The Journal of Applied Pharmacology, 14, 1-10(2006)

Invertebrate Models Used for Characterization of Drug Dependence and Development of Anti-Drug Dependent Agents

Hyunsook CHANG[1], Ha Won KIM[2], and Dong-Hee LEE[2]*

[1]*Department of Child Studies, Korea Nazarene University, Chonan, Korea*
[2]*Department of Life Sciences, University of Seoul, Seoul, Korea*

(Received March 17, 2006; Accepted March 27, 2006)

Abstract – Drug dependence deals a heavy socioeconomic burden to the society. For adolescents, the damage from drug dependence is greater than adults considering their higher susceptibility to drug effect and increasing chance for violence leading to criminal punishment process. Habitual drug use depends on genetic and environmental factors and the complex interactions between the two. Mammalian model systems have been useful in understanding the neurochemical and cellular impacts of abused drugs on specific regions of the brain, and in identifying the molecular targets of drugs. More elucidation is required whether biological effects of drugs actually cause the habitual dependence at the cellular level. Although there is much insight available on the nature of drug abuse problems, none of the systems designed to help drug dependent individuals is efficient in screening functional ingredients of the drug, and thus resulting in the failure of helping drug dependent individuals recover from drug dependence. Alternative model systems draw the attention of researchers, such as the invertebrate model systems of nematodes (*Caenorhabditis elegans)* and fruit flies *(Drosophila melanogaster).* These models should provide new insight into the mechanisms leading to the behavior of drug users (even functional studies analyzing molecular mechanism), and screening useful components to help remove drug dependence among drug users. The relatively simple anatomy and gene expression of the invertebrate model systems should enable researchers to coordinate current knowledge on drug abuse. Furthermore, the invertebrate model systems should facilitate advance in experiments on the susceptibility of specific genetic backgrounds and the interaction between genetic factors to drug dependence.

Keywords ☐ Drug dependence, invertebrate, cocaine, alcohol, dopamine, *Drosophila, C. elegans*

INTRODUCTION

Adolescence is considered as a time period for trial and error on new things. To reduce stress, to feel grown up, or out of pure curiosity, they begin to try alcohol and other drugs from the stage of their life. Nowadays, high school students are facing a wave of new drugs epidemic in many nations. According to the statistics in the US, more than 700,000 incoming high school students find ready access to illegal sources of drug. Over 10% would have tried illegal drugs by the end of their freshmen year. UK's Statistics is less encouraging; 1.5 million pupils have already experienced illegal drugs - 300,000 high school freshmen try them.

Drug dependence is a very serious disease which would deliver devastating effects on individual lives and relationships as most Americans view (Fig. 1). Drug consumption is often linked to violence and physical abuse by adolescents. Drug use

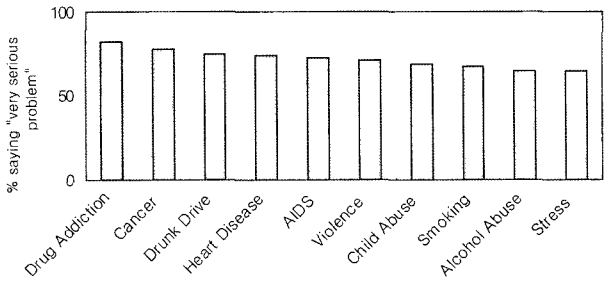

Fig. 1. Americans' views of the seriousness of health problems (top ten of Thirty-Six problems). When asked about the seriousness of health problems, 82% answered that drug addiction was very serious. Sixty five percent answered alcohol abuse, another form of drug abuse, is very serious. (Source: Harvard School of Public Health/Robert Wood Johnson Foundation/ICR, August 2000)

*Corresponding author
Tel: 02-2210-2170, Fax: 02-2210-2888
E-mail: leedh@uos.ac.kr

or dependence undoubtedly deteriorates normal brain processes such as motivation, learning, and memory, which they utilize to gain new knowledge on the environment surrounding them (Martin *et al.*, 1999; Parr *et al.*, 2001; Large *et al.*, 2003). Most drugs serve as psychomotor stimulant and excessive rewarding effects via targeted destruction of dopamine producing cells or neurochemical inhibition of dopamine receptors.

Once exposed to alcohol or drug, many teenager suffer from physico-mental health, or social problems that make their addictive disorders much more difficult to deal with. It is difficult to know which teens will experiment and stop and which will develop serious problems. Compared to adults, teenagers are at a great risk for brain damage and development of drug dependence. Since some genetic backgrounds make individuals more sensitive to particular agents, further mutation studies would lead researchers to a new elucidation of drug dependence and to generation of anti-drug agents designed for teen drug users.

The factors contributing to an increased risk for drug dependence are highly complex and require further dissection of the key components and their interaction. Children who exhibit aggression, a lack of self-control and a difficult temperament may be at greater risk of drug addiction. If you are a victim of an additional emotional problem, such as attention-deficit, depression, hyperactivity, or post-traumatic stress disorder, you are more likely to become dependent on drugs. Particularly for young people, peer pressure is a strong factor in starting to use and abuse drugs. A lack of attachment with your parents may increase the risk of addiction, as can a lack of parental supervision. Drug usage can become a way of temporary relief from these bitter feelings. Some drugs, such as heroin and cocaine, prompt physical addiction than do others.

Drug addiction is more common in some families and likely involves the effects of many genes. If you have family members with alcohol or drug problems, you are at greater risk of developing a drug addiction. Many studies, however, cautiously indicate that genetic factors seem to play a key role in every aspect of drug dependence spanning from the indulgence for drug dependence to the chances of recovery. Study on gene expression responding to ethanol exposure, acute or chronic, may lead to the understanding of the complicated nature of alcohol dependence (Risinger *et al.*, 2000; Rodan *et al.*, 2002). Under circumstances, evidence is now accumulating from behavioral, twin and molecular genetic studies, thus, providing a comprehensive understanding of the genetic basis for drug dependence. Ultimately this should lead to the development of improved methods for assessment and treatment of dependence.

NATURE OF DRUG DEPENDENCE AND DRUG TOLERANCE

Drug abuse is a significant social and economic problem. Scientific dependence research has led experts to conclude that complex genetic and environmental factors contribute to a predisposition for drug dependence. Drug addiction has two aspects, tolerance and dependence. The former refers to decreased drug effect with repeated use which comes from down-regulation of drug receptors. The latter cause withdrawal syndrome when internal drug concentration is low despite up-regulation of drug receptors (Wise and Bozarth, 1987). The genetic contribution is likely multigenic and heterogeneous, making identification of specific "dependence genes" less fruitful to date. Scientists have begun to understand why addicted people may sacrifice everything that is important to them including food, jobs, sex, and social interaction in the quest for a chemical fix.

Many gene products have been proposed to be targets of drugs and alcohol as a result of in vitro experiments; however, no reasonable information is available on which proteins, affected by drugs, are responsible for the behavioral effects (Wise, 2000). Drug dependence is a type of chronic illness like diabetes or hypertension as declared by American Medical Association (AMA) four decades ago. Fig. 2 shows that pathological loss of dopamine transporter during habitual consumption of methamphetamine. As with other diseases, immediate treatment can prevent further complications and may even save a life. Majority of researchers today acknowledge that drug dependence is something different from a compulsive behavior resorting to use and even abuse of drugs such as alcohol and

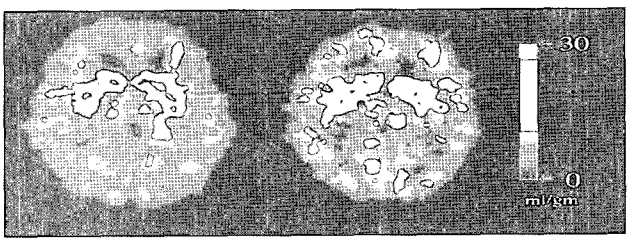

Fig. 2. Loss of dopamine transporter following heavy use of methamphetamine. White portions refer to the area where dopamine transporter have diminished (PET analysis) (*Source: Volkow, N.D. et al., Am J. Psychiatry, 158(3), pp. 377-382, 2001)*

cocaine.

The response to ethanol seems to be greatly affected by genetic components, which serves as a meaningful risk indicator for the dependence on alcohol. Abuse of alcohol and other drugs is one of the most difficult to deal with considering they are being sucked into this vortex of indulgence. It is difficult because no one can not change habitual drug users, only who must be willing to change themselves to overcome the drug problem. Even though you realize that one of your acquaintances may be addicted to an illegal drug it is very difficult to encourage them to seek help immediately. The difficulty comes from a fear of maltreatment when the society respond to them as drug users. Most important, there is lack of reasonable recovery program overcoming drug dependence since drug dependence pathway is insufficiently understood to date.

Behavioral scientists have funneled a great efforts into defining the phenotype and carefully pointing out the variables that have to be considered in describing individual differences in drug dependence and behaviors. Molecular biologists have successfully identified a number of drug receptors, their DNA sequence and genetic locus, and various neurochemicals (particularly dopamine) that may modulate in regulation and activation of behaviors caused by drugs such as alcohol, nicotine and cocaine. Alcoholism is characterized by tolerance, dependence, and unrestrained preference for alcohol (Scholz *et al.*, 2000; Schuckit, 2000). When addicted, they may feel like there is nothing to do except drinking and revolve around alcohol consumption.

In most cases, drug dependence involves the phenomenon of behavioral sensitization. Behavioral sensitization is thought to constitute critical part of drug dependence (McClung and Hirsh J. 1998, 1999). Chronic exposure to cocaine gradually increases psychostimulating effects with subsequent drug input. In this case, the trace of amine tyramine from previous intake, serve as a positive modulator of cocaine sensitization.

USEFULNESS OF INVERTEBRATE TO STUDY DRUG DEPENDENCE

As an efficient substitute for traditional mammal models, invertebrates with certain characteristics shared with mammals, are being employed to study drug dependence problems. Worms and flies feed, reproduce, and alternate between labor and rest periods during the appropriate time of day in a similar fashion with which higher organisms do. Invertebrates' nervous systems are much simpler than those of mammals and invertebrate genetics are comparatively more sophisticated and rapid. Accordingly, researchers have turned their attention to the worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* as animal models for drug dependence researches. Since experimental techniques available for invertebrates still differs from that available in mammals, direct comparisons of the results is very difficult. However, surprising evidences have already begun to emerge at the behavioral, neurochemical, and molecular levels from the invertebrate drug research. Their importance will likely increase in the next few years as the invertebrate studies continue to gain momentum, and as molecules screened through invertebrates are analyzed functionally in mammals.

Undeniably, the major strength of invertebrate systems is the easy accessibility to forward genetic analysis by single gene mutagenesis. Their thorough genome sequences increase a chance for success both in forward and reverse genetics. Due to the large numbers of genetically identical individuals and the short generation length, behavioral analysis reduces variation and empowers many observations into the range of statistical significance. This is important considering many data from mammalian studies are subjected to intense arguments mostly because of insufficient size of study. Their diverse but well defined spectrum of strains permit a study on intergenic interaction among genes affecting drug effects by simple mating of mutants.

Current assays to quantify drug responses and their adaptive changes are considered highly sophisticated and now frequently utilized by researchers. Drug dependence and conditioned disposition assays, however, require further improvement in terms of compilation of sensory input under exposure to drug. Some researchers stick to same animal lines and strains for drug dependence investigation. By studying various strains of invertebrates that vary in their dependence potential for alcohol and the genes responsible for this vulnerability in invertebrates, researchers hope to identify homologous genes in humans that render a person more or less susceptible to alcohol dependence.

C. elegans has a simple nervous system and is a good fit for the studies on specific neurotransmitters, receptors, and signaling molecules function within the context of the nervous system to produce behavior. With recent advances in RNA-mediated gene expression interference (RNAi) technology, reverse genetic analyses became a practicaly viable option to reinforce information from rodents. Along with the single stranded RNAi technology, worm's gene expression can be

modulated with ds-RNAs which can be used for chromosome-wide systematic functional analyses (Xu *et al.,* 1998; Fire *et al.,* 1998; Bargmann, 2001). Combined with the entire genomic sequence data, RNAi allows the directed gene inactivation or any gene having a partial sequence of interest. Utilizing RNAi techniques, a more sophisticated neurobiological study can be pursued in flies which have 300000 neurons while *C. elegans* have only 300. The sizable neuronal network of flies enable researchers to major advances in investigating functional neuronal routes leading to complex behaviors during drug abuse.

UNDERSTANDING OF DRUG DEPENDENCE PROBLEMS USING INVERTEBRATE SYSTEMS

Worms and flies undergo a lower complexity of decision-making situation than that of mammals; however, these organisms have a greater cognitive system than any higher organisms. Proteins implicated in the actions of drug effect require membrane-spanning receptors and transporters such as catecholamine re-uptake transporters, nicotinic acetylcholine receptors, voltage-gated ion channels, and the G protein-mediating K+ channels (Lewohl *et al.,* 1999; Beckman *et al.,* 2000). These serve as the major targets of many drugs such as cocaine and nicotine (Fenster *et al.,* 1999; Gomex *et al.,* 2001). Different from other drugs of abuse, ethanol signaling pathways do not employ specific receptors and, rather, it causes random disturbances in the integrity of neuronal membranes (Kerr *et al.,* 2000; Lev-Ram *et al.,* 2000).

Identification of drug targets

Genetic screens for drug resistance in invertebrates have been useful for defining the direct targets of several drugs with behavioral effects. GABA receptor is the direct target of the drug (Richmond and Jorgensen, 1999). GABA receptors are also abundantly found in worms. Similarly, direct screens for resistance to the paralytic nematocide ivermectin led to the identification of the ivermectin receptor, a glutamate-gated chloride channel. Although some drug targets may not be conserved, a direct search for central receptors of drug effects has been conducted and those bearing sequence similarity has been reported in both organisms.

Nicotine has a definite molecular target for action: nicotinic acetylcholine receptors (nAChR). The mechanisms for nicotine action on nAChRs into nicotine dependence remain to be understood. Considering *Drosophila* do not use acetylcholine at the neuromuscular junction like any insect in general, their nAChRs must be specific to the nervous system. Nicotine does serve as a strong agonist to nAChR for the drug effect and its prolonged exposure results in reinforcing effects, which cause nicotine dependence. PCR-based cloning and genomic search has identified at least ten receptors with homology to nAChRs in *Drosophila.*. Repeated nicotine use results in tolerance, dependence, and frequent administration. *C. elegans* is an attractive model system for identifying the targets of neuroactive compounds regardless of complete difference from rodents at the organism level. *C. elegans* has at least 42 nAchR receptor subunits and this unusual number of receptor subunits strongly implies that the simpler nervous system of the worm has evolved more complex signaling. Recent studies, however, indicates that genetic screens in *C. elegans* serves as an unbiased model in identifying the target of many abused drugs (Fire *et al.,* 1998; Bargmann, 2001).

Pathways involved in drug dependence

Further understanding of drug effects on the mechanisms underlying brain function is essential to develop and improve prevention and treatment strategies against drug addiction (Dudley, 2000; Duerr *et al.,* 2002). Dopamine is believed as the major messenger in any drug-induced behavior in human, mammals, and invertebrates (Andretic and Hirsh J. 2000; Tsai *et al.,* 2000; Depiereux *et al.,* 2000). The extent of reinforcement and reward syndromes depends on the levels of dopamine in critical brain areas named the nucleus accumbens (NAc) following drug consumption. Chronic consumption of cocaine results in a rapid locomotor-stimulation in rodents subsequently challenged with the drug. (Rocha *et al.,* 1998, Kerr *et al.,* 2000; Lev-Ram *et al.,* 2000; Sora *et al.,* 2001). The trace amine tyramine, from previous drug abuse, has been suggested as a positive modulator of cocaine sensitization. The sensitization pathway have been investigated mainly for psychostimuling agents including cocaine and methamphetamine; still, the pathway can be used to explain how acute ethanol exposure enhances dopamine release and locomotor activity.

A complex role for cAMP signaling constitutes consequent drug-induced behaviors. Fig. 3 shows that, at reduced levels of the adenylyl cyclase-stimulating G-protein, following morphine injection, increase sensitivity to drug and decrease voluntary intake. This observation clearly link the cAMP signaling pathway to acute responsiveness under regulation of CREB and PKA which modulate TH (tyramine-hydroxylase) considering TH plays a major role in dopamine biosynthesis. This sig-

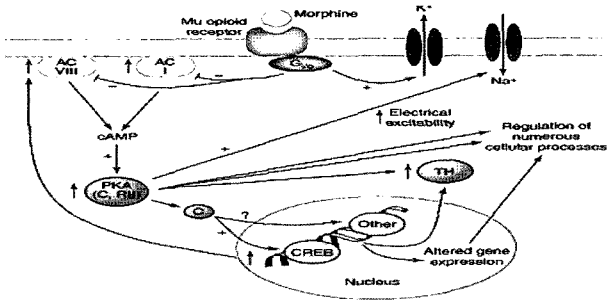

Fig. 3. Pathway involving morphin action. cAMP signaling pathway is used in morphin effect pathway. The adenylyl cyclase (AC)-stimulating G-protein has increased sensitivity to morphine. cAMP signaling pathway to acute responsiveness are regulated via CREB and PKA. PKA has two subunits: C and R (catalytic and regulatory). TH refers to tyramine-hydroxylase. This signaling pathway may mediate many of the addictive behaviors in flies and mice. (Source: Nestler and Malenka, Trends in Pharmacological Sciences, 2004)

naling pathway also mediate majority of the addictive behaviors in drug addiction in invertebrates. Mutagenesis on dopaminergic and serotonergic proteins in flies alter acute cocaine sensitivity (Giorgetti and Zhdanova, 2000). Also, flies treated with dopamine inhibitor, 3-iodotyrosine (3IY) show a minimal sensitivity to cocaine. This resistance can be rescued in the presence of L-Dopa, the immediate product by tyramine-hydroxylase.

Genes affecting vulnerability to drug dependence

Some genetic backgrounds make individuals more susceptible to particular drugs. Further mutation studies, therefore, would enable researchers to further understand the nature of drug dependence and to design anti-drug agents. Because numerous genetic alterations mutually interact at the organism level, identification of genetic factors responsible for drug dependency may be very challenging. Some studies, however, revealed that, in flies, single gene mutants with altered ethanol sensitivity directly affects regulating acute ethanol-induced behaviors (Dubnau, 2001; Kalidas and Smith, 2002).

Genes involving circadian rhythm: *clock, cycle, period, and timeless*

Proteins regulating circadian rhythm are believed to play a major role in cocaine-induced behaviors (Andretic and Hirsh, 2000). Cocaine sensitization disappears in mutants lacking of normal genes for circadian rhythms. In those mutants, induction of tyrosine decarboxylase (TDC) activity is deficient and responsiveness of postsynaptic dopamine receptors ncreased (Park *et al.*, 1999). Whether sensitization itself is controlled according to the circadian rhythm, or vice versa, remains to be further understood. Considering the mutants in *timeless* protein that couples with *period* gene products still sensitize to cocaine, *period* appears to function differently in circadian rhythms and drug sensitization, respectively, via separate molecular mechanisms and spatial commitments.

Protein kinase C related genes: *unc-29, pkc,* and *tpa-1*

C. elegans has a diverse family of nicotinic receptor genes including both neuromuscular and neuronal receptor subtypes. Several of these, including the *unc-29* gene, have been shown to encode functional receptor subunits. Nicotinic receptor agonists have specific and easily effects on *C. elegans'* behavior, including locomotion, feeding, and egg-laying. In nicotine-adapted worms, UNC-29 expression is clearly down regulated and nAChR UNC-29 mutants do not respond to nicotine even though they remain sensitive to further challenge by serotonin when they reach nicotine adaptation. This observation strongly indicates that UNC-29 is likely the major transducer of nicotine-elicition signals. Additionally, UNC-29 expression is unaffected by nicotine in PKC mutants. The gene of *tpa-1* encodes a homolog of protein kinase C (PKC). The *tpa-1* mutant failed to proceed to adaptation to nicotine. After recurrent nicotine exposure; however, they remained sensitive to cholinergic agonists and retained high levels of receptor protein. These results suggest that PKC-dependent signaling pathways may promote nicotine adaptation via regulation the turnover of nicotinic receptor.

Learning and memory genes: *cheapdate and amnesiac; dunce and volado*

Memory genes *cheapdate* and *amnesiac* play a profound role in the drug effect. The mutant, *cheapdate (chpd)*, shows an increased sensitivity to ethanol. The gene of *chpd* is an intragenic counterpart of the *amnesiac* gene, which encodes a neuropeptide participating in the cAMP pathway. Drug dependence accompanies the prolonged neural and behavioral changes following apparently similar tracts used in learning and memory process. Overexpression of *amnesiac* restores the ethanol sensitivity defect in adult *amnesiac* mutant flies although this treatment fails to restore their memory defect. This increased sensitivity to ethanol may be an indication of a failure to adapt during the exposure period because of functional *amnesiac*. Reduced sensitivity, typical for tolerance state,

may reflect an accelerated adaptation to ethanol based upon a blockade in memory process. Genetic defects in ethanol tolerance or ethanol-induced locomotor behaviors has been identified using chemical random mutangenesis on *C. elegans* by EMS, highly toxic mutagen. Among EMS-induced mutants, The genes of *dunce* and *volado* exerted a profound effect on learning and memory process and also, on the promotion of tolerance to ethanol.

Classification of genes for tolerance to drug

There are genes conferring tolerance over susceptibility to drug effect. Mutations or genes that confer drug tolerance were classified into five groups. Although the extent of genetic overlap remains to be further determined, these genes were classified based upon the function in drug-induced gene network. Class I refers to genes whose transcription is induced early and maintained at high level. Class II genes show transcriptional responses late. Class III genes transcription is induced at early time points and then returns back to normal after longer exposure. Class IV genes are repressed at early time points, but whose expression is restored to normal under longer treatment with ethanol. During the drug treatment, Class V genes become repressed at later time points.

Temporal and spatial difference in drug action

The effects of drug on dependence and brain are different in juveniles compared with adults. Many animal studies shows that the young-adolescent brain shows differential sensitivity to alcohol-induced brain damage compared with adults. Significant brain damage was found in both groups. The olfactory bulbs were equally damaged in both groups; however, the associated frontal cortical olfactory regions were damaged only in juveniles. The anterior portions of the piriform and perirhinal cortices also were damaged only in juvenile rats. The damaged area is 4 to 12 times higher in the juvenile rats. Drug sensitivity varies between young and old individuals or among tissues. Expression of alcohol dehydrogenase (ADH), the ethanol detoxifying enzyme, is very high in adults compared than in larva in flies. With more functional ADH, adult flies are well protected from the damage by ethanol. This may explain why juvenile are more affected by alcohol consumption and told not to drink until a certain age. Temporal difference does exist between day/night sensitivity to cocaine. Circadian gene regulation appears to play an integral role in TA biosynthesis and behavioral sensitization (Nikaido *et al.*, 1999; Moriya *et al.*, 1999; Robinson and Berridge 2001).

Utilizing functional expression of temporal and spatial genes, researchers can probe for the potential role of the genes, acute or developmental, and the specific brain area responsible for drug effect. In flies, gene inducible system of GAL4/UAS binary (GUB) expression or the tetracyclin (Tet) induction vector system is frequently used for the conditional overexpression of gene of interest. GUB or Tet vector system is equipped with hormone or Tet-inducible promotor, respectively. These inducible system is very useful when dominant-negative type of transgenes expression is necessary (Osterwalder *et al.*,. 2001). The two vector systems have different perspectives since hormone may affect the heterologous system excessively in terms of physiology. On the other hand, Tet may exert too artificial pressure to the host system. Using the GUB inducible system, cocaine were found to serve as a biphasic rewarding agent when their intra-circulatory infusion was coupled either to any diurnal and nocturnal conditions. Molecular behavioral studies, in spatiotemporal and developmental senses, can provide a complementary information to using other invertebrate species in addiction research.

DRUG EFFECT ON BEHAVIORAL CHANGE IN INVERTEBRATES

Drug abuse and subsequent dependence immensely affect one's behavior. According to studies focusing on family, twin, and adoption, strong evidence points out that genetics contribute to the risk for drug dependence along with environmental factors. Molecular biologists have identified a plethora of drug receptors, the genes of interest and their locations, and other agents that exert a profound effect regulation and activation of nicotine related behavior. Under circumstances, behavioral scientists cautiously suggest that the genetic factors in describing individual differences in drug dependence and behaviors.

Recent investigations in invertebrate neurobiology have opened up a new line of research into the basic behavioral, neurochemical and genomic alterations that accompany psychostimulant drug exposure. Invertebrate drug-behavior researches already reveal that surprising parallels exist between invertebrate and higher organisms at the behavioral and molecular level. Fig. 4 shows behavior dynamic common in mammals and invertebrates. The molecular structure of vertebrate and invertebrate nervous systems is mostly shared. Altered neuronal gene expression, to cope with varied environmental conditions, constitutes complex nature of diverse behaviors.

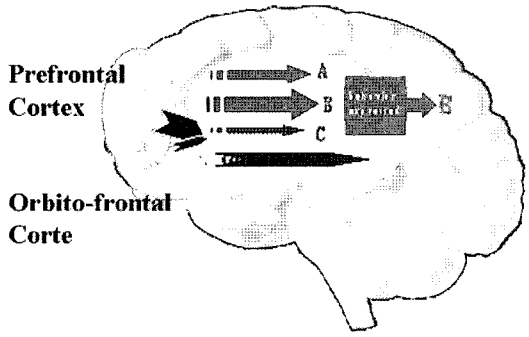

Fig. 4. Principles of behavior dynamics. Activation of dopamine reward pathway initiates a behavior track. Behavior expression is determined by dominance of track, strength of prefrontal cortex to select, and relevance of saliency (orbitofrontal cortex). The longest arrow refers to the initiation action triggered by dopamine. Among three tracks, behavior pattern B moves into the right expression box and finally represents the individual externally. (Source: Miller and Cohen, Annual Review of Neuroscience 24 167 (2001))

Cocaine induced behavior in *Drosophila*

Cocaine, a plant alkaloid, has been used as a powerful psychostimulant and a local analgesic. Cocaine employs a minimal number of transporting machinery such only as the transporters of the catecholamines, dopamine, and serotonin (Morrison *et al.*, 1999; Ranganathan *et al.*, 2001). Consistent with its function in mammalian systems, cocaine functions by inhibiting dopamine re-intake in flies (McClung and Hirsh, 1999). In the presence of volatile free-base cocaine, rodents shows spontaneous locomotion and unusual behaviors such as circling, and incessant movement. Under acute cocaine administration, flies show continuous moving and intermittent bumping against culture dish wall. When flies are treated with cocaine for four days, flies show intense grooming and reduced locomotion at low cocaine doses (less than 5 mM as cellular concentration). Reticent circling and sideway or backward locomotion are streotypical under medium doses (5 to 10 mM). Further increases in dose elicit a series of abnormal hyperactive behaviors, and finally immobility and death.

Ethanol induced behavior

Ethanol is one of the most abused drugs in the world. Still, the mechanisms by which it regulates behavior and brain function is a good subject of inquiry. Key components of ethanol action, such as ligands and channels, are well represented in flies and worms. How ethanol affect these proteins and how these effects relate to ethanol-induced behaviors need further understanding.

In nature, fruit flies virtually live with ethanol vapor. Flies mate, lay their eggs, and feed on fermenting plant debris, which often contain higher levels of ethanol. Recent advances in computer-based video technology has greatly improved the analysis efficiency and resolution of locomotor behaviors. Due to the high spatiotemporal sophistication in the system, researchers are successful in recognition of disctinctive aspects of ethanol-induced locomotor behaviors that had gone unnoticed. Flies show a complex and biphasic behavioral response during exposure to ethanol vapor. As an initial response, fruit flies show uncoordinated locomotion, loss of postural control, and subsequent paralysis. After a brief gap, flies enter a sustained hyperactive phase that levels off gradually as flies undergo a quiescent stage.

Chronic exposure to alcohol results in the tolerance, which initiate enzymatic or cellular functions that accelerate metabolic dispense and cellular discarding of ethanol following consumption of a moderate level of ethanol. This mechanism explains how flies are able to maintain functional integrity at the organism level despite the toxic presence of ethanol. Utilizing the tolerance mechanism, they ingest higher ethanol doses to undergo the similar behavioral experience as past occasions of exposure.

C. elegans have a majority of machinery involved in synaptic vesicle release and recycling receptors, channels for neurotransmission and signal transduction which have been found in mammals. When incubated in ethanol solutions, worm's initial response was a faster movement. Worms respond differently to various ethanol concentrations in incubation media. The higher ethanol concentration, the faster they appear to move, spin and reach an "ecstactic" stage. Following these typical symptoms, worms reach an "unconscious" state where worms lose mobile coordination and eventually motility.

Effects of nicotine on behavior in invertebrates

The monoaminergic regulation of behavior by nicotine has been extensively studied in vertebrates. Recurrent nicotine exposure is believed to affect nAChR, either by causing changes in receptor activity, or in abundance through receptor extrinsic regulation of signaling and dopaminergic systems appear to play a role in nicotine-induced behaviors. Reduction in the cellular dopamine leads to reduced nicotine responsiveness as observed for cocaine and ethanol. When exposed to nicotine, acetylcholine is believed to be the primary excitatory neurotransmitter in flies.

Among invertebrates, nicotine action has been studied most

extensively in the *C. elegans*. The acute effects of nicotine vary with chronic or repeated exposure as in vertebrates. Multitude of gene are post transcriptionally down-regulated, indicating that adaptation is an active process that requires an intracellular response. Under long term nicotine treatment, *C. elegans* gradually attain nicotine tolerance, and appear to require more nicotine intake to achieve normal locomotion after a prolonged exposure. Indeed nicotine-adapted worms become unorganized, showing unstable behaviors when nicotine supply was completely severed. The adaptation mechanism by which *C. elegans* use to cope with chronic nicotine exposure is to control acetylcholine receptor protein levels through a protein kinase C (PKC) dependent pathway. The chronic nicotine exposure appears to affect vulval muscle activity of egg laying whose process is governed by cholinergic and serotonergic balance in the presence of dopamine.

DEVELOPMENT OF ANTI-DRUG DEPENDENCE DRUG AND PROTOCOL

Once individuals are identified as drug users, they are likely to subjected to a harshly punitive measures. If further drug use is detected, punishment surgically increase to longer periods of confinement and even to death punishment in Asian nations of Singapore and China. These punitive anti-drug policies do not appear to be effective, as over recent years the number of drug abuser has been escalating worldwide. Rather, rehabilitation program works to the effectiveness. In the US, for example, several types of drug abuse treatment programs have been utilized for complete recovery from dependency. Short-term rehabilitation program covers less than 6 months and include residential treatment, medication treatment and drug-free outpatient treatment. Longer term treatment may include, methadone maintenance outpatient treatment for opiate addicts and residential therapeutic community treatment. When the addicts have gone through these program, only minimal percentage seek drugs later.

In order to treat or even to prevent drug dependence, basic bio behavioral investigations of the biological, psychological, sociocultural, and genetic factors that influence habitual drug use. *C. elegans* learn to refrain from an attractive odor that had been previously paired with an unpleasant odor. Researchers also have found that drug abusers who have completed treatment successfully knows how to avoid drug indulgence. These processes are believed to play a crucial role in the potential recovery from drug dependence. A genetic, molecular, and neuroanatomical dissection of conditioned reward behaviors in invertebrates may provide further insights into the mechanisms for learning and memory. Therefore, the development of education for recovery from drug dependence should be feasible, and might evolve to rescue many addict completely out of the dependence.

Naloxone-like antidote would be the best example to produce instant sobriety. Recently, methadone has been developed to treat opiate dependence. Studies show that treatment for heroin drug abuse using methadone produce better recovery rate including addiction to heroin and morphine. Adequate dosage application, combined with appropriate behavioral therapy, reduces mortality and health problems associated with drug abuse until recovery and, no doubt, the time taken up to the recuperation. The importance of "Happy Pills" cannot be overemphasized to facilitate drug addiction treatment. Under circumstances, invertebrate mutants having alteration in drug induced behaviors can be used to find novel or unsuspected molecules to rescue drug addict from dependence. The ability to control the activity of such drugs will improve patient outcomes by increasing the safety of patients undergoing treatments. To address the need for safer regulatable therapeutics, a platform to enable generation of drug-antidote pairs should be established. Otherwise, the Happy Pills can become another source of illegal drug.

All the drug treatment program aims at restoring full abstinence by the patient but their immediate goals are to reduce drug use, improve the patient's ability to function, and minimize the medical and social complications of drug abuse. Over the last 25 years, drug abuse treatment have been shown to work to reduce drug abuse and crimes committed by drug abusers.

CONCLUDING REMARKS

One of the most striking findings in neurobiology is the fact that many genes found in the human brain do exists in invertebrates. Considering invertebrates have a simple neuronal structure and network, there are clear experimental advantages to using those organisms for psychostimulant drug studies and, further, their use can provide a diverse and complementary lines of inquiry to research carried out in rodents. Current studies have shown that vast majority of these mechanisms are shared with mammals; thus, invertebrates are ideally suited for bringing studies of psychostimulants to a level of more complex behavioral phenomena. When data from vertebrate and invertebrates are merged, profound overlap is evident in the

psychostimulant's effect on respective neural systems. Psychostimulants serve as strong rewarding agents throughout the animal kingdom and command a much deep-rooted representation in the process of evolution.

In this review we have focused on invertebrate genetic models of drug abuse. Drug dependence is a treatable disorder and recovery from drug dependence can be accelerated when treatment is customized according to individual conditions. Upon successful completion of treatment regimen, patients can immediately return to the society, learn to control their condition, and live normal, productive lives. As is the case with diabetes or heart disease, people in treatment for drug abuse should take medications to accelerate behavioral changes as part of their treatment program. Behavioral treatment can include counseling, psychotherapy, support groups, or family therapy. Treatment medications offer help in suppressing the withdrawal syndrome and drug craving and in blocking the effects of drugs. Selection for antidotes that correct various drug induced behaviors will likely provide novel and promising molecules and mechanisms. The current approach to anti-dependency drug discovery concentrates mostly on inhibiting the expression of dominant or restoring the crippled activity of drug resistant genes. Combination of chemical library screening with *C. elegans* enhance feasibility of the drug discovery scheme, such as target identification, drug hit identification, lead identification and lead optimization.

Development of "Happy Pills" may speed up recovery from drug abuse problems. This must be health-enhancing, long lasting, reversible, and of low cost. The potential happy pills must elicit happy feelings in neutral or unhappy situation. This is especially important when such pills gradually restore a critical balance lost by drug dependence or other clinical treatment. The antidote must cause no damage to any organ or system and no physical dependence or withdrawal. Many therapeutic agents to treat drug dependence are associated with adverse effects in patients. The ability to control the activity of such drugs will improve patient outcomes by increasing the safety of patients undergoing treatments in which such drugs are used. Antidote provides the safest means to overcome drug activity, yet no antidote-controlled drugs are available. Impact of addiction treatment programs and an anti-drug treatment registration would give a clear view of those seeking anti-drug treatment, their characteristic features and the measures taken to solve the problem.

Invertebrates offer more to studies of psychostimulants beyond their tractability in behavioral studies. The consequences of drug abuse are severe and recent findings have corroborated previous work that highlighted the interactions between genetic backgrounds and psychostimulant exposure. Since genomic approaches integrating behavior and gene expression are now feasible, the combined use of forward and reverse genetic approaches should provide many novel insights into the mechanisms by which drugs of abuse alter behavior in upcoming years.

In conclusion, we have clearly demonstrated that invertebrates are scientifically acceptable alternative model system for studying the behavioral and cellular effects of drugs. With the ease of experimental manipulations and the behavioral and genetic information available from invertebrates, further insights into drug addiction may lead to the identification and cloning of human genes for drug sensitivity and dependence. Using invertebrate systems, antidotes to dependency can be more easily selected from natural extracts or from unsuspected over-the-count items thus help drug addicts, especially adolescents, to return to the normal way of life in the society where he used to belong to.

ACKNOWLEDGMENTS

This work was supported by a 2005 University of Seoul Faculty Sabbatical Grant to DH Lee. The authors are grateful to AJ Lee for preparation of the manuscript and meticulous editing.

REFERENCES

Andretic, R. and Hirsh, J. (2000). Circadian modulation of dopamine receptor responsiveness in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **97**, 1873-1878.

Bainton, R. J., Tsai, L. T. -Y., Singh, C. M., Moore, M. S., Neckameyer, W.S. and Heberlein, U. (2000). Dopamine modulates acute responses to cocaine, nicotine, and ethanol in *Drosophila*. *Curr. Biol.* **10**, 187-194.

Bargmann, C. I. (2001). High-throughput reverse genetics: RNAi screens *in Caenorhabditis elegans*. *Genome Biol.* **2,** 1005.1-1005.4.

Berke, J. D. and Hyman, S. E. (2000). Dependence, dopamine, and the molecular mechanisms of memory. *Neuron* **25**, 515-532.

Depiereux, E., Hougouto, N., Lechien, J., Libion-Mannaert, M. and Di Chiara, G. (2000). Role of dopamine in the behavioral actions of nicotine related to dependence. *Eur. J. Pharmacol.* **393**, 295-314.

Dubnau, J., Grady, L., Kitamoto, T. and Tully, T. (2001). Disruption of neurotransmission in *Drosophila* mushroom body blocks retrieval but not acquisition of memory. *Nature* **411**, 476-480.

Dudley, R. (2000). Evolutionary origins of human alcoholism in primate frugivory. *Q. Rev. Biol.* **75**, 3-15.

Duerr, J. S., Gaskin, J. and Rand, J. B. (2002). Identified neurons

in *C. elegans* coexpress vesicular transporters for acetylcholine and monoamines. *Am. J. Physiol. Cell Physiol.* **280**, 1616-1622.

Beckman, M. L., Parker, J. C., Sheffield, E. B., Whitworth, T. L., Quick, M. W. and Lester, R. A. (2000). Regulation of alpha4beta2 nicotinic receptor desensitization by calcium and protein kinase C. *Mol. Pharmacol.* **55**, 432-443.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA *in Caenorhabditis elegans*. *Nature* **391**, 806-811.

Giorgetti, M. and Zhdanova, I. V. (2000). Chronic cocaine treatment induces dysregulation in the circadian pattern of rats°Æ feeding behavior. *Brain Res.* **877**, 170-175.

Gomez, M., De Castro, E., Guarin, E., Sasakura, H., Kuhara, A., Mori, I., Bartfai, T., et al. (2001). Ca2signaling via the neuronal calcium sensor-1 regulates associative learning and memory in *C. elegans*. *Neuron* **30**, 241-248.

Kalidas, S. and Smith, D. P. (2002). Novel genomic cDNA hybrids produce effective RNA interference in adult *Drosophila*. *Neuron* **33**, 177-184.

Kerr, R., Lev-Ram, V., Baird, G., Vincent, P., Tsien, R. Y. and Schafer, W. R. (2000). Optical imaging of calcium transients in neurons and pharyngeal muscle of *C. elegans*. *Neuron* **26**, 583-594.

Kitamoto, T. (2001). Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive shibire allele in defined neurons. J *Neurobiol.* **47**, 81-92.

Lewohl, J. M., Wilson, W. R., Mayfield, R. D., Brozowski, S. J., Morrisett, R. A. and Harris, R. A. (1999). G-protein-coupled inwardly rectifying potassium channels are target of alcohol action. *Nat. Neurosci.* **2**, 1084-1090.

Martin, J-R., Raabe, T. and Heisenberg, M. (1999). Central complex substructures are required for the maintenance of locomotor activity in *Drosophila melanogaster*. *J. Comp. Physiol. A.* **185**, 277-288.

McClung, C. and Hirsh, J. (1998). Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in *Drosophila*. *Curr. Biol.* **8**, 109- 112.

McClung, C. and Hirsh, J. (1999). The trace amine tyramine is essential for sensitization to cocaine in *Drosophila*. *Curr. Biol.* **9**, 853-860.

Mori, I. (1999). Genetics of chemotaxis and thermotaxis in the *nematode Caenorhabditis elegans*. *Annu. Rev. Genet.* **33**, 399-422.

Morrison, G. E., Wen, Y.J., Runciman, S. and van der Kooy, D. (1999). Olfactory associative learning in *Caenorhabditis elegans* is impaired in Irn-1 and Irn-2 mutants. Behav. Neurosci. **113,** 358-367.

Nikaido, T., Moriya, T., Takabayashi, R., Akigama, M. and Shibata, S. (1999). Sensitization of methamphetamine-induced disorganization of daily locomotor activity rhythm in male rats. *Brain Res.* **845**, 112-116.

Osterwalder, T., Yoon, K. S., White, B. H. and Keshishian, H. (2001). A conditional tissue-specific transgene expression system using inducible GAL4. *Proc. Natl. Acad. Sci. USA* **98**, 12596-12601.

Park, S. K., Sedore, S. A., Cronmiller, C. and Hirsh, J. (2000). PKARII- deficient *Drosophila* are viable but show developmental, circadian and drug response phenotypes. *J. Biol. Chem.* **275**, 20588-20596.

Parr, J., Large, A., Wang, X., Fowler, S. C., Ratzlaff, K. L. and Ruden, D. M. (2001). The inebri-actometer: a device for measuring the locomotor activity of *Drosophila* exposed to ethanol vapor. *J. Neurosci. Methods* **107**, 93-99.

Porzgen, P., Park, S. K., Hirsh, J., Sonders, M. S. and Amara, S. G. (2001). The antidepressant-sensitive dopamine transporter in *Drosophila melanogaster*: a primordial carrier for catecholamines. *Mol. Pharmacol.* **59**, 83-95.

Ranganathan, R., Sawin, E. R., Trent, C. and Horvitz, H. R. (2001). Mutations in the *Caenorhabditis elegans* serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. *J. Neurosci.* **21**, 5871-5884.

Richmond, J. E. and Jorgensen, E. M. (1999). One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. *Nat. Neurosci.* **2**, 791-797.

Risinger, F. O., Freeman, P. A., Rubinstein, M., Low, M. J. and Grandy, D. K. (2000). Lack of operant ethanol self-administration indopamine D2 receptor knockout mice. *Psychopharmacology (Berlin)* **152**, 343-350.

Robinson, T. E. and Berridge, K. C. (2001). Incentive-sensitization and dependence. *Addiction* **96**, 103-114.

Rocha, B. A., Fumagalli, F., Gainetdinov, R. R., Jones, S. R., Ator, R., Giros, B., Miller, G. W., et al. (1998). Cocaine self-administration in dopamine-transporter knockout mice. *Nat. Neurosci.* **1**, 132-137.

Rodan, A. R., Kiger, J. A. and Heberlein, U. (2002). Functional dissection of neuroanatomical loci regulating ethanol sensitivity in *Drosophila*. *J. Neurosci., in press.*

Roeder, T. (1999). Octopamine in invertebrates. *Prog. Neurobiol.* 59, 533-561.

Rosay, P., Armstrong, J. D., Wang, Z. and Kaiser, K. (2001). Synchronized neural activity in the *Drosophila* memory centers and its modulation by amnesiac. *Neuron* **30**, 759-770.

Scholz, H., Ramond, J., Singh, C. M. and Heberlein, U. (2000). Functional ethanol tolerance in *Drosophila*. *Neuron* **28**, 261-271.

Schuckit, M. A. (2000). Genetics of the risk for alcoholism. *Am. J. Addict.* 9, 103-112.

Sora, I., Hall, F. S., Andrews, A. M., Itokawa, M., Li, X. F., Wei, H. B., Wichems, C., et al. (2001). Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc. Natl. Acad. Sci. USA* **98**, 5300-5305.

Spanagel, R., Weiss, F.. (1999). The dopamine hypothesis of reward: past and current status. *Trends Neurosci.* 22, 521- 527.

Stebbins, M. J., Urlinger, S., Byrne, G., Bello, B., Hillen, W. and Yin, J. C. (2001). Tetracycline-inducible systems for *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98**, 10775-10780.

Thomas, J. H. (2001). Nematodes are smarter than you think. *Neuron* 30, 7-8.

Torres, G. and Horowitz, J. M. (1999). Cocaetylene synthesis in Drosophila. *Neurosci. Letters* **263**, 201-204.

Torres, G. and Horowitz, J. M. (1999). Drugs of abuse and brain gene expression. *Psychosomatic Medicine* **61**, 630-650.

Wise, R. A. (2000). Dependence becomes a brain disease. *Neuron* **26**, 27-33.

Wise, R. A. and Bozarth, M. A. (1987). A psychomotor stimulant theory of dependence. *Psychol. Rev.* **94**, 469-492.