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# Models for alcohol dependence: A clinical perspective

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**Alcohol use is the third leading modifiable cause of death in the United States. Alcoholism has many genetic, developmental and environmental sources of susceptibility, and recruits extensive neuroadaptation as it develops. Identifying new drugs for alcoholism will be facilitated by the recent emergence of clinically efficacious medications, the identification of neurobiological mechanisms of susceptibility and dependence and the development of animal paradigms which model many of the defining elements of alcoholism.**



## Introduction

Alcohol use is the third leading modifiable cause of death in the United States [1]. Alcohol dependence is a chronic relapsing disorder which shares many characteristics with other chronic relapsing medical conditions including responsiveness to pharmacological treatments. Because of the heterogeneous nature of alcohol dependence, many patients might not respond to the available pharmacological treatments. Producing a wider spectrum of clinically proven medications for alcoholism will improve this outlook. Preclinical tests will facilitate medication development by informing whether lengthy and costly tests in alcohol-dependent patients are warranted. This depends on paradigms which have the ability to identify agents that subsequently alleviate targeted features of alcoholism in a significant number of patients. Many laboratory paradigms model characteristics of alcoholism, and recent evidence is beginning to shed light on their ability to differentiate clinically effective from clinically ineffective medications for alcohol dependence.

## *In vitro* models: focus on neurobiological substrates of alcoholism

From a drug discovery perspective, *in vitro* models are desirable for their simplicity, speed and capacity to test small drug quantities. *In vitro* methods have been used abundantly in the study of acute and chronic alcohol action in the brain, but with rare exceptions, these preparations have not been systematically used to evaluate medications for alcohol dependence. Ethanol has diverse and complex neuropharmacological actions, yielding various potential treatment targets. Ethanol is a positive modulator of $GABA_A$ receptor function and inhibits glutamatergic function at higher doses. These and other neurochemical substrates [e.g. dopamine (DA) and 5-HT] and cellular mechanisms (e.g. CREB protein activation) mediate rewarding and addictive properties of ethanol. Therapeutic mechanisms of action for clinically effective alcoholism medications have been proposed, and even supported to a limited degree. This sets the stage for constructing arrays of predictive *in vitro* preparations. Two examples follow.

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*Dopaminergic modulation*

Acute alcohol administration stimulates dopamine ventral tegmental area (VTA) neurons [2,3]. Clinically, diminished DA neurotransmission is associated with genetic susceptibility to alcoholism, and is observed following a history of chronic alcohol exposure [4,5] implying alcohol might be consumed to alleviate hedonic deficiencies associated with diminished DA tone (i.e. 'relief-seeking'). Electrophysiological studies support these clinical observations; decreased spontaneous firing rates were observed in DA VTA neurons of alcohol-withdrawn animals which persisted after physical withdrawal signs typically subside [6]. That alcohol is consumed to compensate for diminished DA activity is, however, not consistent with the D2 receptor agonist bromocriptine performing no better than placebo on alcohol use measures in clinical studies [7]. Alternatively, chronic alcohol exposure increases sensitivity of DA VTA neurons to alcohol-induced excitation, suggesting heightened rewarding effects [8]. Regardless of the precise mechanism, inhibiting mesolimbic DA neurons through opioid (naltrexone [NTX]), GABA (topiramate, baclofen), 5-$HT_3$ (ondansetron) receptor action or through mechanisms which are not yet clear (acamprosate [ACM]) has been proposed as a significant pharmacotherapeutic action for many clinically effective alcoholism medications. If this action is indeed a significant constituent of alcoholism pharmacotherapy, it would be valuable to show that these drugs block alcohol-induced DA-neuronal activity in cell or slice cultures.

*Glutamatergic hyperexcitability*

Long-term alcohol exposure leads to upregulation of the excitatory glutamatergic system. When alcohol intake is stopped, the hyperglutamatergic state is expressed in the form of physical withdrawal symptoms. Postabstinence brain glutamate levels are higher after cycles of repeated alcohol intoxication and withdrawal, a condition which gives rise to elevated alcohol drinking in rats [9]. Elevated glutamate is also recruited as part of a conditioned anticipatory response to the onset of alcohol ingestion [10]. Thus, in abstinent alcohol-dependent individuals, glutamatergic hyperactivity, particularly in the central nucleus of the amygdala, might engender a relief-driven craving exacerbated even further when environments associated with alcohol intoxication encountered. ACM might diminish alcohol craving and relapse by reducing excessive glutamategic activity. ACM administration blocked elevations in glutamate microdialysate resulting from chronic alcohol intoxication, reduced elevated alcohol drinking and elevated extracellular NAcc glutamate in Per2 null mutant mice to wild-type levels, and diminished behaviors conditioned to alcohol-associated cues [11]. The ability to modulate excitatory amino acid neurotransmission might, therefore, be an important pharmacotherapeutic mechanism of action for treating alcohol dependence. ACM has neuroprotective effects which might be mediated through a glutamatergic mechanism similar to its anticraving effects. Hence, assessing drug effects on alcohol-induced neuroexcitablity and toxicity in brain slices might be a useful screening strategy. To that end, ACM effectively blocked glutamate neurotoxicity during alcohol withdrawal in fetal cortex cultures, and withdrawal-induced neurotoxicity in organotypic hippocampal cultures. ACM also reduced neuronal excitability in the hippocampus by reducing presynaptic glutamate release and shunting the postsynaptic membrane, yielding another potential screening assay [12].

## *In vivo* models: focus on phenotypes

Drinking alcohol in modest amounts is enjoyable for many people, particularly in social settings. Chronic drinking to extreme intoxicating levels induces adaptive responses in the brain leading to tolerance, inability to regulate intake once drinking is initiated, an intense desire to drink alcohol and the manifestation of physical withdrawal symptoms. Whether a person eventually becomes alcohol-dependent is significantly influenced by hereditary factors. Under certain conditions, laboratory animals will voluntarily drink alcohol solutions to pharmacologically relevant levels when water is freely available. As in human social drinking situations, alcohol has positive reinforcing effects under these conditions. Voluntary alcohol drinking and conditioning paradigms are useful tools for studying fundamental processes regulating moderate ethanol drinking and reward. They have limited value for drug discovery, however, unless they model the defining features of alcohol abuse and dependence [13].

In contrast to the exclusively reward-driven moderate drinking models, models of the alcohol dependence phenotype as delineated in Table 1 begin to capture relief-driven, excessive intake described euphemistically as the 'dark side of drug dependence' [14]. As such, these models are predicted to involve biological substrates relevant to therapeutic efficacy. Whether an alcoholism modeling approach promotes the identification of medications for alcohol dependence can be evaluated by studies of the opioid antagonist naltrexone (NTX; Revia®) and the taurine analog acamposate (ACM; Campral®). Meta-analyses of the clinical literature conclude that both effectively treat alcoholism, and both are approved as alcoholism treatments through their reduction of the desire to drink.

**Table 1. Essential characteristics of alcoholism [42]**

- Narrowing of the drinking repertoire
- Salience of drink seeking behavior
- Increased tolerance to alcohol
- Repeated withdrawal symptoms
- Relief or avoidance of withdrawal symptoms by further drinking
- Subjective awareness of compulsion to drink
- Reinstatement after abstinence

*Neuroadaptation models of transition to heavy drinking*

Making rats physically dependent on alcohol increases their alcohol drinking during the first 12 h following withdrawal. Repeated cycles of alcohol vapor exposure and withdrawal over several weeks markedly increases alcohol intake long after abstinence symptoms subside. The neuroadaptively driven transition to a persistent state of high alcohol drinking emulates the clinical indications of alcoholism in that patients are most vulnerable to relapse long after acute withdrawal. ACM administration prevented the transition to high alcohol drinking following intermittent alcohol vapor exposure, but did not reduce moderate drinking levels in rats having no history of alcohol dependence [15]. Selective reduction in dependence-driven heavy alcohol drinking might prove to be a positive signal for clinical efficacy.

*Genetic models of chronic drinking*

Selective breeding increases the frequency of alleles affecting alcohol preference and intake, and presumably engenders vulnerability in neural substrates as they interact with alcohol to create abuse potential. There are several selectively bred alcohol-preferring rat strains. To varying degrees, they exhibit behavioral and physiological characteristics observed in children of alcoholics, alcohol abusers and alcoholics. Most notably, they voluntarily drink alcohol in quantities sufficient to produce blood alcohol concentrations exceeding the legal limit of intoxication in the US. Suppression of alcohol drinking by the opiate antagonist naloxone appeared to be more persistent in alcohol-preferring rats than in outbred rats [16]. A modest ACM dose reduced alcohol intake by three alcohol-preferring rat strains [17], whereas ACM effects in outbred strains with limited alcohol experience are weak [18]. Ritanserin, an ineffective treatment for reducing craving, drinking and relapse in humans, reduced alcohol drinking by nonselected rats, yet failed to reduce alcohol drinking in two selectively bred alcohol-preferring strains [19,20]. These findings support comparing medication effects in animals predisposed toward heavy drinking with those in appropriate control animals.

*Craving and relapse models*

From a clinical perspective, reducing alcohol craving and preventing relapse to heavy drinking are highly desirable therapeutic outcomes. In the reinstatement paradigm, alcohol-seeking responses to stimuli resembling relapse-inducing triggers in humans are measured in trained rats under alcohol-free conditions. The reinstatement models distinguish craving induced by priming doses of alcohol, cues associated previously with alcohol, or negative affect and stress, one of the most common antecedent of relapse in humans [21]. Both NTX and ACM reduced alcohol-seeking precipitated by alcohol-associated cues [22–24].

In a related paradigm, alcohol, rather than a trigger, is presented after forced alcohol abstention producing a transient period of increased drinking relative to baseline. This alcohol deprivation effect (ADE) models aspects of binge drinking following relapse. NTX reduced the ADE more than it reduced baseline drinking [25,26]. ACM administration during alcohol-free periods prevented elevated alcohol drinking following reinstatement [18], and administration during the first 48 h of reinstatement reduced drinking [27]. Mechanisms underlying reinstatement of alcohol-seeking and relapse drinking and those regulating baseline drinking can be dissociated pharmacologically [28]. At present, there is no basis for concluding that medication effects on laboratory craving and relapse measures, but not on drinking itself, predict that craving and relapse will be selectively affected in alcohol-dependent patients.

*Further model validation*

Reasoning that a less severe indication should be more responsive to treatment than a more pronounced manifestation of the same indication implies that an effective medication should more effectively reduce moderate drinking than the heavier drinking associated with alcoholism models. Evidence reviewed above suggests the contrary; alcoholism models appear to have greater predictive validity with respect to medication efficacy. Hence, the ineffective medication ritanserin was only effective when administered to nonselected nondependent rats, whereas ACM has robust effects only in rats with a history of alcohol exposure or dependence. Early human efficacy data for ondansetron, baclofen and topiramate suggest that they could be effective treatments for some alcohol-dependent patients [29–31]. Unfortunately, with the exception of the $GABA_B$ agonist baclofen, they have received limited testing in animal models of alcoholism limiting our ability to confirm the predictive validity of these models as medication screens.

Drugs and targets awaiting clinical verification also demonstrate selective effects in animal models of alcoholism [32]. They include CB1 antagonists (selective breeding models, neuroadaptation models, cue-induced craving, ADE), mGluR5 antagonists (selective breeding models, cue-induced craving, ADE), CRF antagonists (selective breeding models, neuroadaptation models, stress-induced craving), NPY (selective breeding models, neuroadaptation models) and N/OFQ (selective breeding models, neuroadaptation models, cue-induced craving). The ability to assess whether these are efficacious targets in alcohol-dependent patients would further validate selective responses in alcoholism models as a predictor of clinical efficacy.

*Preliminary* in vivo *screens*

Typically, animal models for drug evaluation either function as simple screens which rapidly suggest whether a compound

merits further testing, or as more complex behavioral paradigms with face validity, bearing greater resemblance to the disease phenotype [33]. Fortunately, medication evaluations only require predictive models capable of distinguishing compounds probable to have clinical efficacy, but they need not resemble the etiology or symptoms of human alcoholism. This sole requirement expands the range of possible paradigms to be developed as rapid and inexpensive preliminary assessments while ensuring that a meaningful spectrum of clinically relevant phenotypic characteristics and neural substrates will be assessed.

Animal models are used to examine neuronal actions of alcohol as well as genetic and environmental influences on these actions including behavioral assays of alcohol intoxication, tolerance, sensitization and anxiolytic action [34]. Models of anxiety, epilepsy, depression and impulsivity also represent neurotransmitter systems such as GABA, glutamate, NE and 5-HT that are implicated in alcohol dependence. Medications for which there are human efficacy data must be examined in these paradigms to demonstrate whether positive results in these screens predict efficacy in the complex alcoholism models. Ultimately, model development will prove to be challenging. A definitive pattern of signals on a screening battery which predicts a clinical efficacy might never fully emerge. Rather, a conditionally interrelated profile of signals might emerge predicting clinical efficacy for specific clinical indications, in specific patient populations, through specific mechanisms of action.

## Model comparison

On average, only 5 of every 10,000 compounds investigated are tested in clinical trials. Of those five, only one is ever approved for patient use [35]. Testing compounds in disease models will improve these odds if the models predict clinical efficacy rapidly. Alcohol dependence is heterogeneous, and so is its response to pharmacological treatments. No single model will completely capture the heterogeneity of alcoholism; a range of models is needed. The first step is to validate on the basis of established benchmark compounds. There is some reluctance to view NTX and ACM as comparison standards because they do not cover the full range of potential therapeutic mechanisms, they have small effect sizes in clinical trials, and they are ineffective in some patients. We need only remind ourselves, however, that tricyclic antidepressants were once the 'gold standard' in the development of antidepressant medications [36]. In addition, identifying negative control compounds is not simple. Early failures to show clinical efficacy are unlikely to be followed by further clinical testing, therefore we might be faced with concluding that a medication is ineffective in the absence extensive testing. Nevertheless, SSRIs, bromocriptine and ritanserin have consistently failed to improve drinking outcome measures in alcohol-dependent patients. Despite this, model validation through systematic testing of benchmark compounds remains incomplete. Models must also predict clinical outcomes. Several drug classes yield promising effects in alcoholism models, yet they have not been tested in alcohol-dependent patients. Besides their need for further validation collectively, the models can be compared to one another. *In vivo* models are valuable in terms of their resemblance to behavioral characteristics of alcohol dependence, *in vitro* models in terms of measuring the correct mechanisms of action. A full comparison appears in Table 2.

## Translation to humans

Knowledge of alcoholism subtypes and their differential response to pharmacotherapy provides insights as to how responses in animal models might play out in the clinic. Early onset alcoholism is characterized by high heritability and drinking for the pleasurable actions of ethanol [37]. The 5-$HT_3$ antagonist ondansetron is effective in early- but not late-onset patients [30], and efficacy of NTX is related to a positive family history and possibly to a specific genetic variant of the opioid system [38]. Genetic selection models for high initial ethanol reward are likely to be useful in developing treatments for this patient category. By contrast, reward craving might play little role in the remaining patients. In these patients, successful treatment will rely on suppressing 'relief craving' [39]. Neuroadaptation models, and genetic models emulating these neurochemical changes, will be useful for

**Table 2. Comparison summary table**

| | *In vitro* models | *In vivo* models |
|---|---|---|
| **Pros** | • Emphasizes target mechanism<br>• Relatively rapid<br>• Small drug quantity required | • Models multiple diagnostic characteristics alcohol dependence<br>• Predictive validity partially established |
| **Cons** | • Predictive validity unknown<br>• Simple systems for complex disease | • Time and labor intensive<br>• Requires significant drug quantities |
| **Best use of model** | • Drug screening and discovery | • Secondary and confirmatory assessment. Discovery of new therapeutic targets |
| **How to get access to the model** | • Literature | • Literature, academic suppliers |
| **References** | [2,6,43,44] | [15,19,20,22-28,45] |

identifying such compounds. Finally, relapse-inducing triggers vary greatly within any given individual [40]. This offers the possibility of developing treatments tailored to individual needs. The reinstatement models assesses craving in response to controlled presentation of triggers such as stress, cues associated with alcohol intoxication and alcohol itself. Similar conditions can be arranged in human laboratory settings, enabling testing of medication effects on patients' craving which can be directly translated to findings in animal models and vice versa [41].

## Conclusion

The full utility of specific preclinical models to predict clinical efficacy in alcoholism remains to be proven. This is in large part because a broader range of treatments than NTX and ACM is needed to validate candidate models. However, presently available data allow some conclusions. First, drug effects to modulate spontaneous intake of alcohol in genetically nonselected laboratory rodents are not likely to be very helpful in identifying clinically useful treatments for alcohol dependence. Second, different models will be needed to identify treatments useful for diverse forms of alcohol dependence; some models weighted toward the early-onset, family history positive 'reward cravers' and other models reflective of long-term neuroadaptations resulting from prolonged alcohol use. Finally, reinstatement models will help profiling drug effects to indicate clinical utility in different relapse-prone situations. A systematic use of this range of models is probable to enrich pharmacological alcoholism treatment in the near future, and meet the extensive unmet medical needs.

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