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Caenorhabditis elegans as a Model to Study the Molecular and Genetic Mechanisms of Drug Addiction

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Abstract

Drug addiction takes a massive toll on society. Novel animal models are needed to test new treatments and understand the basic mechanisms underlying addiction. Rodent models have identified the neurocircuitry involved in addictive behavior and indicate that rodents possess some of the same neurobiologic mechanisms that mediate addiction in humans. Recent studies indicate that addiction is mechanistically and phylogenetically ancient and many mechanisms that underlie human addiction are also present in invertebrates. The nematode *Caenorhabditis elegans* has conserved neurobiologic systems with powerful molecular and genetic tools and a rapid rate of development that enables cost-effective translational discovery. Emerging evidence suggests that *C. elegans* is an excellent model to identify molecular mechanisms that mediate drug-induced behavior and potential targets for medications development for various addictive compounds. *C. elegans* emit many behaviors that can be easily quantitated including some that involve interactions with the environment. Ethanol (EtOH) is the best-studied drug-of-abuse in *C. elegans* and at least 50 different genes/targets have been identified as mediating EtOH's effects and polymorphisms in some orthologs in humans are associated with alcohol use disorders. *C. elegans* has also been shown to display dopamine and cholinergic system–dependent attraction to nicotine and demonstrate preference for cues previously associated with nicotine. Cocaine and methamphetamine have been found to produce dopamine-dependent reward-like behaviors in *C. elegans*. These behavioral tests in combination with genetic/molecular manipulations have led to the identification of dozens of target genes/systems in *C. elegans* that mediate drug effects. The one target/gene identified as essential for drug-induced behavioral responses across all drugs of abuse was the cat-2 gene coding for tyrosine hydroxylase, which is consistent with the role of dopamine neurotransmission in human addiction. Overall, *C. elegans* can be used to model aspects of drug addiction and identify systems and molecular mechanisms that mediate drug effects. The findings are surprisingly consistent with analogous findings in higher-level organisms. Further, model refinement is warranted to improve model validity and increase utility for medications development.

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1. INTRODUCTION

Addictions represent a major and growing challenge in our society. Drug addiction takes a massive toll on both direct and indirect human and financial costs, including hundreds of thousands of deaths annually from alcohol and drug-related auto accidents, cancer, and other conditions resulting from alcohol and nicotine addiction, drug-associated homicides, and overdoses. The resulting costs to society are estimated to be hundreds of billions of dollars annually worldwide in lost productivity, drug enforcement, hospitalizations, and treatments for healthcare resulting directly or indirectly, from drug use.[1] Clearly there is an urgent need for effective treatments and prevention strategies that are developed from an understanding of the basic mechanisms that underlie addictive behavior. Much of what we know about the neurobiology of addictions has been either discovered or enhanced through the use of animal models.[2] This includes the discovery and characterization of some of the basic reward circuitry and the development of behavioral measures to model and study human addiction in animals.[3]

Here we discuss a relatively new model system to study addiction based on behavioral measures in a decidedly ancient and simple animal *Caenorhabditis elegans*. The review will briefly introduce animal modeling of addiction in vertebrates and invertebrates to provide some context for the following discussion of *C. elegans* models of addiction. Discussion then turns to specific drugs of abuse including alcohol, nicotine, cocaine, and methamphetamine, their effects in *C. elegans*, and some of the biologic systems and molecular targets identified with these models. Some ideas are presented indicating how *C. elegans* models may be developed and refined in the future to enhance model validity, increase utility for medications development, and improve model value for translational applications.

2. MODELS OF ADDICTION

Through the years, model systems, using primates and other mammals closely associated with humans, have provided essential information—particularly associated with the behavioral effects of drugs.[4,5] However, a review of the literature on animal models of addiction shows that rodents (mainly rats and mice) are a very popular species for addictions research.[2,6] This is due, in large part, to the fact that rodents share basic neurobiologic systems, both structurally and functionally, with humans. In this way, they have enabled the identification of circuitry underlying addictive behavior. In addition, these animals self-administer drugs of abuse including ethanol (EtOH),[6] stimulants including cocaine, nicotine, amphetamine, metham-phetamine,[7,8] opiates such as heroin and morphine,[9] and many other drugs including caffeine and THC.[10,11] Self-administration provides validity to these models, indicating that such models recapitulate some aspects of drug-taking behavior.[12] Similarly, rodent models of addiction also show evidence of construct validity, in which some of the mechanisms and neurobiology that appear to mediate addictive behavior in humans also are present, and function through analogous systems in mammalian models.[12] Finally, rodent models also demonstrate predictive validity, indicating that drugs/treatments that reduce addictive behavior in humans, also show some efficacy in rodents.[13] Overall, these models have, and continue to provide essential information about the neurobiology of

addiction and have been instrumental in the development of the few available pharmacologic treatments.[14] However, relatively little is understood about the molecular foundations of addiction, and animal models that can quickly, efficiently, and systematically examine the underlying mechanisms of addictions have yet to be developed.

3. INVERTEBRATE MODELS

Recent work shows that addiction is a phylogenetically ancient process and indicates that many mechanisms that underlie addictions are present in invertebrates. Elegant behavioral models of addiction such as conditioned place preference (CPP), and other tools historically used to study aspects of addictive behavior in rodent models,[15] have demonstrated that crayfish show drug reward, seeking, and withdrawal to cocaine, amphetamines, and opiates.[16] Similarly, EtOH self-administration and conditioning paradigms have demonstrated that *Drosophila melanogaster* show preference responses to cues that had been previously paired with EtOH.[17] Although some might find it surprising that such a simple animal can be used to model complex behaviors, behavioral models using invertebrates have played a central role in the discovery of the molecular mechanisms that underlie learning and memory.[18] The nematode *C. elegans* is an excellent model organism with conserved neurobiologic systems that is used to model various disease states.[19] It provides the researcher with numerous molecular and genetic tools, including a tractable and fully sequenced genome, the availability of thousands of mutants, and the ability to manipulate genes and their expression through transgenic approaches and RNAi techniques. In addition, a relatively short life cycle and a 3-day generation time from egg to adult can lead to a dramatic increase in the pace of discovery at a fraction of the cost of using higher level organisms. However, to date, there are few established *C. elegans* behavioral models of addiction. We have discovered that, like mammals and other invertebrates, *C. elegans* also develops a conditioned preference for cues after previous parings with methamphetamine or cocaine that is dependent on dopamine neurotransmission.[20] Moreover, with drug pre-exposure they demonstrate sensitization, cross-sensitization, tolerance, and cross-tolerance, all of which are hallmarks of addiction in humans. Validated *C. elegans* behavioral models of addiction designed to enable fast and accurate generation of data would provide the field with valuable and powerful tools to study the molecular mechanisms that underlie addiction, and open new avenues to identify new targets for medications development.

4. *C. ELEGANS* AS A MODEL SYSTEM TO STUDY ADDICTION

C. elegans is an obvious choice as a model system, as the first animal to have its genome completely sequenced,[21] and with approximately 19,000 genes, more is known about the genetics and molecular make-up of *C. elegans* than any other ambulatory organism.[22] *C. elegans* has been used to model many types of disorders in humans,[19] including neurologic and psychiatric disorders ranging from Parkinson's disease to Autism.[23–25] Although rodents have been used to model addiction for many decades,[26–29] the use of *C. elegans* as a model system to study addiction is a relatively recent development.[30–33] Clearly, using *C. elegans* as a model for psychiatric disorders (such as addiction) has some limitations including a lack of some neurotransmitter systems such as norepinephrine. Also, till date, a limited number of valid behavioral models have been developed and characterized to study

the reinforcing properties of drugs. However, accumulating evidence indicates that *C. elegans* is an excellent model to identify molecular mechanisms that mediate drug effects and potential targets for medications development for various addictive compounds. *C. elegans* emit many behaviors that can be easily quantitated such as egg laying and defecation, as well as a host of movement and postural measures, including speed of locomotion and counting the number of body bends per unit time.[34] Other measures document how *C. elegans* interacts with its environment, including chemotaxic behavior,[35] as well as associative and nonassociative learning.[36] Many of these behaviors have been studied for decades and the neurobiologic systems and circuits that mediate them are well described, making *C. elegans* an excellent candidate model system to study the effects of drugs on behavior.

5. DRUGS OF ABUSE

5.1 Ethanol

Investigators have discovered that *C. elegans* can be used to study the effects of EtOH.[37,38] Several studies have established that *C. elegans* display concentration-dependent depression of a variety of behaviors, including locomotion, body bend amplitude, and egg laying, after exposure to EtOH.[38] Importantly, the depressant effects on the locomotor activity of *C. elegans* occur when the internal tissue concentration of EtOH reaches levels that correspond to intoxicating blood alcohol levels in humans.[39] To date, at least 50 genes have been identified that influence EtOH-associated behaviors in *C. elegans*, and several orthologs of these genes have been implicated in alcohol use disorders in humans.[40] As with vertebrates, dopamine systems appear to play a role in EtOH-induced behavioral effects in *C. elegans*. EtOH induces state-dependent learning in *C. elegans* that is absent in animals with functional mutations in the vesicular monoamine transporter (cat-1) or tyrosine hydroxylase (cat-2). In certain experimental paradigms, *C. elegans* also show an EtOH preference response in choice tests that appears to be mediated through the dopamine and serotonin systems.[41] Some of these early observations led to the development of a simple behavioral model to study the effects of EtOH in *C. elegans* and leverage the fully tractable molecular genetics available to researchers using this species. Investigators use some of these behavioral paradigms to identify mutations in individual genes that affect behavioral responses to EtOH in *C. elegans*. Once identified, conserved homologs of these genes may be examined for effects on alcohol-related behaviors in other animal models, and/or polymorphisms of such genes may be assessed in humans for possible roles in alcohol use disorders.

After isolating mutants that showed resistance to the behavioral effects of EtOH, Davies *et al.*[42] found that mutations in the gene slo-1, a highly conserved gene which codes for a BK potassium channel that is homologous to one found in humans, produced resistance to the locomotor effects of EtOH.[33,43] These effects of EtOH were found to be mediated through a direct action at the channel to increase current and to be selective for EtOH.[38] The BK potassium channel appears to subserve behavioral responses across multiple species including humans.[32,33,42] Additional work has identified a specific residue (T381I) on the channel that confers dramatic and selective resistance to the behavioral effects of EtOH.[32]

Thus, the BK potassium channel is a verified mediator of the effects of EtOH across phyla and may serve as a target for the identification and development of new treatments for alcohol use disorders.

An essential characteristic of EtOH effects in animal models is the development of tolerance, which is an adaptation that occurs when the same concentration of EtOH produces a reduced behavioral response after chronic or repeated exposure.[33,44] After continued exposure to EtOH, acute functional tolerance becomes apparent in the Bristol (N2) wild-type *C. elegans* strain. However, such tolerance occurs much more rapidly in the Hawaiian CB4856 wild-type strain.[45] This effect was found to be mediated by a variation in the npr-1 gene. This gene codes for a neuropeptide Y (NPY) receptor homolog in *C. elegans* that was previously shown to underlie differences in social behavior and responses to food.[46] Moreover, NPY is known to regulate EtOH and food intake in vertebrate models.[47,48] Since tolerance is a key feature in the progression to alcohol dependence in humans,[49] NPY appears to be an excellent molecular target for treatment development[50,51] and may be aided through the study of the npr-1 gene in *C. elegans*. More recently, EtOH-induced muscle hypercontraction (EHC) was found to be dependent on cholinergic signaling as the effect was significantly reduced in cholinergic signaling mutants (cha-1 and unc-17) and with exposure of the nonselective nicotinic cholinergic receptor antagonist mecamylamine.[52] Tolerance to EHC was evident in wild-type worms, but was absent in a Na^+/K^+ ATPase mutant eat-6(eg200). Interestingly, cholinergic functioning is also affected by long-chain polyunsaturated fatty acids and mutants deficient in this type of fatty acids show deficits in both the initial sensitivity to EtOH and in the development of acute functional tolerance.[53] These data implicate cholinergic systems, fatty acid metabolism, and Na^+/K^+ ATPase function in the acute activation and/or tolerance effects of EtOH in *C. elegans*, and similar systems may mediate EtOH effects in vertebrates through orthologous mechanisms.

Recent efforts in many research domains have focused on epigenetics. It has become increasingly clear that epigenetic factors such as histone modification play important roles in various aspects of addiction.[54,55] A recent study employing EtOH response behaviors in *C. elegans* demonstrated that genes coding for components of the conserved switching defective/sucrose nonfermenting (SWI/SNF) chromatin-remodeling system are required for the development of acute functional alcohol tolerance in *C. elegans* and/or affect the initial sensitivity to EtOH.[56] This study identified 12 different genes within this system that are involved in mediating these effects in worms. Moreover, allelic variations in SWI/SNF genes (especially in bromodomain containing 7 (BRD7)—homolog to swsn-9 in *C. elegans*) were associated with a diagnosis of alcohol dependence in a human genome-wide association study. Although it is likely that this study only begins to explore epigenetic mechanisms mediating the behavioral effects of EtOH, it does demonstrate the utility of using *C. elegans* models to identify possible epigenetic factors and to identify genes/proteins that may serve as future targets for medications development.

5.2 Nicotine

C. elegans are thought to express at least 27 different nicotinic acetylcholine receptor (nAChR) subunits,[57,58] and thus a rich cholinergic pharmacology. As in higher-level

organisms, acetylcholine in *C. elegans* is critical for many essential behaviors involving muscle contraction, including movement, feeding, and egg laying and several nAChR genes have been identified.[57] Nicotine application induces muscular hypercontraction and egg laying. Continuous exposure to nicotine affects control of egg laying[59,60] which is dependent on the UNC-29 gene.[59] Such exposure also results in tolerance, and nicotine-adapted animals display uncoordinated locomotor activity when removed from nicotine[61] and tolerance is thought to be protein kinase C (PKC) dependent.[59] Further work has demonstrated that nicotine-dependent behavior in *C. elegans* is controlled by transient receptor potential (TRP) proteins TRP-1 and TRP-2.[62] A TRP channel (TRPA1) works to regulate the aversive responses to nicotine and has been identified as a potential target for nicotine pharmacotherapy development in humans.[63] Overall, these studies demonstrate that nicotinic systems subserve many analogous functions in *C. elegans* and nicotine exposure produces behavioral effects that are also consistent with nicotine effects in humans. Furthermore, some specific mediators of nicotine responses appear to be highly conserved in *C. elegans*.

C. elegans have also been shown to display a concentration, time, and age-dependent attraction to nicotine that is reduced by exposure to the nonselective nicotinic receptor antagonists mecamylamine or varenicline.[31] Worms with mutations in genes coding for the dop-1 or dop-2 dopamine receptors, or the acr-5 or the acr-15 nicotinic receptor subunit genes, also showed reduced approach to nicotine. The approach deficit in the acr-15 mutant could be rescued by selective re-expression in neurons but not muscle. *C. elegans* also show “reward-like” cue-conditioned preference for cues previously associated with nicotine which is absent in the acr-5 mutant.[31] Together, these findings provide additional evidence that *C. elegans* may model not only the basic physiologic effects of nicotine, but also the motivational and rewarding properties of nicotine. Use of such models may help to identify the molecular underpinnings of nicotine dependence and identify new targets for the development of new smoking cessation pharmacotherapies.

5.3 Cocaine

C. elegans have conserved monoamine systems and a dopamine system that functions with remarkable similarity to vertebrates, including humans, in terms of signaling.[64] Dopamine is involved in a wide variety of behaviors in the worm, including movement, egg laying, defecation, habituation to touch, as well as sensing and responding to food sources, and copulation behavior in males.[64,65] The dopamine transporter (DAT-1) is sensitive to cocaine[66] and the dopamine neurotoxin 6-hydroxydopamine induces selective dopamine neuronal degeneration in *C. elegans* as it does in vertebrates.[67] Also, cocaine at relatively high concentrations (1.0+ mM) can moderately reduce locomotion velocity and this effect appears to be mediated mainly through the serotonin system.[68] To determine if cocaine could induce reward-like behaviors in *C. elegans*, our group employed a Pavlovian chemosensory cue-conditioning paradigm in which cocaine was paired with an environmental stimulus (a distinctive food or salt cue).[20] After multiple pairings, worms were tested in the absence of cocaine to determine if the history of cocaine coexposure affected the worms’ affinity for the cue. Cocaine pairing (5–50 μM) significantly increased preference for either a salt or food cue. The effect was absent in dopamine-deficient mutants,

including cat-1 (defective in vesicular packaging of monoamines including dopamine) and cat-2 (tyrosine hydroxylase deficient). The cue-conditioned response was rescued in the mutant strains by conducting the conditioning and testing procedures in the presence of exogenous dopamine.[20] In all, these studies suggest that *C. elegans* may be an excellent model system to study the behavioral responses (including the rewarding properties) of cocaine.

5.4 Methamphetamine

Like cocaine, the addictive properties of methamphetamine in mammals are thought to be mediated, in large part, through its effects on dopamine transport.[69] However, in addition to inhibiting uptake, methamphetamine also induces release, and prolonged exposure can induce biogenic amine neurotoxicity.[70] In *C. elegans*, relatively high concentrations of methamphetamine were shown to have effects on egg laying (8.0+ mM), feeding (2.0+ mM), locomotion (16.0 mM), and survival (8.0+ mM) after 1 h of exposure.[71] The lethal effects of methamphetamine (but not its effects on egg laying) were significantly reduced in a mutant strain (nsy-1 [eg691]) that had a single nucleotide mutation in the NSY-1 protein. The mutant also demonstrated resistance to the lethal effects of exposure to high concentrations of dopamine and 3,4-methylenedioxymethamphetamine (MDMA). The nsy-1 gene is associated with sensory neurons,[72,73] has functions in the innate immune response,[74] and appears to be an ortholog of apoptosis signal-regulating kinase-1.[71] Little is known about the relationship between the neurotoxic and appetitive/addictive properties of methamphetamine, and *C. elegans* may serve as an appropriate model system to study this relationship and identify targets for treatments to possibly reduce the neurotoxic effects of methamphetamine.

As with cocaine, our group also investigated reward-like behaviors of methamphetamine in *C. elegans* using the Pavlovian chemosensory cue-conditioning paradigm.[20] Previous methamphetamine pairing (50–500 μM) significantly increased preference for either a salt or food cue. Also similar to cocaine, the effect was absent in both dopamine-deficient mutants cat-1 and cat-2. The methamphetamine cue-conditioned response was also rescued in the mutant strains by conducting the conditioning and testing procedures in the presence of exogenous dopamine.[20] These studies provide additional evidence that, as in mammals methamphetamine is mediating its rewarding effects through the dopamine system, and both the rewarding and neurotoxic effects of methamphetamine can be modeled in *C. elegans*.

6. CONVERGENT MECHANISMS OF DRUGS OF ABUSE IN *C. ELEGANS*

Although the majority of work examining behavioral responses to drugs of abuse in *C. elegans* thus far has been conducted in EtOH research, and additional work is needed with other abused drugs to better characterize the mechanisms of action across drug classes, some consistencies are apparent across the drugs examined in the current manuscript. In reviewing the molecular targets identified in the behavioral paradigms as described in Table 1, monoamine neurotransmission-associated genes are involved in mediating at least some behaviors induced by each drug.[20,31,41,68,75–77] In particular, within this classification, mutation of the tyrosine hydroxylase gene cat-2 resulted in reductions in drug-induced

behaviors for each drug of abuse.[20,76,77] Although this type of mutation is lethal in rodents,[78] drugs of abuse have long been known to function, at least in part, through the dopamine neurotransmitter system[3,27] and all of the addictive drugs examined here have direct or indirect effect on dopamine systems. Similarly, alterations that affect cholinergic neurotransmission also inhibit behavioral responses of *C. elegans* to nicotine.[31,62] In this respect, these findings are consistent with known mechanisms of action of these drugs in higher-level organisms, including humans. Also like vertebrate species, drug effects in *C. elegans* appear to be affected by a wide variety of genes, proteins, and neurobiologic systems that are known to mediate and/or support neuronal function (see Table 1). However, since the majority of these targets to date have been tested in EtOH models, but not with other drugs of abuse, additional studies are needed to determine if these molecular targets are selective for EtOH or represent common mechanisms for multiple drugs of abuse.

7. LEVERAGING *C. ELEGANS* FOR MODEL DEVELOPMENT AND DRUG DISCOVERY

The previous discussion indicates that *C. elegans* can be used to model aspects of drug addiction and identify systems and molecular mechanisms that mediate drug effects. Overall, the findings are surprisingly consistent with analogous findings in higher-level organisms—including humans—and suggest that the effects of addictive drugs are highly conserved. Additional study is needed to better characterize these models and provide a better understanding of how exposure to drugs of abuse can change *C. elegans* neurobiology—driving the animal to seek out further drug exposure. Fortunately, *C. elegans* has a tractable genome that enables both forward and reverse genetics approaches that can be applied to the study of addictions.[33] Additional opportunities for further *C. elegans* model development in the addiction field includes modeling the consequences of drug exposure during critical stages of development. Previous work has described the anatomical effects of embryonic EtOH exposure in *C. elegans* which has some parallels to fetal alcohol syndrome in humans.[86] However, few studies have examined the effects of drug exposure in development on future drug-associated behavior. Initiation of drug taking in humans typically begins in adolescence; thus, models that utilize the exclusive study of the effects of drugs in fully developed adults do not capture the impact of drugs on a developing nervous system. New models that focus on development, and/or take such factors into account, may better model the time course and progression of addiction in humans.

C. elegans are used for screening in a multitude of paradigms. Features such as low cost of maintenance, minimal space requirements, and the availability of image-analysis software for data collection affords advantages to *C. elegans* models in the development of high-throughput assays for medications screening purposes. However, although *C. elegans* are surprisingly highly conserved functionally, differences in some neurotransmitter systems and receptor pharmacology between *C. elegans* and humans could indicate limitations in this approach. For instance, although *C. elegans* make anandamide and 2-arachidonoylglycerol,[87] cannabinoid receptors have yet to be identified in *C. elegans*.[88] Such differences in molecular systems and pharmacology between *C. elegans* and vertebrates could be viewed as a limitation of the model and indeed could limit its application for

certain drug classes. However, as previously discussed, *C. elegans* do respond to many of the common drugs of abuse (e.g., EtOH and the psychomotor stimulants) and the recent discovery of a class of opioid receptors in *C. elegans*[89] may provide a new avenue for drug-abuse modeling and treatment screening in *C. elegans*.

In order to evaluate the possible utility of *C. elegans* models in medications screening, drugs with known efficacy to treat addictions (such as naltrexone) need to be tested to establish predictive validity of the *C. elegans* models. It would be expected that some agents might prove more effective than others based on divergent pharmacology. However, diversity in pharmacology and molecular systems in *C. elegans* may also serve as an important advantage of this model system and provide additional information about how specific compounds may work to reduce drug intake. For instance, topiramate is a compound with a rich pharmacology and many possible mechanisms to reduce EtOH drinking. One possible mechanism is through actions at voltage-sensitive sodium channels,[90] which are absent in *C. elegans*.[91] Should topiramate prove ineffective in reducing the EtOH preference response in *C. elegans*, it would strengthen the argument that these channels may have a role in mediating EtOH drinking/seeking in vertebrates. Thus, the effects of agents to reduce drug intake in these models may be viewed in light of the molecular homology of the systems thought to mediate their respective effects in order to determine the possible influence of divergent pharmacology on the results. Such data might provide important information about the pharmacology and molecular substrates mediating the effects of established pharmacotherapies. Moreover, the advent of gene editing technology (such as CRISPR) offers the potential to replace *C. elegans* receptors with their human orthologs to possibly improve the translational impact and further increase the predictive validity of such models. This could significantly increase the utility of these models to identify new targets/candidate drugs for the treatment of addiction.

8. CONCLUSIONS

Addiction is a worldwide problem with severe consequences to health, relationships, crime, and economics at every level. Unfortunately, there are very few effective pharmacotherapies available to treat addicts. Animal models have been used to better understand the neurobiologic underpinnings of addictive behavior and *C. elegans* has emerged as a viable model system to study addiction. Various drug-induced behaviors in *C. elegans* have been utilized to identify genes and biologic systems that mediate specific behavioral responses (Table 1). Many of these genes/systems have orthologous representation in vertebrate animals and several have been implicated in human addiction. Thus far, EtOH is the best-studied drug of abuse in *C. elegans* behavioral models, and genes affecting alcohol-associated behavior in worms have been linked to polymorphisms in orthologous genes in humans with alcohol-related sensitivity and/or disorders.[40] In general, these genes fall into categories related to (1) alcohol metabolism (i.e., alh-6 and alh-13), (2) neurotransmitter/modulator function including acetylcholine (unc-63), monoamines (cat-1), dopamine (dop-4 and cat-2), serotonin (tph-1), GABA (unc-25), neuropeptide Y (npr-1), (3) cation channels (slo-1, nca-1, and nca-2), and (4) chromatin remodeling complexes (swsn-4 and swsn-9). These data suggest that a number of orthologous neurobiologic systems and molecular mediators of EtOH effects in humans are present and also involved in behavioral responses

to EtOH in *C. elegans*. In addition, the HUMTH01-VNTR polymorphism in the human gene coding for tyrosine hydroxylase is associated with reduced odds of dependent smoking.[92] Although less information is currently available on the precise mechanisms and targets common to both *C. elegans* and humans associated with behavioral responses to other drugs of abuse, the consistent finding of dopamine system involvement (i.e., cat-2) in multiple classes of addictive drugs supports a conserved and central role for dopamine in the effects of drugs of abuse across animal species. The data generated thus far using *C. elegans* models are promising. However, further model development/refinement may enhance the validity of such models, and additional applications of the powerful molecular genetic methodologies used in *C. elegans* may enhance the utility of the species to aid in the identification of new targets and the development of addiction treatments.

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Table 1

Some Addiction Related Targets/Systems/Genes in *C. elegans*

Drugs of Abuse	Category/System	Functional Class	Gene/Mutant	Measure/Effect	References
Alcohol	Aldehyde dehydrogenase	Aldehyde metabolism	*alh-6;alh-13*	EtOH effect on speed+	[39]
	BBS1	Fat storage and others	*bbs-1*	EtOH effect on: IR+; AFT+	[79]
	bHLH transcription factor	Lipid metabolism	*sbp-1*	EtOH effect on: IR−; AFT−	[79]
	C_2H_2 zinc-finger transcription factor	Gene repression; others	*pag-3*	EtOH effect on: AFT−	[79]
	Chloride intracellular channel (CLIC)	Cell tubulogenesis and others	*exc-4*	EtOH effect on: IR−	[80]
			exl-1	EtOH effect on: AFT+	
	Cholinergic signaling	Muscle contraction	*cha-1;unc-17*	EtOH-induced HC−	[52]
	CRF receptor-like GPCR	Peptide neurotransmission	*seb-3*	EtOH effect on: AFT−; W/D elicited tremors− ★GOF mutant: AFT+	[81]
	Delta-5 fatty acid desaturase	Delta-5 fatty acid synthesis	*fat-4*	EtOH effect on: AFT−	[53]
	Delta-6 fatty acid desaturase	Delta-6 fatty acid synthesis	*fat-3*	EtOH effect on: AFT−	[53]
	Diacylglycerol kinase	Synaptic transmission	*dgk-1*	EtOH effect on: IR−	[42,79]
	Dopamine receptor	Dopamine transmission	*dop-4(D1-like)*	EtOH effect to + activity in liquid: +	[75]
	GABA vesicular transporter	GABA neurotransmission	*unc-47*	HC−	[52]
	Glutamic acid decarboxylase	GABA synthesis	*unc-25*	HC−	[52]
	Na^+/K^+ ATPase	Tolerance to muscle contraction	*eat-6*	Tolerance to EtOH- induced HC−	[52]
	NAD-dependent protein deacetylase	Transcription factor	*sir-2.1*	EtOH effect on: IR−; AFT+	[79]
	NALCN-related leak channel	Cation channel function	*nca-1;nca-2*	EtOH effect on: body bend rate+	[82]
	Neuropeptide receptor	Mediates NPY-like effects	*npr-1*	IR−; AFT+	[45,79]
	Nicotinic Acetylcholine receptor	Nicotine neurotransmission	*unc-63*	HC −	[52]
	Nuclear hormone receptor	Regulation of transcription	*nhr-49*	EtOH effect on: AFT−	[79]
	Omega-3 fatty acid acyl desaturase	Polyunsaturated fatty acid synthesis	*fat-1*	EtOH effect on: IR+; AFT−	[53]
	Potassium channel	EtOH mediated K+ current	*slo-1*	EtOH effect on: speed−; eggs−; IR−; AFT−	[32,42]
	Proprotein convertase	Peptide secretion	*egl-3*	EtOH W/D-induced omega turns −	[83]
	RAB3 GTP exchange factor	Synaptic transmission	*aex-3*	EtOH effect on speed: −	[84]
	SM protein	Trafficking, synaptic function	*unc-18*	EtOH effect on swimming behavior−	[85]
	Small molecular wt GTP-binding protein	Synaptic transmission	*rab-3*	EtOH effect on: speed−; movement to food−	[84]
	SWI/SNF complexes	Chromatin remodeling	*swsn-9;swsn-4; swsn-1;swsn-2.1; swsn-2.2 swsn-3; swsn-6;pbrm-1;*	EtOH-induced HC and/or tolerance to HC; varied effects	[56]

Drugs of Abuse	Category/System	Functional Class	Gene/Mutant	Measure/Effect	References
			swsn-7;phf-10; let-526;dpff-1		
	Transcriptional corepressor	Transcription	*ctbp-1*	EtOH effect on: speed−; IR+; AFT−	[79]
	Triacylglycerol lipase	Hydrolase (predicted)	*lips-7*	EtOH effect on: IR−; AFT+	[79]
	TRPV channel	Cation transport	*osm-9*	Inhibits npr-1 enhanced AFT induced with EtOH	[45]
	Tryptophan hydroxylase	Serotonin synthesis	*tph-1*	EtOH preference−	[41]
	Tyrosine hydroxylase	Catecholamine synthesis	*cat-2*	EtOH-induced SDL: −	[76]
	Vesicular monoamine transporter	Monoamine packaging	*cat-1*	EtOH-induced SDL: −	[76]
	Voltage insensitive leak channel modulator	Modulates channel subunit protein levels; others	*unc-79* *unc-80*	EtOH-induced effects on: immobility− Swimming+ Swimming+	[37,82] [82]
Nicotine	Aromatic amino acid decarboxylase	Monoamine synthesis	*bas-1*	NIC effect on gustatory plasticity−	[77]
	Cholinergic receptor subunit	Acetylcholine neurotransmission	*acr-5*	NIC preference− NIC cue conditioning−	[31]
	Cholinergic receptor subunit	Acetylcholine neurotransmission	*acr-15*	Nicotine preference− NIC cue conditioning− NIC locomotion stimulation−	[31] [62]
	Cholinergic receptor subunit	Acetylcholine neurotransmission	*acr-15*	NIC locomotion stimulation−	[62]
	Dopamine receptor	Dopamine neurotransmission	*dop-2*	Nicotine preference−	[31]
	Dopamine receptors	Dopamine neurotransmission	*dop-1/dop-2 (doublemut)*	Nicotine preference−	[31]
	Phospholipase C beta	Second messenger system	*egl-8*	NIC locomotion stimulation−; dependent behavior−	[62]
	TRPC channels	Variable nonselective cation channel activity	*trp-1;trp-2*	NIC locomotion stimulation−	[62]
	Tyrosine hydroxylase	Dopamine neurotransmission	*cat-2*	NIC effect on gustatory plasticity−	[77]
Cocaine	Aromatic amino acid decarboxylase	Monoamine synthesis	*bas-1*	Cocaine-induced hypoactivity−	[68]
	Serotonin-gated chloride channel	Serotonin neurotransmission	*mod-1*	Cocaine-induced hypoactivity−	[68]
	Serotonin transporter	Serotonin neurotransmission	*mod-5*	Cocaine-induced hypoactivity−	[68]
	Tryptophan hydroxylase	Serotonin synthesis	*tph-1*	Cocaine-induced hypoactivity−	[68]
	Tyrosine hydroxylase	Dopamine neurotransmission	*cat-2*	Cue-conditioning to cocaine−	[20]
	Vesicular monoamine transporter	Monoamine neurotransmission	*cat-1*	Cue-conditioning to cocaine−	[20]
Methamphetamine	MAP kinase (MAP3K)	Neuronal signaling	*nsy-1*	MAP-induced toxicity−	[71]
	Tyrosine hydroxylase	Dopamine neurotransmission	*cat-2*	Cue-conditioning to MAP−	[20]
	Vesicular monoamine transporter	Monoamine neurotransmission	*cat-1*	Cue-conditioning to MAP−	[20]

Abbreviations: +, increased; −, reduced; AFT, acute functional tolerance; eggs, egg-laying behavior; IR, initial response to drug; HC, hypercontraction; MAP, methamphetamine; SDL, state-dependent learning; W/D, withdrawal. All observations were made from genetic manipulations using loss of function mutations or RNA interference, except ★GOF gain of function mutation.