroin (0.075, 0.25, 0.75 mg/kg, s.c.) were also tested (n = 4 punishment; n = 7 extinction).	Priming injections of heroin or lorazepam induce relapse to remifentanil seeking after punishment.
3	Economidou et al. (2009)	Rat strain and sex Outbred Lister hooded males. Impulsivity assessment Rats were assigned to high- or low-impulsivity groups based on training on a five-choice serial reaction-time task. Drug training 0.25 mg/infusion of cocaine under a FR1-RI120 second-order reinforcement schedule for 10–15 sessions. Sessions ended when 11 cocaine infusions were earned. Punishment 50% of seeking responses now punished by 0.5-s foot shock. Sessions lasted 2 h and rats were trained for eight sessions. Extended access FR1 for cocaine for 12 sessions. Sessions lasted for 6 h or when 150 infusions were earned. Retraining for drug and punishment reassessment Retraining on FR1-RI120 schedule for cocaine for four sessions (as described in drug training section) followed by retraining on punishment for eight sessions (as described in punishment section). Forced abstinence Rats returned to home cage for 7 days.	Relapse Test for relapse under the FR1-RI20s schedule for 1 h (n = 9 high impulsive; n = 12 low impulsive). Relapse with atomoxetine 7 days after punishment, rats were given pretreatment of atomoxetine (3.0 mg/kg, i.p.) 20 min prior to relapse test (n = 10 high impulsive; n = 10 low impulsive).	Extended-access cocaine self-administration increases relapse after punishment plus 7 days of forced abstinence in the high-impulsivity group; this effect is decreased by atomoxetine.
4	Pelloux et al., (2013)	Rat strain and sex Outbred Lister hooded males. Drug training 0.25 mg/infusion of cocaine under an FR1 schedule for five to seven sessions. Sessions lasted for 2 h or when 30 cocaine reinforcers were earned. Followed by training on FR1-RI120-s schedule for nine sessions. Sessions ended when 11 cocaine infusions were earned.	Reinstatement Lever presses under the second-order schedule without cocaine or punishment (n = 10–16 per brain region).	Dorsal mPFC lesions decrease relapse to cocaine seeking, whereas AI lesions increase relapse after punishment plus 7 days of forced abstinence.

(*continued*)

TABLE 3—*Continued*

No.	Reference	General Training Procedures	Test	Major Findings
		Motivational assessments Training under progressive ratio for two sessions followed by probe of seeking under extinction for one session and finally baseline second-order retraining for four sessions. Punishment 50% of seeking responses now punished by 0.5-s foot shock. Sessions lasted 2 h for eight sessions. Forced abstinence Rats returned to home cage for 7 days.		
5	Marchant et al. (2013a)	Rat strain and sex Alcohol-preferring males. Home-cage alcohol intake Two-bottle choice for 20% v/v alcohol versus water every other day (12 × 24-h sessions). Drug training Context A: 0.1 ml 20% alcohol (FR1 to FR5) for seven sessions (2 h, no limit noted) followed by VI30 for four sessions (2 h, no limit noted). Punishment group Context B: 50% of responses for alcohol paired with 0.5-s foot shock (starting at 0.45 mA to 1.09 mA) for three to seven sessions. Extinction group Context B: Responses on active lever that met the VI30 requirement led to presentation of light-tone cue but not alcohol or foot shock for 13 sessions (2 h).	Relapse test Test of relapse in context A and context B (counterbalanced) for 30 min (n = 15 punished; n = 11 unpunished; n = 8 noncontingent).	Re-exposure to self-administration context (context A) induces relapse to alcohol seeking after punishment or extinction in context B. The study introduces the new model.
6	Krasnova et al. (2014)	Rat strain and sex Sprague-Dawley males. Drug or food training FR1 for 0.1 mg/kg/infusion methamphetamine for 14 sessions (9 h, limited to 35 infusions/3 h) or FR1 for five pellets for 14 sessions (9 h). Punishment 50% of responses for methamphetamine or food paired with 0.5-s foot shock (0.12 mA, increased to 0.6 or 0.66 mA) for 9–10 sessions (9 h). Forced abstinence Rats returned to home cage until relapse test.	Relapse tests Test for methamphetamine or food seeking for 1 h on abstinence day 2 and 21 (n = 26 punished methamphetamine; n = 20 unpunished methamphetamine; n = 24 punished food; n = 22 unpunished food).	Incubation of methamphetamine and food craving is observed after punishment plus 21 days of home-cage forced abstinence.
7	Marchant et al. (2014)	Rat strain and sex Alcohol-preferring males. Home-cage alcohol intake Two-bottle choice for 20% v/v alcohol versus water every other day (12 × 24-h sessions). Drug training Context A: 0.1 ml 20% alcohol (FR1) for six sessions (2 h, no limit noted) followed by VI30 for six sessions (2 h, no limit noted). Punishment Context B: 50% of responses for alcohol paired with 0.5-s foot shock (starting at 0.3 mA to 0.7 mA) for three to seven sessions (2 h).	Relapse test Test of relapse in context A and/or context B for 90 min for Fos (n = 8 punishment context B; n = 8 alcohol training context A; n = 5 home cage) and Fos + CTb labeling (n = 8 punishment context B; n = 8 alcohol training context A; n = 6 no test) or for 30 min with infusions of muscimol + baclofen (0.06 + 0.6 mM or 3.6 + 64.1 ng/0.5 μl/side) nto LH (n = 8 vehicle; n = 10 muscimol + baclofen in LH; n = 7 muscimol + baclofen in dorsal to LH).	Context-induced relapse of alcohol seeking after punishment in male rats is associated with increased Fos expression in LH and selective activation of NAc shell neurons projecting to LH. Inactivation of LH using muscimol + baclofen decreases context-induced relapse in alcohol-preferring P rats.
8	Marchant and Kaganovsky (2015)	Rat strain and sex Alcohol-preferring males. Home-cage alcohol intake Two-bottle choice for 20% v/v	Relapse test Test of relapse in context A and/or context B for 90 min with systemic (n = 7, 5 μg/kg;	Systemic and NAc shell and core injections of the Drd1 receptor antagonist SCH 23390 decreases context-induced

(*continued*)

TABLE 3—*Continued*

No.	Reference	General Training Procedures	Test	Major Findings
		alcohol vs. water every other day (12 × 24-h sessions). Drug training Context A: 0.1 ml 20% alcohol (FR1) for six sessions (2 h, no limit noted) followed by VI30 for six sessions (2 h, no limit noted). Punishment Context B: 50% of responses for alcohol paired with 0.5-s foot shock (starting at 0.1 mA to 0.7 mA) for 7–11 sessions.	n = 9, 10 μg/kg SCH 23390; n = 9, vehicle) or microinfusions of Drd1 receptor antagonist SCH 23390 (n = 9, 0.6 μg/side SCH 23390 and n = 15, vehicle in shell; n = 8, 0.6 μg/side SCH 23390, and n = 12 vehicle in core).	relapse to alcohol seeking after punishment in alcohol-preferring P rats.
9	Marchant et al. (2016)	Rat strain and sex Alcohol-preferring males. Home-cage alcohol intake Two-bottle choice for 20% v/v alcohol vs. water every other day (12 × 24-h sessions). Drug training Context A: 0.1 ml 20% alcohol (FR1) for six sessions (2 h, no limit noted) followed by VI30 for six sessions (2 h, no limit noted). Punishment Context B: 50% of responses for alcohol paired with 0.5-s foot shock (starting at 0.1 mA to 0.7 mA) for six to seven sessions.	Relapse test Test of relapse in context A and/or context B for 30 or 90 min with infusions of muscimol + baclofen (0.06 + 0.6 mM or 3.6 + 64.1 ng/0.5 μl/side) into vSub (n = 7 per test group in muscimol + baclofen).	Context-induced relapse is associated to alcohol seeking is associated with selective activation of vSub→NAc shell projection. Inactivation of vSub using muscimol + baclofen and DREADD inhibition of vSub→NAc projection decreases relapse.
10	Torres et al. (2017)	Rat strain and sex Sprague-Dawley males. Drug training 0.1 mg/kg/infusion methamphetamine or saline control (FR1) for 22 sessions (9 h, limited to 35 infusions/3 h). Punishment 50% of responses for methamphetamine paired with 0.5-s foot shock (0.18 mA, increased 0.06 mA each session until 0.42 mA) over 13 sessions (9 h). Saline rats had yoked foot-shock delivery. Forced abstinence After voluntary abstinence rats were returned to home cage for 21 days of forced abstinence.	Relapse tests Test for methamphetamine seeking for 1 h on abstinence day 2 and 21 (n = 9 shock sensitive; n = 7 shock; n = 5 saline control).	Incubation of methamphetamine craving after punishment and forced abstinence is greater in punishment-resistant rats.
11	Krasnova et al. (2017)	Rat strain and sex Sprague-Dawley males. Drug training 0.1 mg/kg/infusion methamphetamine or saline control (FR1) for 20 sessions (9 h, limited to 50 infusions/3 h). Punishment 50% of responses for methamphetamine paired with 0.5-s foot shock (0.18, increased 0.06 mA each session until 0.30 mA) over five sessions (9 h). Saline rats had yoked foot-shock delivery. Forced abstinence After voluntary abstinence, rats were returned to home cage for 30 days of forced abstinence.	Relapse tests Test for methamphetamine seeking for 1 h on abstinence day 2 and 30 (n = 8 shock sensitive; n = 9 shock resistant; n = 8 saline).	Incubation of methamphetamine craving after punishment plus forced abstinence in male rats is associated with upregulation of several genes in striatum (e.g., oxytocin in NAc and CARTpt in dorsal striatum) in punishment-resistant rats.
12	Pelloux et al. (2018a)	Rat strain and sex Sprague-Dawley males. Drug training Context A: 0.75 mg/kg/infusion cocaine (FR1) for six sessions (6 h, no limit noted) followed by VI30 for six sessions (6 h, no limit	Relapse tests Test for cocaine seeking in contexts A and B for 60 min (n = 8 paired; n = 6 unpaired) or 90 min in context A, context B, or home cage for Fos expression (n = 5–7 per context).	Context-induced relapse of cocaine seeking after punishment is associated with selective activation of dorsal and ventral mPFC, AI, dorsal striatum, BLA, PVT, LHb, SN, vSub, and

(*continued*)

TABLE 3—*Continued*

No.	Reference	General Training Procedures	Test	Major Findings
		noted). Punishment Context B: 50% of responses for cocaine paired with 0.5-s foot shock (0 mA, increased by 0.1 mA each session until 0.5 or 0.7 mA) for eight sessions (6 h, VI30). Rats divided into two groups: paired (foot shock paired with drug delivery) and unpaired (yoked foot-shock delivery).		DR, but not NAc, CeA, LH, VTA, and other brain regions.
13	Pelloux et al. (2018b)	Rat strain and sex Sprague-Dawley males. Drug training Context A: 0.75 mg/kg/infusion cocaine (FR1) for six sessions (6 h, no limit noted) followed by (VI30) for six sessions (6 h, no limit noted). Punishment Context B: 50% of responses for cocaine paired with 0.5-s foot shock (0 mA, increased by 0.1 mA each session until 0.5 or 0.7 mA) for eight sessions (6 h, VI30). Extinction Context B: Responses on active lever led to presentation of light-tone cue but not cocaine or foot shock.	Relapse tests Test for cocaine seeking for 1 h in context A or B with injections of muscimol + baclofen (50 + 50 ng/0.5 μl/side) into BLA (n = 11 punishment; n = 12 extinction) or CeA (n = 14 punishment; n = 16 extinction).	BLA inactivation with muscimol + baclofen increases context-induced relapse of cocaine seeking after punishment but not after extinction in context B. BLA or CeA inactivation with muscimol + baclofen induces relapse in context B after punishment in this context.
14	Campbell et al. (2018)	Rat strain and sex Alcohol-preferring males. Home-cage alcohol intake Intermittent two-bottle access of 20% v/v alcohol and water (three to four times per week) for eight sessions (24 h). Drug training Context A: 0.1 ml 20% alcohol (FR1) for seven sessions (20 min, no limit noted), followed by (VI30) for six sessions (20 min, no limit noted). Voluntary abstinence Context B: 50% of responses for alcohol paired with 0.5-s foot shock (0.2 mA, increased by 0.2 mA each session until 0.6 or 0.7 mA) for six sessions (20 min, VI30).	Relapse tests Test for alcohol seeking in context A or context B for 20 min for effect of paternal alcohol exposure (n = 6 control-sired offspring; n = 24 alcohol-sired offspring).	Paternal alcohol exposure decreases context-induced relapse after punishment in F1 offspring.
15	Farrell et al. (2019)	Rat strain and sex Long-Evans males and females. Drug training Context A: 0.58 mg/kg/infusion (male rats) or 0.66 mg/kg/infusion cocaine (female rats) (FR1) for five sessions (2 h, no limit noted) followed by three sessions VI5 (no limit noted), three sessions VI10 (no limit noted), and three to six sessions VI15 . Voluntary abstinence Context B: 50% of responses for cocaine paired with 0.5-s foot shock (0.3 mA) for three to four sessions (2 h, VI30), followed by increasing shock 0.15 mA every two sessions, up to 0.75 mA.	Relapse tests Test for cocaine seeking for 2 h in context A (no cue, cue, no cue + 10 mg/kg cocaine priming) or B (cue) with DREADD inhibition of VP (n = 10 males and n = 10 females in control; n = 26 males and n = 20 females in Gi-DREADD).	Chemogenetic inhibition of VP decreases cocaine priming and context-induced relapse to cocaine seeking after punishment. No sex differences were observed in cocaine self-administration, voluntary abstinence, or reinstatement, but female rats show greater cocaine-induced locomotion.
16	Campbell et al. (2019a)	Rat strain and sex Alcohol-preferring males. Home-cage alcohol intake Intermittent two-bottle access of 20% v/v alcohol and water (three to four times per week) for 8–12 sessions (24 h). Drug training	Relapse tests Test for alcohol seeking in context A or context B for 20 min on abstinence day 1 (n = 12 per context) and 30 (n = 21–23 per context) with Fos expression associated with incubation of alcohol craving (n = 6–8 per context) and muscimol + baclofen (50 + 50 ng/0.5 μl/	Incubation of alcohol craving after punishment plus forced abstinence selectively occurs in context B but not context A; AI inactivation with muscimol + baclofen decreases incubation in context B.

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TABLE 3—*Continued*

No.	Reference	General Training Procedures	Test	Major Findings
		Context A: 0.1 ml 20% alcohol (FR1) for seven sessions (20 min, no limit noted), followed by (VI30) for six sessions (20 min, no limit noted). Punishment Context B: 50% of responses for alcohol paired with 0.5-s foot shock (0.2 mA, increased by 0.2 mA each session until 0.6 or 0.7 mA) for six sessions (20 min, VI30). Forced abstinence After voluntary abstinence rats were returned to home cage for 28 days of forced abstinence.	side) inactivation in AI (n = 8–10 per condition, testing only in context B on abstinence day 30).	
17	Campbell et al. (2019b)	Rat strain and sex Alcohol-preferring males. Home-cage alcohol intake Intermittent two-bottle access of 20% v/v alcohol and water (three to four times per week) for 10 sessions (24 h). Drug training Context A: 0.1 ml 20% alcohol (FR1) for seven sessions (20 min, no limit noted), followed by (VI30) for six sessions (20 min, no limit noted). Punishment Context B: 50% of responses for alcohol paired with 0.5-s foot shock (0.2–0.7 mA) for six sessions (20 min, VI30). Forced abstinence After voluntary abstinence, rats were returned to home cage for 30 days of forced abstinence.	Relapse (extinction) tests Test for alcohol seeking in context A or context B for 20 min on abstinence days 1 and 32 for effect of environmental enrichment (n = 8–10 per context and enrichment group).	Enriched environment for 30 days in home cage decreases context A–induced relapse to alcohol seeking after punishment.
18	Hu et al. (2019)	Rat strain and sex Sprague-Dawley males. Drug training 0.1 mg/kg/infusion methamphetamine or saline control (FR1) for 20 sessions (9 h, no limit noted). Voluntary abstinence 50% of responses for methamphetamine or saline control paired with 0.5-s foot shock (0.18, 0.24, 0.3, 0.3, and 0.3 mA) over five sessions (9 h). Forced abstinence After voluntary abstinence, rats were returned to home cage for 30 days of forced abstinence.	Relapse tests Test for methamphetamine seeking for 30 min on abstinence days 3 and 30 (n = 11 shock sensitive; n = 7 shock resistant; n = 11 saline control).	fMRI signaling increases in OFC-DMS circuitry and decreases in dorsal mPFC-NAc circuitry after abstinence in rats that are more resistant to punishment. No differences in incubation of methamphetamine craving after punishment plus forced abstinence between punishment-resistant versus punishment-sensitive rats.

CARTpt, the gene encoding cocaine- and amphetamine-regulated transcript protein; DR, dorsal raphe; FI, fixed interval; FR, fixed ratio; LHb, lateral habenula; RI, random interval; SN, subtantia nigra; VI, variable interval; VR, variable ratio.

expression in different subregions of VP. The authors also divided the rats into punishment-sensitive and punishment-resistant groups based on the maximal shock intensity that induced abstinence in context B. They found that context-induced relapse was stronger when lever presses were reinforced with the discrete cues and that individual differences in punishment responding are associated with increased context-induced relapse but not cocaine priming–induced relapse. DREADD inhibition of VP decreased both context- and drug-priming–induced relapse after punishment. Finally, in agreement with Pelloux et al. (2018a), VP Fos expression was similar after exposure context A versus context B. Together, the results of this study indicate a role of VP in both context- and cocaine priming–induced relapse of cocaine seeking after punishment.

c. Relapse to drug seeking after punishment and home-cage forced abstinence. Several studies investigated mechanisms of relapse to drug seeking after punishment-imposed abstinence and subsequent home-cage forced abstinence. Early studies reported that, like extinction (Bouton and Swartzentruber, 1991), the punishment-suppressed conditioned response spontaneously recovers with passage of time (Azrin and Holz, 1966). Thus, this variation of the punishment-induced

abstinence model mimics features of the human condition of resumption of drug taking over time after termination of the punishment contingencies that have maintained abstinence (e.g., termination of mandatory urine samples in which drug detection results in loss of employment or visitation rights of one's children).

i. Alcohol Studies. Campbell et al. (2019a) used alcohol-preferring male rats and the ABA punishment-based procedure (Marchant et al., 2013a) to investigate the time course of relapse at different days after punishment cessation. They found that after 1 day of home-cage abstinence, relapse to alcohol seeking is context-specific and only occurs in context A, replicating previous findings (Marchant et al., 2013a, 2014, 2016). In contrast, after 30 days of home-cage abstinence, relapse reliably occurred in both context A and context B (punishment context), reflecting spontaneous recovery or incubation of the conditioned response in the punishment context (Krasnova et al., 2014). In follow-up experiments, they found that relapse in context B after 30 days of home-cage abstinence is associated with increased Fos expression in AI but not in other brain regions. Additionally, on abstinence day 30, muscimol + baclofen inactivation of AI decreased incubated relapse in context B. In contrast, on abstinence day 1, AI inactivation had no effect on context-induced relapse in context A or nonincubated low responding in context B. A question for future research is whether AI activity also plays a role in relapse in context A after prolonged home-cage abstinence.

Finally, in another study using a similar experimental design, Campbell et al. (2019b) first trained alcohol-preferring male rats to self-administer alcohol in context A. Next, the rats underwent punishment in context B and tested for relapse in context A or B. The authors then exposed groups of rats to either standard paired housing or enriched environment (large cages with enrichment items) for 31 days and retested them for relapse in both contexts on day 32. They reported that enrichment decreased relapse in both contexts. However, unlike the authors' previous findings (Campbell et al., 2019a), incubation of lever presses in context B did not occur in the standard paired-housing group because of the large individual differences in context B responding both 1 and 32 days after punishment. The reasons for the large individual differences in context B responding are unknown.

ii. Cocaine Studies. Economidou et al. (2009) investigated whether individual differences in impulsivity (assessed in the five-choice serial reaction-time task) and cocaine exposure history (extended versus limited access) predict relapse 7 days after punishment-induced abstinence. The authors screened male rats for high and low impulsivity on the five-choice serial reaction-time task, and the impulsive phenotype was determined with respect to their total number of premature responses (responses made before the onset of the target stimulus) during challenge sessions in which the intertrial interval period was increased from 5 seconds to 7 seconds. Next, the authors trained the rats to self-administer cocaine under a *second-order reinforcement schedule*. During punishment, foot shock was probabilistically paired with the seeking response on 50% of the trials, and on these trials, cocaine was not delivered. They found that relapse was higher in the high-impulsivity rats and that a history of extended-access cocaine self-administration selectively increased responding in these rats. They also reported that acute pretreatment with the norepinephrine uptake release inhibitor atomoxetine decreases cocaine relapse. Atomoxetine is a medication for attention deficit/hyperactivity disorder that decreases impulsivity in animal models and humans (de Wit, 2009).

In a follow-up study using the same experimental procedure, Pelloux et al. (2013) investigated in male rats the effect of permanent pretraining excitotoxic lesions of anterior cingulate, prelimbic, infralimbic, OFC, or AI cortices on relapse 7 days after punishment-induced abstinence. They found that prelimbic lesions decrease relapse, whereas AI lesions increase relapse; lesions of the other areas had no effect. Additionally, the cortical lesions had no effect on cocaine self-administration or punishment-induced abstinence. In contrast, BLA lesions had no effect on cocaine self-administration but blocked punishment-induced abstinence. These rats were not tested for relapse. However, a limitation of the study is the use of pretraining permanent lesions. Thus, a question for future research is whether similar results would emerge after reversible inactivation of the different regions immediately prior to relapse tests. Based on a recent study showing that acute reversible inactivation of AI decreases relapse to alcohol seeking after punishment plus prolonged home-cage abstinence in alcohol-preferring male rats (Campbell et al., 2018), we suspect that the results will be different.

iii. Methamphetamine Studies. Krasnova et al. (2014) investigated incubation of methamphetamine and palatable food seeking after extended-access self-administration (9 hours per day for 14 sessions) and punishment-induced suppression of drug or food self-administration (9 days) in male rats. Punishment-induced suppression of self-administration was achieved in all rats by increasing the daily shock level from 0.12 mA to 0.6 mA. Next, the authors tested the rats for relapse to drug or food seeking after 2 and 21 days of home-cage forced abstinence. The authors found time-dependent increases in both methamphetamine and food seeking (incubation of craving after punishment). As in previous studies (Li et al., 2015b, 2018; Grimm, 2020), they also found incubation of methamphetamine and food seeking after home-cage forced abstinence.

Torres et al. (2017) modified the incubation of drug craving after punishment procedure to investigate whether individual differences in punishment suppression correlate with incubation of methamphetamine seeking in male rats. In the modified procedure, shock level was progressively increased from 0.18 to 0.42 mA, resulting in two groups of rats: punishment-resistant (~45% suppression) and punishment-sensitive (~90% suppression). The authors found that incubation of methamphetamine seeking was higher in the punishment-resistant rats.

Krasnova et al. (2017) used a variation of the punishment procedure described above (shock levels were increased from 0.18 to 0.3 mA) to investigate gene expression changes in dorsal and ventral striatum after incubation of drug seeking in punishment-resistant (~15%–20% suppression) and punishment-sensitive (~80%–90% suppression) male rats. They used Affymetrix array platform containing 68,842 probes and measured different gene transcripts 24 hours after the day 30 relapse test. They replicated the findings that incubation of methamphetamine seeking after punishment is stronger in punishment-resistant rats. They also reported many gene expression differences between the punishment-resistant and punishment-sensitive rats in dorsal (e.g., CARTpt, the gene encoding cocaine- and amphetamine-regulated transcript protein) and ventral (e.g., oxytocin) striatum. However, the relevance of these correlational findings to mechanisms of incubation of methamphetamine craving after punishment is unknown because the authors have not followed up on the correlational results with causal role site-specific pharmacological or viral gene knockdown manipulations.

Hu et al. (2019) used the same punishment-resistance/sensitive experimental procedure used by Krasnova et al. (2017) and included a rat fMRI procedure to longitudinally investigate circuit connectivity changes during methamphetamine self-administration, punishment responding in punishment-resistant and punishment-sensitive male rats, and incubation of drug seeking after punishment. They reported that over time fMRI signaling increases in OFC-medial striatum projection and decreases in dorsal mPFC-NAc projection in punishment-resistant but not punishment-sensitive rats. These are interesting correlational data, but most likely they are not relevant to understanding of incubation because, unlike the studies described above, no differences in incubation of methamphetamine seeking were observed between punishment-resistant and punishment-sensitive rats. Additionally, even within the context of circuit changes that control resistance to punishment (Vanderschuren et al., 2017), the results should be interpreted with caution in the absence of projection-specific inhibition/activation experiments to determine the projections' role in punishment responding. This is because an inherent confound in the authors' experimental procedure is that punishment-resistant rats are exposed to both more methamphetamine and foot shock than the punishment-sensitive rats. Thus, the observed circuit changes may be due to group differences in both drug and stress exposure, both of which cause long-lasting brain changes (Kalivas and Stewart, 1991; Nestler and Aghajanian, 1997; McEwen et al., 2016).

3. Conclusions. Investigators have developed experimental procedures to investigate mechanisms of relapse after punishment-induced abstinence. The different procedural variations of the punishment-induced abstinence model can be used to study different forms of relapse of drug seeking. In one procedural variation, punishment can be used as a substitute for extinction to investigate the effect of manipulations traditionally used in reinstatement studies (discrete cue, context, and drug priming) on relapse after punishment-induced abstinence (Panlilio et al., 2005; Marchant et al., 2019). In another procedural variation, investigators can study relapse after punishment-induced abstinence and different periods of home-cage abstinence (incubation of drug craving after punishment) (Krasnova et al., 2014).

A main question for future research is whether mechanisms of relapse are similar after punishment-induced abstinence versus extinction-induced abstinence. The data indicate both similarities and differences. Heroin priming injection induces relapse after either punishment or extinction, whereas lorazepam's effect is selective to punishment (Panlilio et al., 2005). LH, vSub, NAc shell, vSub to NAc shell projections are critical for context-induced relapse after either punishment (Fig. 2) or extinction (Marchant et al., 2019). In contrast, reversible inactivation of BLA has opposite effects on context-induced relapse after punishment (potentiation) versus extinction (inhibition), whereas CeA inactivation decreases relapse after extinction but not punishment (Pelloux et al., 2018b). Another question for future studies is whether the findings discussed above generalize to female rats. To date, with the exception of Farrell et al. (2019), who used both males and females, all studies using the punishment-induced abstinence model only included male rats.

B. Electric Barrier–Induced Voluntary Abstinence Model

1. Overview. The procedure includes three phases: drug self-administration, electric barrier–induced voluntary abstinence, and relapse tests. During the training phase, laboratory animals are trained to self-administer a drug; each drug delivery is paired with a discrete cue. Next, during the voluntary abstinence phase, drug-taking behavior is suppressed by introducing an electric barrier of increasing intensity in

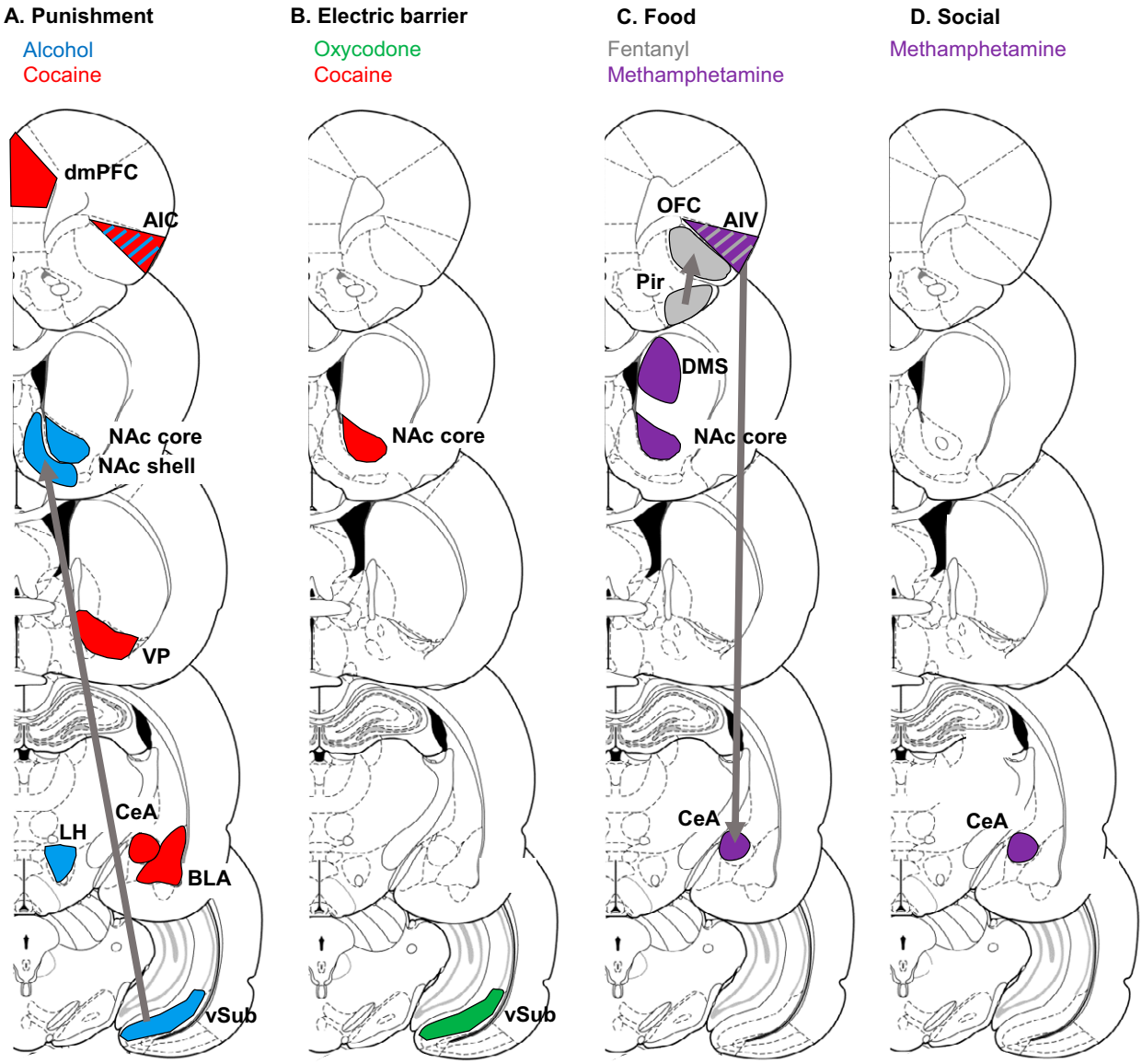

Fig. 2. Brain regions and projections involved in relapse after voluntary abstinence induced by adverse consequences (A-B) or food and social choice (C-D) for different addictive drugs. AIC, AI cortex; BLA, basolateral amygdala; CeA, central nucleus of amygdala; dmPFC, dorsomedial prefrontal cortex; DMS, dorsomedial striatum; LH, lateral hypothalamus; NAc, nucleus accumbens; OFC, orbitofrontal cortex; Pir, piriform cortex; vSub, ventral subiculum.

front of the drug-paired lever; the rats must cross this barrier to gain access to the drug (Cooper et al., 2007). During testing, typically performed 1 day after the completion of voluntary abstinence, relapse is assessed in the presence or absence of the electric barrier (Cooper et al., 2007). As with punishment-induced abstinence, relapse to drug seeking after electric barrier–induced abstinence can be tested after different abstinence periods to investigate incubation of drug craving (Fredriksson et al., 2020).

In the electric barrier–based relapse model, the negative consequences are associated with *drug seeking* (the electric barrier is adjacent to the drug-paired lever) prior to drug taking. The model attempts to mimic abstinence in humans due to negative consequences of drug seeking (e.g., securing money to obtain drug, stressful interactions with a drug dealer, adverse interactions with law enforcement) (Cooper et al., 2007). The electric barrier–based relapse model [also termed a conflict model (Cooper et al., 2007)] is based on the Columbia obstruction box method that was used many years ago to assess rats' motivation to obtain rewards under different deprivation conditions in the presence of an electric barrier (Jenkins et al., 1926; Warden, 1931). Relapse after electric barrier–induced abstinence has been studied in rats trained to self-administer cocaine, heroin, and oxycodone and has only been used to investigate discrete cue–induced relapse and incubation of drug craving (Table 4).

2. Review of Studies. a. Discrete cue–induced relapse in the presence of the electric barrier. In an initial study, Cooper et al. (2007) trained male rats to lever press for cocaine infusions that were paired with a discrete light cue. Next, they introduced an electric barrier and increased the barrier intensity over days from 0.25 mA up to 0.45 mA until the rats voluntarily abstained from cocaine self-administration for 3 days. During the subsequent relapse tests, they exposed the rats to intermittent noncontingent light-cue presentations (every 5 minutes for 20 seconds) and measured resumption of lever responding in the presence of the electric barrier intensity that led to 3 voluntary abstinence days. During testing, lever presses led to contingent light-cue presentations but not cocaine. The authors found that noncontingent cue exposure led to resumption of lever presses during the relapse tests, with large individual differences in responding in the rats who crossed the barrier during testing. The large individual differences during the relapse test agrees with other studies in which electric shock was used to suppress cocaine-taking behavior (Deroche-Gamonet et al., 2004; Pelloux et al., 2007). In these studies, only a relatively small proportion of the rats (less than 25%) continued to engage in drug-taking behavior in the presence of the aversive stimulus.

In a follow-up study, Barnea-Ygael et al. (2012) used a similar experimental procedure, except that they tested the male rats for relapse in the presence of either maximal-intensity electrical barrier or 85% of this intensity, measured relapse either 1 or 14 days after the last electric barrier exposure, and compared cocaine relapse to sucrose relapse. They reported that relapse responding was higher when electric barrier is at 85% intensity, responding for the cocaine cue was higher than responding for the sucrose cue, and responding was lower after 14 days of home-cage forced abstinence. The latter finding was unexpected based on the findings from many studies on incubation of cocaine craving after home-cage forced abstinence (Grimm et al., 2001; Wolf, 2016; Dong et al., 2017). A potential reason for this discrepancy is the development of time-dependent sensitization to electric barrier exposure (a stress condition) and cues associated with the electric barrier (Antelman et al., 2000) under the authors' experimental conditions. This can result in the development of incubation of conditioned fear (Pickens et al., 2009), which competes with the development of incubation of cocaine craving. Indeed, when the authors performed a second relapse test in the absence of the electric barrier during early (day 2) and late abstinence (day 15), responding was somewhat higher during late abstinence.

Peck et al. (2013) used the procedure developed by Cooper et al. (2007) to compare cue-induced relapse after electric barrier–induced voluntary abstinence in male rats trained to self-administer cocaine or heroin. One procedural difference was that during the relapse tests, the rats were only exposed to noncontingent cue presentations every 5 minutes, but lever presses were not reinforced by the cue. They reported that although cue-induced relapse was observed in all heroin-trained rats (10 of 10), responding was much more variable in the cocaine-trained rats (only 3 of 8), confirming the observations of large individual differences observed in the studies of Cooper et al. (2007) and Barnea-Ygael et al. (2012).

Together, heroin-trained male rats appear more vulnerable to cue-induced relapse after electric barrier–induced abstinence. A possible explanation for greater relapse in heroin- versus cocaine-trained rats might be the different motivational effects of heroin- and cocaine-associated cues during both self-administration and electric barrier suppression (see section IV.B for discussion).

Saunders et al. (2013) measured cue-induced relapse to cocaine seeking in male rats, identified as sign-trackers and goal-trackers based on their behavioral response in a Pavlovian conditioned approach procedure in which a lever extension (the conditioned stimulus) predicts the delivery of food (unconditioned stimulus) to a nearby receptacle (Peterson et al., 1972). Sign-trackers are rats that respond to the lever conditioned stimulus by interacting with it (e.g., licking, biting); goal-trackers are rats that respond to the lever conditioned stimulus extension by approaching the location where the food unconditioned stimulus would be delivered (i.e., the food receptacle) (Peterson et al., 1972). The authors reported that the sign-trackers showed higher cue-induced relapse to cocaine seeking after electric barrier–induced abstinence, a finding that agrees with the authors' previous finding of higher discrete cue–induced reinstatement after extinction in these rats (Saunders and Robinson, 2010). The authors also reported that NAc core injections of the nonselective dopamine receptor antagonist flupentixol decrease cue-induced relapse to cocaine seeking after electric barrier–induced abstinence, whereas local amphetamine injections increase relapse. In both cases, the effects were more pronounced in sign-trackers than in goal-trackers. These results demonstrate a critical role of NAc core dopamine in cue-induced relapse to cocaine seeking after electric barrier–induced abstinence (Saunders et al., 2013).

Finally, in a more recent study, Ewing et al. (2021) used a variation of the electric barrier model to study the effect of systemic injections of a Drd3 receptor antagonist (NGB 2904) and a Drd1 receptor partial agonist (SKF 77434) on cue-induced relapse to heroin seeking in the presence of the electrical barrier (set to

TABLE 4
Relapse after electric barrier–induced voluntary abstinence: summary of main findings

No.	Reference	General Training Procedures	Test	Major Findings
1	Cooper et al. (2007)	Rat strain and sex Sprague-Dawley males. Drug training 0.5 mg/kg/infusion of cocaine (FR1 to FR2) for 10–13 sessions (3 h, limit of 35 infusions per session). Electric barrier Application of an electric barrier that covers two-thirds of the chamber closest to lever that results in a continuous foot shock if entered (0.25 mA, increasing to 0.45 mA).	Relapse test 30-min sessions of noncontingent drug-cue exposure while the electric barrier was active (n = 24).	Large individual differences in cue-induced relapse to cocaine seeking after electric barrier–induced abstinence; relapse tests were performed in the presence of the barrier in this study and in studies 2–4 below.
2	Barnea-Ygael et al. (2012)	Rat strain and sex Sprague-Dawley males. Drug training 0.5 mg/kg/infusion of cocaine (FR1 to FR2) for 11–15 sessions (3 h, limit of 35 infusions per session). Electric barrier Application of an electric barrier that covers two-thirds of the chamber closest to lever that results in a continuous foot shock if entered (increasing intensity until three consecutive sessions of abstinence). Forced abstinence Rats undergoing electric barrier–induced abstinence returned to home cage for 14 days of forced abstinence.	Relapse test Cue-induced reinstatement for 3 h conducted 1 or 14 days after three consecutive sessions of abstinence with electric barrier active or at 85% intensity (n = 11 day 1; n = 10 day 14).	Cue-induced relapse to cocaine seeking after electric barrier–induced abstinence is lower after 14 days of home-cage forced abstinence than after 1 day.
3	Peck et al. (2013)	Rat strain and sex Long-Evans males. Drug training 0.5 mg/kg/infusion of cocaine or 0.05 mg/kg/infusion of heroin (FR1) for 15 sessions (3 h, no limit noted). Electric barrier Application of an electric barrier that covers two-thirds of the chamber closest to lever that results in a continuous foot shock if entered (0.25 mA, increasing by 0.04 mA intensity until three consecutive sessions of abstinence).	Relapse test 30-min sessions of noncontingent drug-cue exposure while the electric barrier was on (n = 10 heroin noncontingent cue; n = 10 heroin no noncontingent cue; n = 8 cocaine noncontingent cue).	The proportion of heroin-trained male rats that demonstrate discrete cue–induced drug seeking after electric barrier–induced abstinence is higher than that of cocaine-trained rats.
4	Saunders et al. (2013)	Rat strain and sex Sprague-Dawley males. Pavlovian training Rats were screened for sign tracking or goal tracking prior to drug training phase, according to three measures of Pavlovian conditioned approach following CS-US pairings (VT90, 25 trials). Drug training 0.4 mg/kg/infusion cocaine (FR1) for 10 infusions (three sessions), then 20 infusions per session (three sessions), and finally 40 infusions per session (five sessions). Rats were further divided into two groups: paired (drug delivery paired with light cue) and unpaired (cue light presented randomly). Electric barrier Application of an electric barrier that covers two-thirds of the chamber closest to lever that results in a continuous foot shock if entered and continuous mild foot shock (0, 0.15, 0.20, 0.25 mA, or increased by 0.05 mA) until they earned fewer than five infusions (30-min session).	Relapse tests Test for cocaine seeking for 30 min with foot shock on but at 50% intensity each rat had reached during training. Cocaine seeking (n = 10 sign-trackers and n = 10 goal-trackers for paired; n = 8 sign-trackers and n = 7 goal-trackers for unpaired) with additional tests for effect of vehicle or flupentixol (20 μg/0.5 μl/side; n = 8 sign-trackers and n = 6–7 goal-trackers per condition), or vehicle or amphetamine (10 μg/0.5 μl/side; n = 7–8 sign-trackers and n = 6–7 goal-trackers per condition).	Cue-induced cocaine seeking after electric barrier–induced abstinence is stronger and more reliable in sign-tracking male rats than in goal-tracking rats; NAc core injections of Drd1-Drd2 antagonist (flupentixol) and amphetamine decrease and increase, respectively, cue-induced cocaine seeking.

(*continued*)

TABLE 4—*Continued*

No.	Reference	General Training Procedures	Test	Major Findings
		Forced abstinence Rats undergoing electric barrier–induced abstinence returned to home cage for 2 weeks of forced abstinence.		
5	Fredriksson et al. (2020)	Rat strain and sex Sprague-Dawley males and females. Drug training 0.1 mg/kg/infusion oxycodone (FR1) for 14 sessions (6 h, limited to 15 infusions per hour). Electric barrier Application of an electric barrier that covers two-thirds of the chamber closest to lever that results in a continuous foot shock if entered and continuous mild foot shock (0 mA, increased by 0.1 mA until 0.4 mA) for 14 or 28 sessions (2 h). Forced abstinence Separate group of home-cage forced abstinence for 14 or 28 days.	Relapse tests Test for oxycodone seeking for 30 min on abstinence day 1, and 15 or 30 with foot shock off for effect of incubation of oxycodone craving (n = 14–28 males and n = 12–25 females per abstinence condition and day) with additional tests of (−)-OSU6162 (vehicle, 7.5 or 15 mg/kg, s.c.) after electric barrier–induced abstinence (n = 9–13 males and n = 9–15 females per dose and abstinence day) or after forced abstinence (n = 10–11 males and n = 9 females per dose).	Male and female rats show stronger incubation of oxycodone craving after electric barrier–induced abstinence than after home-cage forced abstinence. Systemic injections of (−)-OSU6162, a dopamine stabilizer, decrease incubated oxycodone seeking in both male and female rats after electric barrier–induced abstinence but only in male rats after forced abstinence. No sex differences were observed in oxycodone self-administration or electric barrier–induced abstinence.
6	Ewing et al. (2021)	Rat strain and sex Long-Evans males Drug training 0.05 mg/kg/infusion of heroin (FR1) for 15 sessions (3 h). Electric barrier Application of an electric barrier that covers two-thirds of the chamber closest to lever that results in a continuous foot shock if entered and continuous mild foot shock (0.25 mA, increasing by 0.07 mA intensity until three consecutive 30-min sessions of abstinence).	Relapse test Test for cue-induced heroin seeking in 30-min sessions (noncontingent heroin-cue exposure and contingent heroin-cue exposure (FR2) with electric barrier set to 25% intensity). 30-min pretreatment (intraperitoneal) with NGB 2904 (n = 45 vehicle, 0.25, 1, 1.5, or 2 mg/kg), SKF 77434 (n = 40 vehicle, 0.25, 1, or 2 mg/kg), or NGB 2904 + SKF 77434 combination (n = 42 0.25 + 0.25, 0.25 + 0.5, 1 + 0.25, or 1 + 0.5 mg/kg).	Systemic injections of a combination of low doses of NGB 2904 (Drd3 receptor antagonist) and SKF 77434 (Drd1 receptor partial agonist) decrease cue-induced heroin seeking more effectively than either compound alone.
7	Fredriksson et al. (2021)	Rat strain and sex Sprague-Dawley males and females. *Fos-lacZ* males and females. Drug training 0.1 mg/kg/infusion oxycodone (FR1) for 14 sessions (6 h, limited to 15 infusions per hour). Electric barrier Application of an electric barrier that covers two-thirds of the chamber closest to lever that results in a continuous foot shock if entered and continuous mild foot shock (0.4 mA, starting at 0.0 mA increasing by 0.1 mA) for 13 or 16 days. Daun02 induction Short (15 min) oxycodone context or novel context exposure followed by injections of vehicle or Daun02.	Relapse tests Test for oxycodone seeking for 30-min or 90-min sessions on abstinence day 1, 15, or 18 with foot shock off for vSub Fos expression (n = 6–7 per no test versus test), vehicle or muscimol + baclofen after electric barrier (50 + 50 ng/0.5 μl/side) injections in vSub (n = 10–14 per day 1 versus day 15), vehicle or muscimol + baclofen forced abstinence (50 + 50 ng/0.5 μl/side) injections in vSub (n = 16–18 per dose), and Daun02 (0.4 μg/side) inactivation (n = 12–17 per context and dose).	Relapse to oxycodone seeking is associated with increased vSub Fos expression. vSub injections of muscimol + baclofen or Daun02 decrease incubation of oxycodone seeking after electric barrier–induced abstinence but not forced abstinence.

CNO, clozapine-N-oxide; CS, conditioned stimulus; FR, fixed ratio; US, unconditioned stimulus; VT, variable time.

25% of the intensity for each rat during abstinence) after electric barrier suppression of heroin self-administration in male rats. They reported that a combination of low doses of each drug, which were independently ineffective, decreased cue-induced heroin seeking after electric barrier–induced abstinence.

b. Incubation of opioid craving. Fredriksson et al. (2020) modified the electric barrier-relapse model to study incubation of opioid (oxycodone) craving after voluntary abstinence due to negative consequences of drug seeking. An important difference between this procedural variation of the electric barrier model is that testing is performed in the absence of the electric barrier and active lever presses during testing lead to contingent presentation of a compound discrete cue (tone and light). In an initial study, Fredriksson et al.

(2020) compared incubation of oxycodone seeking after electric barrier–induced abstinence with incubation after home-cage forced abstinence. The authors trained male and female rats to self-administer oxycodone (6 hours per day) for 14 days. They then exposed them to either home-cage forced abstinence or voluntary abstinence induced by an electric barrier of increasing intensity (from 0.1 mA to 0.4 mA) near the drug-paired lever. On abstinence days 1, 15, or 30, the authors tested the rats for oxycodone seeking without shock and drug. They found that, independent of sex, the time-dependent increase in oxycodone seeking after cessation of opioid self-administration (incubation of opioid craving) was stronger after electric barrier–induced abstinence than after forced abstinence (Fredriksson et al., 2020) (Fig. 3). These results suggest that abstinence due to negative consequences would increase relapse vulnerability. The reasons for this potentiated effect are unknown, but the authors speculated that a potential reason might be due to stress exposure during the electric barrier phase. This speculation agrees with a previous study showing that repeated restraint stress exposure during forced abstinence increases incubation of cocaine seeking (Glynn et al., 2018).

Fredriksson et al. (2020) also determined whether the dopamine stabilizer (−)-OSU6162 [a potential addiction treatment medication (Khemiri et al., 2015)] would decrease incubation of oxycodone seeking after forced or voluntary abstinence in male and female rats. (−)-OSU6162 was developed by Carlsson and colleagues, and the drug can stimulate or inhibit dopamine-related behaviors depending on dopaminergic tone (Sonesson et al., 1994; Natesan et al., 2006; Rung et al., 2008). The authors found that (−)-OSU6162 decreases incubation of oxycodone seeking after voluntary abstinence, an effect that was stronger in male rats. (−)-OSU6162 also decreased incubation of oxycodone seeking after forced abstinence in males but not females. (−)-OSU6162's effect on oxycodone seeking was only observed on abstinence day 15 but not day 1, suggesting a selective effect on incubated opioid seeking that, at least in males, is independent of the method used to achieve abstinence (Fredriksson et al., 2020). The reasons for the lack of effect in female rats are unknown. The authors suggested that the sex differences in (−)-OSU6162's effect may be due to sex differences in dopamine function and responses to dopaminergic drugs (Robinson and Becker, 1986; Roth et al., 2004; Becker and Hu, 2008) or potential sex differences in (−)-OSU6162 pharmacokinetics.

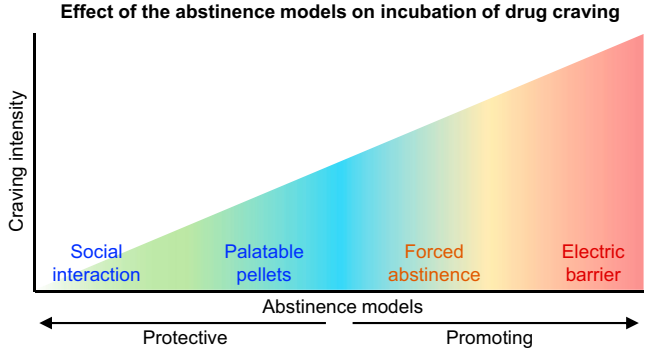

Fig. 3. Schematic representation of the effect of different abstinence models on incubation of drug craving. Drug-seeking intensity during relapse tests after prolonged abstinence is represented as an increasing spectrum ranging from white (low drug seeking) to red (high drug seeking). The figure depicts how positive alternative rewards during abstinence (e.g., social interaction or palatable food pellets) tend to be protective (left portion). Conversely, no alternatives (e.g., forced abstinence) or negative alternatives (e.g., electric barrier–induced voluntary abstinence) tend to promote drug seeking (right portion).

In another study, Fredriksson et al. (2021) determined the role of vSub in incubation of oxycodone seeking after electric barrier–induced abstinence in male and female rats. They tested the rats for relapse to oxycodone seeking on abstinence day 15 and extracted their brains for Fos immunohistochemistry or tested the rats after vSub injections of vehicle or muscimol + baclofen on abstinence day 1 or day 15 after forced or electric barrier–induced abstinence. The authors found that relapse after electric barrier–induced abstinence was associated with increased Fos expression in vSub and that local inactivation of vSub decreases incubated oxycodone seeking on day 15. In contrast, local inactivation of vSub on abstinence day 1 or day 15 had no effect on nonincubated oxycodone seeking or incubated oxycodone seeking after forced abstinence. In the same study, the authors used the-*Daun02 chemogenetic inactivation procedure* (Koya et al., 2009; Bossert et al., 2011) in Fos-LacZ transgenic rats to selectively inactivate the relapse (incubation) test-activated Fos-expressing neurons in vSub. They found that Daun02 inactivation decreased incubated oxycodone seeking, indicating a role of vSub neural ensembles in incubation after electric barrier–induced abstinence.

3. Conclusions. We and others developed experimental procedures to investigate mechanisms of relapse after electric barrier–induced abstinence. As with the punishment-based procedures, electric barrier can be used to investigate the effect on drug seeking of manipulations traditionally used in reinstatement studies (discrete cue, context, and drug priming) on relapse (Cooper et al., 2007) or to investigate incubation of drug craving (Fredriksson et al., 2020) after abstinence due to negative consequences of drug seeking.

In the electric barrier model, relapse can be assessed either in the presence or the absence of the electric barrier, and this methodological parameter can have a significant effect on relapse behavior. For example, large individual differences in relapse vulnerability are observed in the presence of the electric

barrier (Cooper et al., 2007; Barnea-Ygael et al., 2012) but not in the absence of the barrier (Fredriksson et al., 2020). These individual differences can be explained in part by pre-existing individual differences in responding to food reward conditioned stimuli in classic Pavlovian approach procedures (Saunders et al., 2013). Another potential reason for the individual differences observed in the presence of the electric barrier (Cooper et al., 2007; Barnea-Ygael et al., 2012) is the use of a single noncontingent cue versus a contingent compound cue (tone + light) in the Fredriksson et al. (2020) study. Previous studies have shown that noncontingent cues do not reliably reinstate cocaine seeking after extinction and that a compound discrete cue is more effective than a single cue (See et al., 1999; Kruzich et al., 2001).

Additionally, time-dependent decreases in relapse to drug seeking during abstinence are observed in the presence of the barrier (Barnea-Ygael et al., 2012), whereas the opposite occurs in the absence of the barrier (Fredriksson et al., 2020). However, a potential alternative explanation for the lack of incubation in the Barnea-Ygael et al. (2012) study is the use of a short-access training procedure (3 hours per day) versus a long-access training procedure (6 hours per day) in the Fredriksson et al. (2020) study. Indeed, Lu et al. (2004) reported that incubation of cue-induced reinstatement of cocaine seeking after extinction is more robust after extended-access (6 hours per day) versus limited-access (2 hours per day) cocaine self-administration.

Finally, results from neuropharmacological studies indicate that dopamine is critical to both cue-induced relapse to cocaine and heroin seeking and incubation of oxycodone seeking after electric barrier–induced voluntary abstinence (Saunders et al., 2013; Fredriksson et al., 2020; Ewing et al., 2021). In the case of cue-induced relapse to cocaine seeking, a critical brain region is the NAc core (Saunders et al., 2013). Additionally, recent data indicate a role of vSub in potentiated incubation of opioid seeking after electric barrier–induced abstinence (Fig. 2) (Fredriksson et al., 2020). However, to date, very few neuropharmacological studies assessed relapse after electric barrier–induced abstinence, and only one peer-reviewed study included females. In this study, females were less sensitive than males to the antirelapse effects of the dopamine stabilizer, (−)-OSU6162 (Fredriksson et al., 2020), which might be due to sex differences in dopamine function and responses to dopaminergic drugs (Robinson and Becker, 1986; Roth et al., 2004; Becker and Hu, 2008).

III. Relapse after Abstinence Induced by Availability of Alternative Nondrug Rewards

A. Food Choice–Induced Voluntary Abstinence Model

1. Overview. The procedure includes four phases: food and drug self-administration, early abstinence relapse test, food choice–induced voluntary abstinence, and late abstinence relapse test (Caprioli et al., 2015a). During the training phase, laboratory animals are first trained to self-administer palatable food pellets and then to self-administer a drug; each reward is paired with unique discriminative and discrete cues. During the early abstinence relapse test, the subjects are tested for drug seeking (lever presses are reinforced by the drug-associated discrete cues but not the drug, extinction conditions). During the voluntary abstinence phase, drug self-administration is suppressed by providing the subjects multiple daily mutually exclusive choices between the drug and the palatable food (Fig. 1). During the late abstinence relapse test, performed 1 day after the completion of the voluntary abstinence phase, the subjects are tested again for relapse to drug seeking. During the relapse tests, the food reward alternative is not available.

The procedure can be used to investigate incubation of drug craving after food choice–induced voluntary abstinence (Caprioli et al., 2015a; Venniro et al., 2017b) or relapse after voluntary abstinence when drug seeking is not assessed during early abstinence (Venniro et al., 2017a; Reiner et al., 2020) (Table 5). The development of the food choice voluntary abstinence/relapse model was based on previous food choice studies using nonhuman primates (Nader and Banks, 2014; Banks and Negus, 2017). These choice studies were primarily used to study pharmacological mechanisms of drug reinforcement, but Gasior et al. (2004) suggested that these models can also be used to study relapse to drug use. The food choice voluntary abstinence model (Caprioli et al., 2015b) was also inspired by studies of the Ahmed group showing that rats strongly prefer saccharin or sucrose over cocaine or heroin (Lenoir et al., 2007; Lenoir et al., 2013; Ahmed, 2018). Caprioli et al. (2015b) independently replicated this effect and reported that male rats show strong preference for palatable high-carbohydrate food pellets over methamphetamine under multiple experimental conditions.

From a translational perspective, the food choice–induced abstinence model attempts to mimic some aspects of abstinence in humans that is achieved due to availability of nondrug rewards that are chosen over the addictive drug (Caprioli et al., 2015a). This principle is exemplified by *contingency management*, a behavioral treatment in which small prizes or monetary vouchers can maintain abstinence for many months; however, when contingency management is discontinued, humans often relapse to drug use (Preston et al., 2002; Higgins et al., 2004).

2. Review of Studies. a. Methamphetamine studies. In the first study, Caprioli et al. (2015a) trained male rats to self-administer palatable food (six

sessions, 9 or 3 hours per day) and then to self-administer methamphetamine under two different self-administration procedures that are widely used to model drug addiction: extended-daily-access drug self-administration procedure (12 sessions, 9 hours per day) (Ahmed and Koob, 1998) and the *three-criteria DSM-IV model* (Deroche-Gamonet et al., 2004), used to identify "addicted" rats after long-term training (50 sessions, 3 hours per day). The authors then assessed methamphetamine seeking in relapse tests after 1 or 21 abstinence days. Between tests, the rats underwent either home-cage forced abstinence or voluntary abstinence for 19 days (achieved via a discrete-choice procedure between methamphetamine and palatable food; 20 trials per day). The authors found that under both training protocols (short-term extended daily access or long-term limited daily access) and abstinence conditions (forced and voluntary), methamphetamine seeking in the relapse tests was higher after 21 abstinence days than after 1 day (incubation of methamphetamine craving). These results indicate that incubation after food choice–induced voluntary abstinence is as robust as incubation after forced abstinence. In the same study, Caprioli et al. (2015a) also determined the effect of the novel $mGluR_2$ (metabotropic glutamate receptor 2) positive allosteric modulator, AZD8529 (Justinova et al., 2015), on relapse to methamphetamine seeking after forced or voluntary abstinence in male rats. AZD8529 decreased methamphetamine seeking on day 21 but not day 1 of forced or voluntary abstinence, indicating a specific effect of AZD8529 on incubated methamphetamine seeking that is independent of abstinence condition (voluntary or forced).

In a follow-up study, Venniro et al. (2017b) used a similar experimental procedure (except that the duration of the training sessions was 6 hours per day) to determine the generality of the voluntary abstinence choice model to female rats and to determine whether there are sex differences in incubation of drug seeking after food choice–induced abstinence. The authors found that incubation of methamphetamine seeking generalizes to female rats and that there are no sex differences in incubation of methamphetamine seeking. However, the authors observed a small decrease in food choice in female rats trained to self-administered methamphetamine (but not heroin) compared with male rats.

In the first study on brain mechanisms of incubation of methamphetamine craving after food choice–induced abstinence, Caprioli et al. (2017) explored the role of DLS and DMS in this incubation in male rats. The authors chose these striatal subregions because Li et al. (2015b) previously found that their activity is critical to incubation of methamphetamine seeking after home-cage forced abstinence. Using RNAscope, the authors found that incubation of methamphetamine seeking after food choice–induced abstinence is associated with increased *Fos* in DMS but not DLS and that *Fos* was coexpressed on both *Drd1* and dopamine D2 receptor (*Drd2*)-expressing cells. Next, the authors found that DMS injections of a Drd1 antagonist (SCH 39166) or a Drd2 antagonist (raclopride) selectively decrease incubated relapse on abstinence day 21 but not nonincubated relapse on day 1. Finally, in the same study, Caprioli et al. (2017) used the Daun02 chemogenetic inactivation procedure (Koya et al., 2009; Bossert et al., 2011) in Fos-LacZ transgenic rats to selectively inactivate the relapse test-activated Fos-expressing neurons in DMS to demonstrate a causal role of these neurons (the putative *neuronal ensembles*) in incubation of methamphetamine seeking. Together, the results of this study demonstrate a role of DMS Drd1- and Drd2-expressing neurons in incubation of methamphetamine seeking after voluntary abstinence and that DMS neuronal ensembles, which comprise both neuronal populations, are critical for this incubation.

In a follow-up study, Rossi et al. (2020) investigated the role of NAc core and shell Drd1 and Drd2 in incubation of methamphetamine craving after food choice–induced abstinence in male rats. They used experimental procedures similar to those used in the studies described above except that the alternative nondrug reward was a palatable solution (sucrose 1% + maltodextrin 1%) and incubation was assessed after 15 abstinence days. Using RNAscope, they reported that incubation of methamphetamine seeking is associated with increased *Fos* in *Drd1*- and *Drd2*-expressing cells in NAc core but not shell. Additionally, NAc core but not shell injections of muscimol + baclofen, flupentixol, SCH 39166, and raclopride decreased incubated methamphetamine seeking after 15 abstinence days. Together, these results indicate that dopamine transmission through Drd1 and Drd2 in NAc core is critical to incubation of methamphetamine seeking after voluntary abstinence.

Finally, Venniro et al. (2017a) used the food choice–induced voluntary abstinence model to investigate relapse after limited-access (2-hour sessions per day) methamphetamine self-administration in male rats. Under these conditions, drug seeking does not incubate over time (unpublished data). The goal of the study was to determine the role of CeA and glutamatergic projections to this region in relapse after 15 days of food choice–induced abstinence. The authors found that relapse after voluntary abstinence was associated with higher *Fos* expression in CeA *Drd1*-expressing neurons than in *Drd2*-expressing neurons, with a similar pattern of activation in lateral (CeL) and medial (CeM) parts. Additionally, systemic injections of a Drd1 antagonist (SCH 39166) decreased

TABLE 5
Relapse after food choice–induced voluntary abstinence: summary of main findings

No.	Reference	General Training Procedures	Test	Major Findings
1	Caprioli et al. (2015a)	Rat strain and sex Sprague-Dawley males. Food training Five palatable food pellets per delivery for six sessions (9 h). Drug training with choice probes 0.1 mg/kg/infusion methamphetamine (FR1) for 12 sessions (9 h, limited to 15 infusions per hour) with choice probes every 3 days. Food choice–induced abstinence Choice between five food pellets and methamphetamine (0.1 mg/kg/infusion) for 20 trials per session for 19 sessions. Forced abstinence Separate group of home-cage forced abstinence for 20 days.	Relapse tests 3-h pretreatment of AZD8529 (0, 20, 40 mg/kg, s.c.) on abstinence day 1 or day 21. Test for methamphetamine seeking for 30 min (n = 8–9 per AZD8529 dose after forced abstinence; n = 14–16 per AZD8529 dose after voluntary abstinence).	Incubation of methamphetamine craving is observed after food choice–induced voluntary abstinence. Systemic injections of the positive allosteric modulator of metabotropic glutamate receptor 2, AZD8529, decrease incubated methamphetamine seeking.
2	Caprioli et al. (2017)	Rat strain and sex Sprague-Dawley males. *Fos-lacZ* females. Food training Five palatable food pellets per delivery for six sessions (9 h). Drug training with choice probes 0.1 mg/kg/infusion methamphetamine (FR1) for 12 sessions (9 h, limited to 15 infusions per hour) with choice probes every 3 days. Food choice–induced abstinence Choice between five food pellets and methamphetamine (0.1 mg/kg/infusion) for 20 trials per session for 14 or 19 sessions. Forced abstinence Separate group of home-cage forced abstinence for 20 days. Daun02 induction Short (15 min) methamphetamine or food seeking session followed by injections of vehicle or Daun02 75 min later on abstinence day 18.	Relapse tests Test for methamphetamine seeking for 60- or 90-min sessions on abstinence day 1 or day 21 for Fos expression during relapse (n = 5 day 1; n = 5 day 21; n = 6 no test), or effect of injections into DMS of vehicle or Drd1 (SCH 39166) or Drd2 (raclopride) antagonists (1.0 μg/0.5 μl/side) 10 min before test sessions (n = 6–7 per dopamine receptor antagonist) or effect of Daun02 (4.0 μg/1.0 μl/side) manipulation (n = 13–14 female Fos-lacZ per Daun02 or vehicle).	Incubated methamphetamine seeking is associated with increased Fos expression in DMS Drd1 and Drd2 neurons. Injections of Drd1 or Drd2 antagonists and Daun02 inactivation in DMS decrease methamphetamine seeking.
3	Venniro et al. (2017a)	Rat strain and sex Sprague-Dawley males. Food training Five palatable food pellets per delivery for six sessions (2 h). Drug training with choice probes 0.1 mg/kg/infusion methamphetamine (FR1) for 21 sessions (2 h, limited to 15 infusions per hour). Food choice–induced abstinence Choice between five food pellets and methamphetamine (0.1 mg/kg/infusion) for 20 trials per session for 14 sessions. Forced abstinence Separate group of home-cage forced abstinence for 14 days.	Relapse tests Test for methamphetamine seeking for 60- to 120-min sessions on abstinence day 15. Fos expression, n = 13–14 per dose with systemic SCH 39166 (0 or 20 μg/kg s.c.) injections, plus n = 8 per group no-test condition; RNAscope, n = 5–6 per condition; Fos + CTb labeling, n = 4; CeA or BLA SCH 39166 or raclopride (0, 0.5, or 1.0 μg/0.5 μl/side) injections, n = 7–9 per group; CeA CNO (0 or 1.0 mM/0.5 μl/side) injections, n = 15 group; AIV or OFC muscimol + baclofen i (0 or 50 + 50 ng/0.5 μl/side) injections, n = 8–12 per group.	Relapse to methamphetamine seeking is associated with increased Fos expression in CeA Drd1 neurons and projections from AIV to CeA in male rats. Injections of a Drd1 antagonist in CeA, muscimol + baclofen injections in AIV, and DREADD inhibition of projections from AIV to CeA decrease relapse.
4	Venniro et al. (2017b)	Rat strain and sex: Sprague-Dawley males and females. Food training Five palatable food pellets per delivery (FR1) for six sessions (6 h, limited to 15 deliveries per hour). Drug training with choice probes 0.1 mg/kg/infusion	Relapse tests Test for methamphetamine or heroin seeking for 30 min on abstinence day 1 and 120 min on abstinence day 21 (n = 10 males and n = 10–11 females per abstinence condition for methamphetamine; n = 11–15 males and n = 15–16 females	Incubation of methamphetamine craving after food choice–induced abstinence generalizes to female rats. Rats with a history of heroin self-administration do not show incubation of heroin craving after food choice–induced voluntary abstinence. No sex differences were observed in

(*continued*)

TABLE 5—*Continued*

No.	Reference	General Training Procedures	Test	Major Findings
		methamphetamine or heroin (FR1) for 12 sessions (6 h, limited to 15 infusions per hour) with choice probes every 3 days. Food choice–induced abstinence Choice between five food pellets and methamphetamine or heroin (0.1 mg/kg/infusion) for 20 trials per session for 20 sessions. Forced abstinence Separate group of home-cage forced abstinence for 14 days.	per abstinence condition for heroin).	any of the behavioral measures, except for a small decrease in food choice in female rats trained to self-administer methamphetamine compared with male rats.
5	Reiner et al. (2020)	Rat strain and sex Sprague-Dawley males and females. Food training Five pellets per delivery (FR1) for six sessions (6 h, limited to 12 total deliveries per hour). Drug training with choice probes 2.5 μg/kg/infusion fentanyl (FR1) for 12 sessions (6 h, limited to 12 infusions per hour) with choice probes every 3 days. Food choice–induced abstinence Choice between five food pellets and 2.5 μg/kg/infusion fentanyl for 20 trials per session for 10–14 sessions.	Relapse tests Test for fentanyl seeking for 30 min on abstinence day 1 and 60 min on abstinence day 14 (n = 22 males; n = 20 females) or 3-h test on abstinence day 15 for Fos expression associated with fentanyl relapse (n = 7 per test condition), vehicle, or muscimol + baclofen (50 + 50 ng/0.5 μl/ side) injections in OFC (n = 10–17 per dose), AIC (n = 13 per dose), and Pir (n = 14–15 per dose), or OFC-Pir disconnection test (n = 5–11 per dose).	Rats with a history of fentanyl self-administration do not show incubation of fentanyl craving after food choice–induced abstinence. Relapse to fentanyl seeking is associated with increased Fos expression in OFC, AIC, Pir, and projections from Pir to OFC. Injections of muscimol + baclofen in these regions and asymmetric anatomic disconnection between Pir and OFC decrease relapse. No sex differences were observed for any of the behavioral measures except for voluntary abstinence in one experiment in which females had a lower food preference. However, when data from all experiments are combined, this sex difference is not observed.
6	Rossi et al. (2020)	Rat strain and sex Sprague-Dawley males. Food training 0.4 ml delivery of 1% sucrose + 1% maltodextrin solution (FR1) for six sessions (6 h, limited to 120 deliveries per session). Drug training with choice probes 0.1 mg/kg/infusion methamphetamine (FR1) for 12 sessions (6 h, limited to 120 infusions per session) with choice probes every 3 days. Food choice–induced abstinence Choice between 1% sucrose + 1% maltodextrin solution and 0.1 mg/kg/infusion methamphetamine for 20 trials per session for 14 sessions.	Relapse tests Test for methamphetamine seeking for 60 min on abstinence day 1 or 15 for Fos expression associated with incubated methamphetamine seeking (n = 3–5 per test condition); muscimol + baclofen (50 + 50 ng/0.5 μl/ side) injections in NAc core or shell (n = 6–8 per dose); Drd1-Drd2 antagonist (flupentixol, 10 μg/0.5 μl/side, n = 7 per dose) injections in NAc core; Drd1 antagonist (SCH 39166, 1.0 μg/ 0.5 μl/ side, n = 6–8 per dose) and Drd2 antagonist (raclopride, 1.0 μg/0.5 μl/side, n = 6–8 per dose) in NAc core.	Incubated methamphetamine seeking is associated with increased Fos expression in NAc core (but not shell) Drd1 and Drd2 neurons. Injections of muscimol + baclofen, a Drd-Drd2 antagonist (flupentixol), or selective Drd1 (SCH 39166) and Drd2 (raclopride) antagonists in NAc core decrease relapse.

AIC, AI cortex; CNO, Clozapine-N-oxide; FR, fixed ratio.

both relapse and relapse-associated increases in CeA activity. The systemic effect of SCH 39166 was mimicked by CeA drug injections; in contrast, CeA injections of a Drd2 antagonist (raclopride) or BLA injections of SCH 39166 were ineffective. Furthermore, relapse after voluntary abstinence was associated with selective activation of the ventral AI (AIV) projection to CeA but not projections from vSub, PVT, vmPFC, and BLA. Reversible inactivation of the AIV (but not the nearby OFC) and chemogenetic inhibition of the AIV→CeA projection decreased relapse, and inhibition of the AIV→CeA projection also decreased CeA Fos expression. Finally, in the same study, Venniro et al. (2017a) used electron microscopy and electrophysiology to demonstrate that AIV vGluT1 (vesicular glutamate transporter 1)-expressing projection neurons preferentially innervate the CeL subregion of CeA and form monosynaptic glutamatergic asymmetric synapses on CeA cells. Together, these results demonstrate a critical role of Drd1-mediated neuronal activity in CeA, which is controlled by AIV→CeA glutamatergic projections, in relapse to methamphetamine seeking after food choice–induced abstinence.

b. Opioid studies. Venniro et al. (2017b) compared incubation of heroin seeking in male and female rats after food choice–induced abstinence versus home-cage forced abstinence. The experimental

procedure was identical to the one used in the methamphetamine studies described above except that lever presses were reinforced with heroin during the 6-hour drug self-administration sessions. As in previous studies (Shalev et al., 2001; Airavaara et al., 2011), the authors found reliable incubation of heroin seeking after forced abstinence (higher drug seeking on abstinence day 21). In contrast, food choice–induced voluntary abstinence prevented the emergence of incubation of heroin seeking in both male and female rats. Finally, under the authors' experimental conditions, there were no sex differences in the strong preference for the palatable food over heroin, incubation of heroin seeking after forced abstinence, or the prevention of incubation of heroin seeking after food-induced abstinence (Venniro et al., 2017b).

In a recent study, Reiner et al. (2020) showed that the inhibitory effect of food choice–induced voluntary abstinence on incubation of heroin seeking generalizes to the synthetic opioid fentanyl. In this study, the authors investigated the role of OFC and its afferent projections in relapse to fentanyl seeking after 2 weeks of food choice–induced abstinence. The authors studied the OFC because, in a previous study, Fanous et al. (2012) demonstrated a role of this brain region in incubation of heroin seeking after forced abstinence. Reiner et al. (2020) found that in both male and female rats, relapse after food choice–induced abstinence is associated with increased Fos expression in OFC and that reversible inactivation of OFC with muscimol + baclofen decreases relapse. The authors then determined projection-specific activation of OFC afferents during the relapse test by using Fos plus CTb (injected into OFC). They found that relapse to fentanyl seeking is associated with increased Fos expression in piriform cortex (Pir) neurons projecting to OFC but not in projections from BLA and thalamus. Next, the authors found that inactivation of Pir with muscimol + baclofen decreases relapse. This effect was mimicked by anatomic disconnection of Pir from OFC by unilateral muscimol + baclofen injections into Pir in one hemisphere plus unilateral muscimol + baclofen injections into OFC in the contralateral side. Finally, relapse to fentanyl seeking after food choice–induced abstinence was associated with increased Fos in AI, and reversible inactivation of the AI decreased relapse. Together, the results identify a role of OFC, Pir, AI, and Pir-OFC projections in relapse to fentanyl seeking after food-induced abstinence.

3. Conclusions. Caprioli et al. (2015a) established a reliable procedure to study mechanisms of relapse to psychostimulant and opioid seeking after food choice–induced voluntary abstinence in male and female rats. At the behavioral level, no sex differences were observed in the strong preference for the palatable food over the drugs or in relapse after food choice–induced abstinence except for a small decrease in food choice in female rats trained to self-administered methamphetamine (but not heroin) compared with male rats.

An unexpected finding from the studies discussed above is that food choice–induced abstinence has no effect on incubation of methamphetamine seeking but prevents the emergence of incubation of opioid (heroin and fentanyl) seeking. The reasons for the selective effect of food choice on heroin versus methamphetamine relapse are unknown and a question for future research.

Finally, mechanistic studies demonstrate a role of DMS (but not DLS) and NAc core (but not shell) in incubation of methamphetamine seeking after food choice–induced abstinence. We speculate that this common role suggests that relapse after choice-induced abstinence reflects goal-directed (devaluation sensitive) behavior for which activity of both DMS and NAc core (but not DLS) is critical (Balleine and O'Doherty, 2010; Parkes et al., 2015). There is also evidence for a role of CeA Drd1, AIV (but not OFC) activity, and the projection from AIV to CeA in relapse to methamphetamine seeking after food choice–induced abstinence (Fig. 2). Finally, the recent study by Reiner et al. (2020) indicates a role of OFC, AI, Pir, and projections between Pir and OFC in relapse to fentanyl seeking after food choice–induced abstinence (Fig. 2). The selective role of OFC in relapse to fentanyl but not methamphetamine seeking suggests that the brain circuits that control relapse to psychostimulant versus opioid drugs after food choice–induced abstinence are not the same.

B. Social Choice–Induced Voluntary Abstinence Model

1. Overview. The procedure includes four phases: social interaction and drug self-administration, early abstinence relapse test, social choice–induced voluntary abstinence, and late abstinence relapse test (Venniro et al., 2018; Venniro and Shaham, 2020). During the training phase, laboratory animals are first trained to lever press for access to social interaction with a peer and then to self-administer a drug; each reward is paired with unique discriminative and discrete cues. During the early abstinence relapse test, the subjects are tested for drug seeking (lever presses are reinforced by the discrete drug-associated cues but not the drug). During the voluntary abstinence phase, drug self-administration is suppressed by providing the subjects daily mutually exclusive choices between the drug and social interaction (social reward). During the late abstinence relapse test, performed 1 day after the completion of the voluntary abstinence phase, the subjects are tested again for relapse to drug seeking. During the relapse tests, the

social-reward alternative is not available (Venniro and Shaham, 2020).

Venniro et al. (2018) developed the social choice procedure because the use of palatable food as the nondrug reward may limit the clinical translation of choice models. In most human drug users, the rewards that compete with drugs are primarily social (family and employment) (Stitzer et al., 2011). From a translation perspective, the social choice model attempts to mimic some aspects of human behavioral treatments such as the *community reinforcement approach* and the therapeutic work place, which promote prolonged abstinence by offering volitional social interactions with social reinforcers such as support groups and positive work environments (Hunt and Azrin, 1973; Silverman et al., 2012). A major finding from the initial study introducing the social choice model was that rats strongly prefer social interaction over drugs and that this effect was independent of "addiction score," drug (opioid versus psychostimulant), dose, self-administration procedure, housing conditions (single versus paired housing), sex, and duration of time in home cage (forced abstinence) (Venniro et al., 2018) (see Table 6 for a detailed description). Below, we describe results from the recent studies on incubation of drug craving after social choice–induced voluntary abstinence (Table 7).

2. Review of Studies. In the first study, Venniro et al. (2018) trained rats to lever press for social interaction (six sessions, 2 hours per day, 60 trials per session) or palatable food (6 hours per day). Next, the authors trained the rats to lever press for methamphetamine for 12 sessions (6 hours per day). The authors then compared different groups of male and female rats for incubation of methamphetamine seeking after social choice–induced abstinence versus incubation after food choice–induced abstinence or forced abstinence. As in previous studies (see above), the authors found reliable incubation of drug seeking after either food choice–induced voluntary abstinence or forced abstinence. In contrast, incubation of methamphetamine seeking was prevented by social choice–induced voluntary abstinence (Fig. 3); this inhibitory effect persisted for an additional 30 days of home-cage forced abstinence (Venniro et al., 2018).

In two follow-up correlational experiments Venniro et al. (2018) used double and triple immunohistochemistry and RNAscope in situ hybridization and found that the protective effect of social choice–induced abstinence on incubation of methamphetamine seeking is associated with activation (assessed by Fos) of inhibitory protein kinase-Cδ (PKCδ)-expressing neurons in CeL and decreased activity of output neurons in CeM. In contrast, the strong incubation of drug seeking after forced abstinence was associated with activation of CeL-expressing somatostatin (SOM) neurons and CeM output neurons. The protective effect of social choice–induced abstinence on incubation was also associated with decreased activity (Fos expression) of AIV and dorsal AI, but not anterior cingulate, dorsal and ventral mPFC, lateral and medial OFC, and BLA (Venniro et al., 2018).

In a recent study, Venniro et al. (2020b) determined the causal role of CeL PKCδ and SOM in inhibition of incubation of methamphetamine seeking after social choice–induced abstinence and expression of incubation of drug seeking after home-cage forced abstinence, respectively. For this purpose, the authors used male rats and developed short-hairpin RNAs against PKCδ or SOM. In initial slice in vivo electrophysiology experiments, the authors found that viral knockdown of the PKCδ enzyme or SOM peptide in CeL inhibits neuronal activity (decreased firing in response to depolarizing current injections) in PKCδ-expressing or SOM-expressing cells, respectively. Next, in the behavioral experiments, the authors found that viral knockdown of PKCδ enzyme in CeL decreases Fos in CeL PKCδ-expressing neurons, increases Fos in CeM output neurons, and reverses the inhibitory effect of social choice–induced abstinence on incubation of methamphetamine seeking. In contrast, viral knockdown of SOM CeL injections decreased Fos in CeL SOM-expressing neurons, decreased Fos in CeM output neurons, and decreased incubation after forced abstinence (Venniro et al., 2020b). Together, these results demonstrate a causal role of CeL PKCδ activity in the protective effect of social choice–induced abstinence against the development of incubation of methamphetamine seeking and CeL SOM activity in the expression of incubation of methamphetamine seeking after home-cage forced abstinence.

Finally, in another study, Venniro et al. (2019) determined the generality of the protective effect of social choice–induced abstinence to incubation of heroin seeking. In the first experiment, the authors trained male and female rats for social self-administration (6 days) and then for extended-access heroin self-administration (12 days). Next, the authors determined incubation of heroin seeking after social choice–induced abstinence or forced abstinence. The authors found that incubation of heroin seeking was observed under both experimental conditions, but it was lower after social choice–induced abstinence than after forced abstinence. In the second experiment, the authors replicated the findings of incubation of heroin seeking after social choice–induced abstinence and also showed that this incubation occurs under conditions of more limited social interaction via a screen that separates the drug self-administration chamber and the social peer chamber; the screen allows

physical contact but prevents rats from crossing between the chambers. Venniro et al. (2019) developed the fully automatic screen model to eliminate procedural limitations of the original semiautomatic model: intense workload and repeated physical interaction between the experimenter and rats, which can introduce experimenter-related confounds and cause rodent-related allergies to the experimenter (Venniro and Shaham, 2020).

3. Conclusions. Venniro et al. (2018) established a reliable procedure to study relapse to psychostimulant and opioid seeking after social choice–induced voluntary abstinence. As with the palatable food choice–induced abstinence model, they observed no sex differences in the strong preference for social interaction over the drugs or in relapse after social choice–induced abstinence. At the behavioral level, there are two main findings. The first is the unexpected strong inhibitory effect of social choice–induced abstinence on incubation of methamphetamine seeking but not incubation of heroin seeking. This finding was unexpected because this pattern of results is opposite from what was observed after food choice–induced abstinence: prevention of incubation of heroin seeking and no effect on incubation of methamphetamine seeking. The reasons for this double dissociation between the drug type and the alternative reward are unknown. At this time, we feel it is too early to speculate about potential reasons. We hope that future mechanistic studies, particularly on mechanisms of incubation of drug craving after social versus food choice–induced abstinence across drug classes, will identify factors contributing to this double dissociation.

Finally, the mechanistic studies demonstrate a critical role of PKCδ-expressing neurons and the PKCδ enzyme in CeL and neuronal activity in yet-to-be-identified cell type(s) in CeM in the protective effect of social choice–induced abstinence on incubation of methamphetamine seeking (Fig. 3). A question for future research is what afferent and efferent CeL projections contribute to this protective effect. Based on the correlational Fos data showing that the prevention of incubation of social choice–induced abstinence is associated with decreased activity of AIV neurons, we speculate that decreased activity of the AIV→CeL glutamatergic projection (Venniro et al., 2017a) to

TABLE 6
The impact of social reward on drug self-administration in different animal models of addiction

Addiction Model	Behavioral Results
Escalation model	In the study introducing the social self-administration and choice model (Venniro et al., 2018), the authors first used the established extended-access (6 h/day) escalation model of addiction (Ahmed and Koob, 1998) to determine whether methamphetamine (0.05, 0.1, 0.2, 0.4 mg/kg/infusion) or heroin (0.05, 0.1, 0.1 mg/kg/infusion) self-administration would be prevented by operant access to social interaction. The authors then devalued the social reward by either increasing the delay after social-lever press or by punishment of 50% of social-lever presses with foot shock of increasing intensity (0.0 to 0.5 mA). Social reward prevented methamphetamine and heroin self-administration independent of drug unit dose. Methamphetamine or heroin self-administration resumed only if there was a long delay before social reward or if social-lever presses were punished. Rats preferred social interaction over methamphetamine even after either 15 or 30 days of forced abstinence.
Three-criteria DSM-IV–based model	Subsequently, the authors performed a more stringent test of the effect of social reward using rats identified as addicted in the three-criteria DSM-IV–based model (Deroche-Gamonet et al., 2004). In this experiment, they trained rats for methamphetamine self-administration in 50 daily sessions that included three 40-min drug periods separated by two 15-min nondrug periods (during which we measured nonreinforced active lever presses). The authors then determined the rats' addiction score by measuring 1) total nonreinforced lever presses during two daily nondrug periods under the fixed-ratio reinforcement schedule, 2) number of drug rewards earned under a progressive-ratio reinforcement schedule, and 3) punishment responding. They classified rats as highly addicted, or High (≈19%); moderately addicted, or Medium (≈21%); and mildly addicted, or Low (≈60%). Finally, we trained some or all rats from each group (High, Medium, Low) for social self-administration (six sessions) and then determined drug versus social-reward preference in five discrete-choice sessions. The main finding was that the rats strongly preferred social interaction over methamphetamine and that this effect was independent of addiction-score group.
Intermittent-access drug self-administration model	Next, the authors determined whether rats with high addiction scores would be more vulnerable to reversal of their preference for social over drug reward. In this experiment, they trained rats to self-administer methamphetamine first using the escalation model (Ahmed and Koob, 1998) (9 days, 6 h/day) and then using the intermittent-access drug self-administration model (Zimmer et al., 2012). They determined the rats' addiction score, which included the number of drug rewards earned under the progressive-ratio reinforcement schedule and punishment responding. The authors classified rats as High (≈22%), Medium (≈30%), and Low (≈48%). They trained some rats from each group (High, Medium, Low) for social self-administration (four sessions) and ran discrete-choice sessions using delay or punishment of social reward. As in the previous experiments, the rats strongly preferred social interaction over methamphetamine, and this effect was independent of the addiction-score group. Additionally, high addiction scores did not predict lower social preference.

TABLE 7
Relapse after social choice–induced voluntary abstinence: summary of main findings

No.	Reference	General Training Procedures	Test	Major Findings
1	Venniro et al. (2018)	Strain and sex Sprague-Dawley males and females. Social self-administration training 60 s access to social partner (FR1) for six sessions (20 or 60 trials; 40 or 120 min). Drug training Methamphetamine or heroin (0.05–0.1 mg/kg/infusion) training (FR1) for 3–50 sessions (6 h, limited to 15 infusions per hour). Methamphetamine self-administration under extended access (6 h/day, 9 days, FR1) and intermittent schedules (9 days of 12 daily sessions of 5 min ON, 25 min OFF) of drug reinforcement. Food training Five pellets per delivery (FR1) for six sessions (6 h). Social choice–induced abstinence Choice between 60 s access to social partner or five food pellets and 0.1 mg/kg/infusion methamphetamine for 15 trials per session for 14 sessions. Forced abstinence Separate group of home-cage forced abstinence.	Relapse tests Test for methamphetamine seeking during 30-, 60-, or 90-min sessions on abstinence days 1, 15, 30, and 45 (n = 42 after three-criteria–based training; n = 27 after escalation and intermittent training; n = 10–12 males after choice-induced abstinence incubation; n = 6 males per group and n = 6 females per group for incubation after forced versus social choice–induced abstinence comparison; neurobiological assessments (immunohistochemistry: no test, n = 15 males; day 1, n = 16 males; day 15 forced and social abstinence, n = 7 males per group. RNAscope: no test, day 15 forced and social abstinence, n = 7 males per group).	Rats trained in established addiction models—escalation (both males and females), three-criteria DSM-IV–based, and intermittent access—voluntarily abstain when given mutually exclusive choices between methamphetamine or heroin versus social interaction. Social choice–induced abstinence prevents incubation of methamphetamine craving. This protective effect is associated with activation (assessed by the activity marker Fos) of inhibitory CeA PKCδ-expressing neurons and decreased neuronal activity in AIC.
2	Venniro et al. (2019)	Strain and sex Sprague-Dawley males and females. Social self-administration training 60 s access to social partner (FR1) for six sessions (20 or 60 trials; 40 or 120 min). Drug training 0.1 mg/kg/infusion heroin training (FR1) for 12 sessions (6 h, limited to 15 infusions per hour). Social choice–induced abstinence Choice between 60 s access to social partner or five food pellets and 0.1 mg/kg/infusion heroin for 15 trials per session for 10 sessions. Forced abstinence Separate group of home-cage forced abstinence.	Relapse tests Test for heroin seeking during 30-min session on abstinence day 1 or 15 (n = 16 males and females for forced abstinence; n = 18 males and females for voluntary). Test for heroin seeking during 30-min session on abstinence day 1 or 15 with semiautomatic and fully automatic procedure (n = 4 males and 3 females for semiautomatic; n = 7 males and 8 females for fully automatic).	Social choice–induced abstinence decreases incubation of heroin craving. There are no differences in social self-administration, social choice–induced abstinence, and incubation of craving in rats trained in the standard semiautomatic procedure versus the newer fully automatic procedure. No sex differences were observed in any behavioral measure.
3	Venniro et al. (2020b)	Strain and sex Sprague-Dawley males. Social self-administration training 60 s access to social partner (FR1) for six sessions (20 trials, 40 min). Drug training 0.1 mg/kg/infusion methamphetamine (FR1) for 12 sessions (6 h, limited to 15 infusions per hour). Social choice–induced abstinence Choice between 60 s access to social partner and 0.1 mg/kg/infusion methamphetamine for 15 trials per session for 10 sessions. Forced abstinence Separate group of home-cage forced abstinence for 14 days.	Relapse tests Test for methamphetamine seeking for 30 min (abstinence day 1) or 90 min (abstinence day 15). Effect of CeL PKCδ knockdown on methamphetamine incubation after social choice–induced abstinence (n = 11–12 per virus condition). Effect of CeL SOM knockdown on incubation of methamphetamine craving after forced abstinence (n = 13 per virus condition).	The protective effect of social reward on incubation of methamphetamine craving is mediated by activation of CeL PKCδ, leading to inhibition of CeM output neurons. The study introduces novel AAV shRNAs to selectively knockdown PKCδ or SOM in wild-type rodents.

AAV, adeno-associated virus; AIC, AI cortex; FR, fixed ratio; shRNA, short hairpin RNA.

drug-associated cues during the late abstinence relapse tests contributes to this protective effect.

IV. Conclusions and Clinical Implications

In this review, we describe different animal models that assess relapse to drug seeking after voluntary abstinence achieved either by introducing adverse consequences to the drug taking (punishment) or seeking (electric barrier) or by introducing operant nondrug rewards (palatable food or social interaction) in a mutually exclusive discrete-choice procedure. We also reviewed studies on behavioral and neuropharmacological mechanisms identified in studies using these voluntary abstinence relapse models. Below, we discuss similarities and differences in mechanisms of relapse after voluntary abstinence versus forced abstinence, similarities and differences in mechanisms of relapse after voluntary abstinence across drug classes, and the role of sex as a biological variable in relapse after voluntary abstinence, and we conclude by briefly discussing clinical implications.

A. Similarities and Differences between Forced and Voluntary Abstinence Relapse Models

A question that has guided recent studies has been whether the new voluntary abstinence relapse models would lead to the identification of brain mechanisms of relapse that are distinct from those identified from studies using the classic extinction-reinstatement and home-cage forced abstinence models. The limited literature to date indicates some similarities but also, importantly, some notable differences. Below, we discuss several examples. A methodological caveat in the analysis below is that some comparisons were made across studies in which investigators used different experimental procedures (e.g., different drug unit dose or alcohol concentrations, reinforcement schedules, session durations, and durations of self-administration training and abstinence), which can potentially impact behavior during the relapse tests beyond the impact of the abstinence-inducing manipulation.

1. Punishment and Electric Barrier–Induced Abstinence. In the case of drug priming, injections of both heroin and the anxiolytic drug lorazepam provoke relapse to remifentanil seeking after punishment. In contrast, heroin but not lorazepam priming reinstates remifentanil seeking after extinction (Panlilio et al., 2005). Future studies are needed to determine whether this antipunishment effect of benzodiazepines generalizes to other addictive drugs. However, the selective effect of lorazepam in the punishment-based relapse model extends previous studies on increased operant responding by anxiolytic drugs in the punishment component of the classic Geller-Seifter conflict model (Geller et al., 1962). A potential clinical implication of the lorazepam priming results is that drugs that induce relapse after punishment in the animal model (Panlilio et al., 2005) may provoke relapse in drug users who abstain due to adverse consequences of drug use.

In the case of context-induced reinstatement/relapse after extinction versus punishment, there is evidence for both similarities and differences. Inhibition of LH, vSub, NAc shell, and vSub-to-NAc shell projection decreases context-induced relapse to alcohol seeking after either extinction or punishment (Marchant et al., 2019).

In contrast, inhibition of BLA (but not CeA) increases context-induced relapse of cocaine seeking after punishment, whereas inhibition of either BLA or CeA activity decreases context-induced reinstatement after extinction (Pelloux et al., 2018b). The reasons for the dissociable roles of the amygdala subregions in context-induced relapse after extinction versus punishment are unknown. In both the extinction- and punishment-based ABA renewal models (Marchant et al., 2019; Bouton et al., 2020), drug seeking is induced by exposure to drug-associated contexts and tests occur under extinction conditions. However, an important difference is that extinction-induced abstinence occurs in the absence of the drug, whereas punishment-induced abstinence occurs in the presence of the drug (Marchant et al., 2019). Thus, the two abstinence-induced manipulations involve different forms of new learning: responding does not result in drug delivery versus responding results in drug delivery plus adverse consequences (Marchant et al., 2019). As speculated elsewhere, these and other differences in learning and psychologic processes involved in extinction versus punishment may recruit different brain mechanisms of renewal or context-induced reinstatement/relapse for both drug and nondrug rewards (Marchant et al., 2019; Bouton et al., 2020).

In the case of the electric barrier–induced abstinence model, much less is known about the brain areas controlling relapse in this model. Fredriksson et al. (2021) showed that inhibition of vSub decreases incubated oxycodone seeking after electric barrier–induced abstinence. This finding agrees with previous studies showing that inhibition of vSub decreases context-induced reinstatement of heroin seeking (Bossert and Stern, 2014) after extinction and context-induced relapse of alcohol seeking after punishment (Marchant et al., 2016). Thus, it appears that across drug classes and relapse models, vSub activity is critical for relapse/reinstatement independent of the method to achieve abstinence. However, the lack of effect of vSub inactivation on incubation of oxycodone seeking after forced abstinence suggests a more selective role of vSub in relapse after extinction-, punishment-, or

electric barrier–induced abstinence but not forced abstinence.

Finally, Fredriksson et al. (2020) recently showed that incubation of oxycodone seeking is potentiated after electric barrier–induced abstinence compared with home-cage forced abstinence. This finding suggests that the method used to achieve abstinence can also modulate the magnitude of incubation of drug craving (Fig. 3). The data discussed below further support this notion.

2. Food and Social-Choice–Induced Abstinence. Differences in the magnitude and expression of incubation of drug seeking have also been shown in studies using the food and social choice–induced abstinence models. Thus, incubation of heroin seeking is observed after forced abstinence or social choice–induced abstinence but not after food choice–induced abstinence (Venniro et al., 2017b, 2019). Additionally, incubation of methamphetamine seeking is observed after forced abstinence or food choice–induced voluntary abstinence but not after social choice–induced voluntary abstinence (Fig. 3) (Caprioli et al., 2015a; Venniro et al., 2018). As discussed above, dissociable central amygdala mechanisms contribute to the inhibitory effect of social choice on incubation of methamphetamine seeking (CeL PKCδ activity) and to the expression of incubation of drug seeking after home-cage forced abstinence (CeL SOM activity) (Venniro et al., 2020b).

Finally, in the case of similarities in mechanisms between voluntary and forced abstinence, a role of OFC in relapse to opioid seeking has been demonstrated after both forced (Fanous et al., 2012; Altshuler et al., 2021) and food choice–induced abstinence (Reiner et al., 2020).

3. Conclusions. Recent studies indicate some behavioral and neurobiological similarities in relapse after forced versus voluntary abstinence. More importantly, these studies also indicate that the methods used to achieve abstinence can lead to both quantitative (e.g., potentiation of incubation of opioid seeking after electric barrier–induced abstinence) and qualitative (e.g., emergence of incubation of methamphetamine seeking after forced and food choice–induced but not social choice–induced abstinence) differences in relapse-related behaviors. Additionally, the methods used to induce abstinence can lead to recruitment of different neuronal circuits that control inhibition or potentiation of relapse-related behavior (e.g., opposite role of BLA activity in context-induced relapse after extinction versus punishment).

B. Similarities and Differences of Drug Classes within Voluntary Abstinence Models

Studies using the reinstatement and incubation of drug craving after forced-abstinence models have shown both similarities and differences in brain mechanisms of drug seeking across drug classes (Badiani et al., 2011; Bossert et al., 2013). Evidence from recent studies suggests that this is also the case for voluntary abstinence relapse models. Thus, inhibition of vSub decreases both context-induced relapse to alcohol seeking after punishment and incubation of opioid seeking after electric barrier–induced abstinence (Marchant et al., 2016; Fredriksson et al., 2021). Additionally, inhibition of AI decreases relapse to both fentanyl and methamphetamine seeking after food choice–induced abstinence (Venniro et al., 2017a; Reiner et al., 2020). In contrast, inhibition of OFC decreases relapse to fentanyl but not methamphetamine seeking after food choice–induced abstinence (Venniro et al., 2017a; Reiner et al., 2020).

Several behavioral observations also suggest that different brain mechanisms control relapse after voluntary abstinence across drug classes. For example, food choice–induced abstinence prevents the emergence of incubation of heroin or fentanyl seeking but not methamphetamine seeking (Caprioli et al., 2015a; Venniro et al., 2017b; Reiner et al., 2020). Additionally, social choice–induced abstinence prevents the emergence of incubation of methamphetamine seeking and only modestly decreases incubation of heroin seeking (Venniro et al., 2018, 2019). Finally, heroin-trained male rats appear more vulnerable to cue-induced relapse after electric barrier–induced abstinence than cocaine-trained male rats (Peck et al., 2013). One reason for these differences is the different motivational effects of heroin- and cocaine-associated cues during both self-administration and electric barrier suppression. Specifically, we speculate that during the relapse test, the cocaine cues induce higher anxiogenic-like responses that summate with the anxiogenic-like responses induced by the electric barrier, resulting in inhibition of cocaine seeking in most rats. In this regard, elegant studies using the runway model (Ettenberg, 2009) showed that, like food, heroin-reinforced responding is manifested as "pure" approach behavior (decreased run time to the goal box over time). In contrast, like food paired with shock, cocaine-reinforced responding is manifested as approach-avoidance behavior (increased run time to the goal box over time). For additional discussion of these differences and their implications for behavioral and neurobiological mechanisms of cocaine and heroin self-administration and relapse, see Badiani et al. (2011).

In conclusion, results from several studies suggest both similarities and differences in brain mechanisms of relapse after voluntary abstinence across drug classes. However, the profound differences between heroin and methamphetamine in the behavioral effects of food and social choice–induced abstinence on incubation of drug seeking suggest that additional

differences in mechanisms of relapse after voluntary abstinence across drug classes are likely to be identified, as has been the case for relapse after forced abstinence or extinction (Badiani et al., 2011; Bossert et al., 2013).

C. Sex as a Biological Variable in Voluntary Abstinence Relapse Models

Over the last several years, we have included both male and female rats in recent studies using voluntary abstinence relapse models. Unexpectedly, under the experimental conditions described in Tables 4, 5, and 7, we found little evidence for sex differences in methamphetamine or opioid (oxycodone and fentanyl) self-administration, voluntary abstinence induced by electric barrier, food choice, or social choice and no evidence for sex differences in relapse/incubation of drug seeking across drug classes (Venniro et al., 2017b, 2019; Fredriksson et al., 2020; Reiner et al., 2020). This contrasts with results from previous studies with cocaine on sex differences and a role of ovarian hormones in drug-priming–induced reinstatement and incubation of drug craving after forced abstinence (Carroll et al., 2004; Kerstetter et al., 2008; Nicolas et al., 2019). The reasons for these differences are unknown but potential reasons might be the different procedures used to achieve abstinence or the different drugs used in the studies (cocaine versus opioids or methamphetamine). Additionally, it should be noted that the experimental conditions described in Tables 4, 5, and 7 were not optimal to determine sex differences in opioid or methamphetamine self-administration and choice because of the use of a single-unit dose of heroin or methamphetamine and choice procedures that biased choice toward the alternative reward and generated low variability within each sex and between sexes. However, these studies were both designed and powered to detect sex differences in incubation of drug craving or electric barrier suppression if such differences exist (Tables 4, 5, and 7).

Nevertheless, these studies provide a strong rationale for including females in relapse-related studies using both forced and voluntary abstinence–based models. This inclusion will allow to both increase the generality of the preclinical results to humans who use drugs and, equally important, to test medications and study neuropharmacological mechanisms in both males and females. In this regard, we recently reported that females are less sensitive than males to the antirelapse effects of potential medications in both the context-induced reinstatement model (Bossert et al., 2020) and the electric barrier–induced abstinence relapse model (Fredriksson et al., 2020).

In conclusion, despite evidence for lack of sex differences for opioid and psychostimulant seeking in the new voluntary abstinence models, we argue that it is critical to include females in such studies. The inclusion of both sexes will improve the generality of the behavioral findings, determine whether there are sex differences in the mechanisms underlying relapse in the new models, and lead to identification of sex-dependent effects of potential medications (Bossert et al., 2020; Fredriksson et al., 2020).

D. Clinical Implications

In the final section of the review, we discuss two potential clinical implications of the voluntary abstinence relapse models. The first implication is for medication development, and the second implication is for the understanding of behavioral and neuropharmacological mechanisms of human drug relapse.

1. Implications for Medication Development. Since the 1990s, numerous studies have used the extinction-reinstatement model to identify novel relapse-prevention medications (Shalev et al., 2002; Spencer and Kalivas, 2017; Reiner et al., 2019). From a translational perspective, the model has shown good postdictive validity with effective human medications (naltrexone, acamprosate, buprenorphine, methadone, or varenicline) decrease reinstatement of drug seeking in the rat model. However, with two exceptions (Kowalczyk et al., 2015; Sinha et al., 2020), the model has yet to show "true" predictive validity (medications identified in the model decrease drug relapse in humans) (Venniro et al., 2020a). Additionally, many "promising" medications identified in the reinstatement model did not decrease drug relapse in humans (Table 2 in Venniro et al. (2020a)).

The weak evidence for the prospective predictive validity of the reinstatement model and similar weak evidence from other animal models (Tables 1 and 3; Venniro et al. (2020a)) have led us and other investigators to develop alternative voluntary abstinence–-based relapse models that incorporate critical features of human addiction: abstinence induced by negative consequences of drug use or by the availability of competing alternative nondrug rewards (Marlatt, 2002; Epstein et al., 2006; Ahmed, 2010). A question for future research is whether the voluntary abstinence models will improve the predictive validity of rat relapse models. We hope that this is the case, but it is important to note that improved homology between the animal model and the human condition does not guarantee improved predictive validity (Sarter and Bruno, 2002; Epstein et al., 2006). Therefore, an important initial step in establishing the newer relapse models will be to demonstrate their postdictive validity with FDA-approved medications.

Additionally, although the voluntary abstinence models more closely mimic the human condition than extinction or home-cage abstinence models, they do not fully capture the complex nature of human abstinence. One important difference is that in the rat

models, the adverse consequences of drug taking and seeking, or the availability of nondrug rewards, occur in close temporal proximity to the drug-taking or drug-seeking behaviors. In contrast, in humans, both the adverse consequences of using drugs or the availability of nondrug reward when abstaining from drug use are often delayed (Cooper et al., 2007; de Wit et al., 2018).

The voluntary abstinence models were used to identify potential novel pharmacological targets in two studies. In the first study, using male rats, Caprioli et al. (2015a) reported that the mGluR2 (metabotropic glutamate receptor 2) positive allosteric modulator AZD8529 has no effect on nonincubated drug seeking on abstinence day 1 but decreases incubated methamphetamine seeking on abstinence day 21 after forced or food choice–induced voluntary abstinence. These results indicate that the effect of AZD8529 on incubated methamphetamine seeking is independent of the conditions used to achieve abstinence.

In the second study, using both sexes, Fredriksson et al. (2020) reported that in males, the dopamine stabilizer (−)-OSU6162 decreases incubated (but not nonincubated) oxycodone seeking after either electric barrier–induced or forced abstinence. In contrast, in females, (−)-OSU6162 modestly decreased incubated oxycodone seeking after electric barrier–induced abstinence but not forced abstinence. The apparent sex-specific effect of (−)-OSU6162 on incubated oxycodone seeking highlight the importance of testing medications in both males and females.

Together, these results indicate that AZD8529 and (−)-OSU6162 may serve as treatments to prevent relapse in males who use methamphetamine or opioids, respectively. We hope that our review will inspire investigators both to test approved FDA-medications and to identify novel pharmacological targets using the newer voluntary abstinence models. A question for future research is whether the results from the newer relapse models will better translate to humans.

2. Implications for Mechanisms of Human Drug Relapse. A question for future research is whether the use of the newer voluntary abstinence relapse models will result in new insights into behavioral and neuropharmacological mechanisms of drug relapse. Several recent results suggest that this is likely going to be the case. At the behavioral level, perhaps the most important general finding is that, compared with relapse after forced abstinence, relapse after voluntary abstinence induced by adverse consequences is potentiated (Fredriksson et al., 2020), whereas relapse after voluntary abstinence induced by providing alternative food or social reward is inhibited (Fig. 3). This pattern of results from the rat models supports the notion that relapse prevention is more likely to succeed by introducing alternative nondrug rewards than by punishment or incarceration (Hunt and Azrin, 1973; Higgins et al., 1991; Silverman et al., 2012).

At the neuropharmacological level, as discussed above, there is evidence for dissociable abstinence-dependent brain mechanisms of relapse induced by the same relapse-provoking stimulus. Perhaps the most striking example is that BLA activity plays opposite roles in context-induced reinstatement after extinction (inhibition) versus context-induced relapse after punishment (potentiation). These results demonstrate that the amygdala's role in relapse depends on the method used to achieve abstinence and highlights the importance of studying relapse under different experimental conditions that mimic abstinence conditions that occur in humans who use drugs.

E. Concluding Remarks

In closing, we hope that our review will inspire addiction researchers to incorporate animal models of relapse after voluntary abstinence into their studies. We also hope that more widespread use of these models will improve the predictive validity of the relapse models and our understanding of behavioral and neuropharmacological mechanisms of human drug relapse. However, we do not know whether the use of the newer models will improve the predictive validity of relapse models because, to date, there are no published studies showing postdictive validity of the newer models with FDA-approved medications. And until there is conclusive predictive validity–related evidence that some relapse models are superior to others, we recommend that investigators use a relapse model that best fits their research question.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Fredriksson, Venniro, Reiner, Chow, Bossert, Shaham.

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