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# Nematodes feel a craving - Using *Caenorhabditis elegans* as a model to study alcohol addiction

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Alcohol is the most frequently-used addictive drug. However, the mechanism by which its consumption leads to addiction remains largely elusive. Given the conservation of behavioral reactions to alcohol, *Caenorhabitis elegans* (*C. elegans*) has been effectively used as a model system to investigate the relevant molecular targets and pathways mediating these responses. In this article, we review the roles of BK channels (also called SLO-1), the lipid microenvironment, receptors, the synaptic machinery, and neurotransmitters in both the acute and chronic effects of alcohol. We provide an overview of the genes and mechanisms involved in alcoholism-related behaviors in *C. elegans*.

Keywords: C. elegans; substance abuse; ethanol; BK channel

# Introduction

Substance abuse refers to the harmful or hazardous use of psychoactive substances including alcohol and illicit drugs. Due to its sophisticated genetics, *Caenorhabditis elegans* (*C. elegans*) has provided novel insights into the mechanisms of substance abuse. Unbiased forward genetic screening for drug-resistant or hypersensitive mutants permits the identification of new addiction-related molecules, and RNA interference (RNAi)<sup>[1, 2]</sup> allows the targeted inactivation of any gene. More importantly, the *C. elegans* nervous system is similar to that in mammals, including ion channels, signal pathways, synaptic machinery, and neurotransmitters, so using this model may lead to a better understanding of the molecular and neurobiological factors that underlie substance abuse.

Alcohol consumption is common in many societies. It is associated with an increased risk of acute and chronic health conditions related to its intoxicating, toxic and dependence-inducing properties (World Health Organization). Despite the prevalence of alcohol consumption and its propensity for abuse, the molecular targets and physiological mechanisms underlying intoxication and abuse remain elusive. In humans, acute exposure to alcohol causes hyperactivity at low doses and physical impairment of coordination and balance, sedation, and even death at high doses. Interestingly, the same internal concentrations of alcohol cause incoordination and sedation in *C. elegans*. Moreover, both the metabolic enzymes for alcohol degradation and the molecular/physiological pathways mediating the actions of alcohol are similar in *C. elegans* and mammals, which suggest conserved function of alcohol targets in the nervous system. Thus, *C. elegans* has been widely used as a genetic model organism to identify the effectors of intoxication. Genetic studies in worms have provided a more complete understanding of alcohol preference, tolerance, and withdrawal.

*C. elegans* exhibits acute behavioral responses to ethanol comparable to those in higher species<sup>[3]</sup>. Acute exposure causes a dose-dependent depression in locomotion and egg-laying behaviors of *C. elegans* at the same internal concentration of ethanol (20 to 30 mmol/L) that induces intoxication in humans and other mammals, while acute tolerance is induced by continuous exposure<sup>[4]</sup>. Here, we summarize recent insights into the behavioral actions of ethanol in *C. elegans* and the diverse molecular effectors involved.

# Ion Channels

BK (or SLO-1) channels play an important role in acute behavioral responses to ethanol. These channels, which are activated by both intracellular Ca<sup>2+</sup> and membrane depolarization, play a prominent role in coupling intracellular Ca<sup>2+</sup> with cellular excitability<sup>[5]</sup>. In the nervous system, BK channels function as an important regulator of neural transmission and network activity<sup>[6-8]</sup>. Ethanol reduces neuronal excitability by activating BK channels. This altered BK channel activation has the potential to limit neuropeptide and growth hormone release, nociception, and cerebrovascular tone.

A role for BK channels in the regulation of ethanol actions was first identified in a screen for ethanol-resistant C. elegans mutants by Davies et al.<sup>[3]</sup>. slo-1 loss-of-function mutants are strongly resistant to the sedative effects of the drug, while slo-1 gain-of-function mutants display depression of locomotion and egg-laying behaviors to a degree similar to ethanol-treated wild-type animals<sup>[3]</sup>. The absence of BK channel activity in *slo-1* mutants provides a mechanism for resistance to the behavioral effects of ethanol. Furthermore, in vivo electrophysiological recording from dopaminergic CEP (cephalic) neurons in C. elegans shows that ethanol activation of SLO-1 does not require cytosolic factors and is not due to increased internal Ca2+ levels<sup>[3]</sup>. Ethanol activates BK channels, hyperpolarizing the neurons and inhibiting neuronal excitability, which is a major cause of the acute responses in worms.

#### Lipid Microenvironment

C-terminal-binding protein 2 (CTBP2), the mammalian homolog of CTBP-1, acts as a transcriptional repressor and shows a significant association with alcoholdependence in a genome-wide association study of an Australian population<sup>[9]</sup>. In *C. elegans*, two transcriptional co-repressors (*ctbp-1* and *pag-3*) have been identified by a genetic screen for mutations that result in the defective development of acute functional tolerance<sup>[10]</sup>. Bettinger *et al.* found that transcriptional repression of the levels of the triacylglycerol lipase LIPS-7 modifies the phenotype of gain-of-function mutations in the BK channel<sup>[10]</sup>. This study suggests that the lipid microenvironment tunes the neuronal effects of ethanol, including the initial sensitivity as well as the development of acute tolerance. Taken together, these findings suggest that the mechanisms of ethanol action on BK channels may be conserved between *C. elegans* and higher organisms. Overall, a variety of factors can fine-tune the action of ethanol on BK channels and result in changes in channel activity<sup>[11]</sup>.

# **Presynaptic Proteins**

It is believed that the synapse is the most ethanol-sensitive element in the central nervous system<sup>[12]</sup>. At the level of the synapse, ethanol indirectly inhibits neurotransmitter release through modulation of neuronal activity in *C. elegans* and vertebrates<sup>[3, 13, 14]</sup>.

# RAB-3/RAB3A

The *rab3* gene encodes a small G-protein that interacts directly with synaptic vesicles to regulate their release<sup>[15-17]</sup>. Genetic disruption of the function of presynaptic RAB-3/A protein alters ethanol-related behaviors. Loss-of-function mutations in RAB-3 and the RAB-3 exchange factor AEX-3 confer resistance to the locomotor effects of ethanol in *C. elegans*<sup>[18]</sup>. Similarly, mice lacking one or both copies of *Rab3A* are resistant to the ataxic and sedative effects of ethanol, whereas *Rab3A* haploinsufficiency increases voluntary ethanol consumption<sup>[18]</sup>. These data suggest a conserved role of RAB3/A-dependent neurotransmitter release in behavioral responses to ethanol. However, it remains unclear whether the resistance to ethanol in both species is the result of altered acute sensitivity or the abnormal development of tolerance.

# Munc18-1

The synaptic protein Munc18-1 interacts with the SNARE protein syntaxin-1 and functions in exocytosis. A genetic study of two mouse strains with different ethanol preference indicated a correlation with a polymorphism (D216N) in Munc18-1<sup>[19]</sup>. Interestingly, *munc18-1* transgenic mutant worms (D214N) are strongly resistant to both the stimulatory and sedative effects of acute ethanol. Analysis of an alternative Munc18-1 mutation (I133V) supports the link between reduced SNARE complex binding and ethanol resistance<sup>[20]</sup>. The interaction between the SNARE complex and Munc18-1 may also be a target for the transduction of effects. This study pinpoints a role of ethanol at the level of vesicle fusion, whereby its acute effects are ameliorated by point mutations in UNC-18. Hence, vesicle recruitment and docking might be potential sites for the neuronal action of ethanol.

# **Neurotransmitters**

Lee *et al.* created an ethanol-preference assay and found that *C. elegans* develops a preference for or attraction to ethanol as a result of prolonged pre-exposure to the drug<sup>[24]</sup>. *cat (catalase)-2* mutant worms (deficient in the synthetic enzyme for dopamine) fail to develop a preference for ethanol, suggesting that dopamine is required. *tph (tryptophan hydroxylase)-1* mutant worms that have defects in the synthetic enzyme for serotonin are also defective in ethanol preference, indicating that serotonin also plays a role. These results suggest that dopamine and serotonin are required for this form of behavioral plasticity.

#### Receptors

#### NPR-1

In vertebrates, neuropeptide Y (NPY) receptor genes play a role in alcohol addiction<sup>[21, 22]</sup>. The *npr-1* gene encodes an NPY-like receptor protein in *C. elegans*<sup>[23]</sup>. Acute tolerance develops more rapidly in *npr-1* loss-of-function mutants, and the mutant *npr-1* negatively regulates acute ethanol tolerance<sup>[4]</sup>, which indicates that NPR-1 is implicated in the development of acute tolerance.

# SEB-3

Jee *et al.* found that *seb-3* in *C. elegans*, which encodes a CRF (corticotropin-releasing factor) receptor-like G-protein-coupled receptor, contributes to acute tolerance to ethanol and to the development of tremor during ethanol withdrawal. Similarly, a specific CRF receptor antagonist reduces acute functional tolerance to ethanol in mice<sup>[25]</sup>.

#### **Conclusion and Perspectives**

*C. elegans* has been widely used as a model system to study the mechanisms of addiction-related behaviors, such as acute responses, tolerance, sensitization, withdrawal, and dependence. Current studies in *C. elegans* indicate that the behavioral responses induced by ethanol are regulated by various factors and take place at presynaptic as well as postsynaptic sites. Ethanol activates presynaptic BK channels in *C. elegans*, causing a large efflux of K<sup>+</sup>, hyperpolarizing the neuron, depressing neuronal excitability, and inhibiting neurotransmitter release (Fig. 1). Additional factors, including posttranslational modification

and alternative splicing of SLO subunits<sup>[26]</sup>, BK channel assembly with accessory subunits<sup>[27, 28]</sup>, and the lipid milieu, may modulate BK channel activity<sup>[10]</sup>. Downstream of BK and Ca<sup>2+</sup> channel function, ethanol may act on additional presynaptic effectors, such as Rab3 and Munc18, which function in vesicle fusion and recruitment/docking respectively, to modulate neurotransmitter release <sup>[18, 20, 29, 30]</sup> (Fig. 1).

The fact that the ethanol responses of genes and the mechanisms involved in *C. elegans* contribute to a large extent to our molecular understanding of ethanolinduced behavior in mammals underlines the existence of conserved targets and pathways in vertebrates and invertebrates. It is worth noting that the direct and indirect interaction of ethanol with these targets may result in effects through subsequent gene expression and synaptic activity. Moreover, chronic ethanol treatment may induce long-lasting alterations in neuronal networks and behavioral plasticity that could underlie compulsive alcohol consumption and drug-seeking behavior.

#### **Recent Technological Advances**

The community of investigators using C. elegans possesses a wealth of tools to investigate the neuronal and molecular mechanisms underlying the effects of various drugs. Combinations of forward and reverse genetic approaches<sup>[42]</sup> provide a means of understanding the correlation of genes and drug-induced behavior. The existence of numerous well-defined promoters can be used to regulate gene expression spatially and temporally. The role and dissection of neuronal circuits in drug-related behaviors can be achieved through laser ablation of specific sensory neurons and interneurons. Patch-clamp recording from neurons and neuromuscular junctions *in vivo*<sup>[43]</sup> is a powerful tool for exploring neuronal activity. The ability to perform Ca<sup>2+</sup> imaging and optogenetic manipulations on free-moving worms<sup>[44, 45]</sup> permits the monitoring of neuronal activity in response to acute or chronic drug exposure. Strikingly, whole-brain imaging in *C. elegans*<sup>[46]</sup> is becoming feasible and will hopefully emerge for future studies of drug abuse.

# **Other Addictive Drugs**

In addiction to cocaine and amphetamine, the dopamine



Fig. 1. Ethanol action on BK channels and the modulation of exocytosis in *C. elegans*. Ethanol activates presynaptic BK channels in *C. elegans*, causing a large efflux of K<sup>+</sup>, hyperpolarizing the neuron, depressing neuronal excitability and inhibiting neurotransmitter release. Ethanol may act on additional presynaptic effectors (such as Rab3 and Munc18 that function in vesicle fusion and vesicle recruitment/docking respectively) to modulate neurotransmitter release.

reuptake transporter is believed to be a critical molecular target. Behavioral assays have been developed to assess responses and/or adaptation to cocaine and amphetamine in *C. elegans*<sup>[31, 32]</sup>. Acute cocaine treatment changes locomotor activity, and the neurotransmitter serotonin is required for the cocaine response in *C. elegans*<sup>[31]</sup>.

Nicotine, the primary addictive substance in tobacco, acts on the brain through neuronal nicotinic acetylcholine receptors (nAChRs). There are 42 different predicted nAChR subunits in *C. elegans*<sup>[33]</sup>. Worms exhibit behavioral responses to nicotine including an acute response<sup>[34, 35]</sup>, tolerance<sup>[36]</sup>, withdrawal, and sensitization<sup>[37]</sup>. These nicotine responses require nAChRs, suggesting that they are functionally conserved. Importantly, mutant worms lacking

TRPC (transient receptor potential, canonical) channels are defective in their response to nicotine, which can be rescued by a human TRPC channel. These results have uncovered a novel role for TRPC channels in regulating nicotine-dependent behavior<sup>[37]</sup>.

Although no opioid and cannabinoid receptors have been identified so far<sup>[38-41]</sup>, *C. elegans* serves as a useful tool to characterize the functions of known genes as well as to identify new genes that are involved in the regulation of addiction to other drugs. The fact that some substances are often used together makes studies of addiction more challenging. For instance, dependence on alcohol is correlated with dependence on tobacco. Revealing the underlying mechanism of the interactions between these substances and their addictions will lead to a better understanding of co-occurring addiction and its treatment. *C. elegans* also holds the potential for further use in the screening of therapeutic targets and compounds for treating alcoholism.

The continued use of *C. elegans*, a simple, yet powerful *in vivo* model system, may help us to uncover the mysterious mechanisms by which drug exposure induces changes in synaptic and behavioral plasticity.

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