Psihologijske teme, 25, 2016 (2), 245-262

Pregledni rad – UDK – 613.83
612.821
159.9:612

From Flies to Humans: Circadian Genes in the Neurogenetics of Addiction

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Abstract

Drug addiction is a persistent brain disease with severe and sometimes fatal consequences. Addictive drugs induce long-lasting neuroadaptations in the functioning of the nervous system and currently there is no efficient pharmacological treatment that can successfully prevent or reverse these changes. As a consequence, addicted individuals often suffer from recurrent relapses, sometimes triggered by environments, situations or stressors that have previously been associated with drug taking.

Behavioral neuroscience uses animal models to understand the neurobiological mechanisms which cause and correlate with the development of addiction, and recently the emphasis is on animals that are genetically tractable, such as mice (*Mus musculus*) and fruit flies, (*Drosophila melanogaster*). In spite of many obvious differences between humans and *Drosophila*, similarities at the genetic level and in the basic neuronal physiology, make *Drosophila* an excellent model organism for the study of many complex human behaviors, including addiction. Discovery that circadian genes influence the development of behavioral sensitization to cocaine in *Drosophila* led to numerous studies about the role of circadian genes in the regulation of drug-induced behaviors in laboratory mammals and humans. Results show that circadian genes are involved in regulating the behavioral and molecular response to different classes of drugs of abuse. Furthermore, studies in humans show the interconnectivity between the regulation of circadian behavior, mental diseases and addiction, and suggest that behavioral interventions aimed at improving the quality of the circadian behavior will be important in prevention and treatment of addictive behaviors.

Keywords: addiction, circadian, model organisms, *Drosophila melanogaster*, genes

Introduction

The idea that neurogenetic studies using fruit fly might help in devising better behavioral treatments for human addicts is not immediately obvious. However, last decade and a half of genetic, neurophysiological and behavioral work identified

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circadian genes as an important genetic factor in the development of addiction, and, as a consequence, has produced results that could help psychologists and other specialists to devise a behavioral treatment to prevent and treat addiction.

Addiction remains a poorly understood brain disease, a consequence of a very complex interaction between numerous environmental and social factors and their effect on brain chemistry and functioning. At the level of the brain, response to drug consumption is determined by the sequence of molecular and neurophysiological changes that are driven by the unique genetic architecture of an individual. Understanding which genes and gene variants influence the cascade of changes in the brain physiology will be important for the understanding of the addicted brain.

This review explains the importance of model organisms in the study of complex behavior such as addiction. The emphasis is both on *Drosophila* - an ideal organism for the forward genetic approaches aimed at identifying genes, and rodents - animals with high similarity in brain anatomy with humans, where the gene functions and mechanisms of action can be further investigated. Numerous genes correlated with addicted behavior have been identified through the use of association studies in humans and genomic techniques in laboratory animals, however, their actual role and function in the neurological changes induced by drug taking are often unclear. In contrast, application of forward genetic approaches in laboratory animals can lead to the identification of genes with clear, significant effect on behavior under investigation.

This review focuses on a group of genes, named circadian genes, whose role in drug-induced behavior has been identified in *Drosophila*. Circadian genes were found to be pleiotropic, as in addition to circadian behavior, they modulate a wide array of cellular and behavioral events that lead to addiction. Ultimately, the work on model organisms led to studies in humans that further emphasized the circadian role, and are now suggesting a direction for behavioral intervention in prevention and treatment. The cure for drug addiction does not exist in spite of decades of research. Use of model organisms for forward genetic approaches, and application of fast-evolving new techniques for measuring neuronal activity, will remain important reductionist approach in tackling this complex brain disease.

Behavioral Aspects of Addiction

In humans, the path from the initial drug taking to the development of addiction has several phases, characterized by a particular neural process being triggered or modified by drug taking. Most often, the initial phase is characterized by a pleasurable experience induced by the drug, as drug acts on the reward centers in the brain (Hyman, Malenka, & Nestler, 2006; Nestler, 2004). In doing so, the drug reinforces drug taking behaviors, and for most drugs, this is mediated by increased activity of dopamine signaling within the reward pathway (Kelley & Berridge, 2002). The change in the dopaminergic activity leads to neuromodulation in the reward

center causing craving, drug-seeking and drug-taking in spite of negative consequences, and over time becomes habit-forming. Relapse is common among addicts who stop taking drugs because drugs cause long-lasting changes by engaging mechanisms of neuronal plasticity, which results in a change in the brain functioning (Russo et al., 2010). In particular, associations between environmental and emotional cues and drug-taking that are formed in the brain are such that an addict learns to connect pleasurable effects of the drug with places or situations. Formation of such associations engages similar types of molecules and networks as associative conditioning, and memories which are formed, i.e. the neuromodulation of the neural networks, are particularly resistant to extinction (Hyman et al., 2006). Because long-term memory is only one form of long-term changes in neuronal functioning induced by addictive drugs, current therapies for addiction, which mostly combine medication with counseling, show only partial short-term success, as the relapse is common and can occur even years after drug taking has ceased.

Animal models enable experimental approaches that are ethically or technically not feasible in humans. Because addiction is a multistep process, it is too complex to be studied in model organism as one behavior. Instead, simpler behaviors, or phenotypes with relevance for the neurophysiology of human addiction, are commonly quantified and studied in the laboratory environment. To this end, scientists have developed a number of administration procedures that enable them to study tolerance, sensitization, dependence, craving, reward or relapse (Sanchis-Segura & Spanagel, 2006).

Drug-induced behaviors in animal models are then used for molecular studies to correlate and causally link changes in the behavior with those at the molecular level (Spanagel & Heilig, 2005). This involves establishing causation between an inactivated or modified version of a gene, or gene product, and related effect on the brain morphology, physiology, and, ultimately, behavior. Currently, there is no other model organism where the ease and sophistication of genetic techniques is present in a small animal with complex behavioral repertoire, as is the case with *Drosophila melanogaster*. This means that neuronal architecture of complex behaviors, such as response to drugs of abuse, or learning and memory, can be investigated, and molecularly and genetically dissected, to get an understanding about how do genes affect neurons, and how do neurons interact to produce a behavior.

One type of drug-induced behavior that has been extensively studied in animal models is behavioral sensitization (Vanderschuren & Pierce, 2010). It refers to the progressive increase in the species-specific behavioral responses to repeated drug administrations, and in rodents it can persist even months after withdrawal, suggesting long-term changes in the neuronal functioning (Vanderschuren et al., 1999). Behavioral sensitization is often induced by psychostimulants, cocaine and amphetamine, which have a pronounced motor-activating component, leading to the increase in the amount of locomotor activity, easily observed and measured in laboratory animals. In humans, the sensitization of the incentive value of a drug is

observed as an increased craving. Because behavioral sensitization represents a relatively simple form of behavior based on long-term changes in the neuronal circuitry, it is akin to changes in the brain functioning induced by repeated drug consumption that ultimately leads to addiction. Behavioral sensitization can be induced even in *Drosophila*, suggesting that neuronal architecture upon which it is based is present even in a much simpler brain that is easier to investigate.

Neurobiological Aspects of Addiction

Understanding the functioning of the brain reward pathway after drug consumption will be essential for the understanding of the neurobiology of addiction. In mammals, particular importance is given to signaling from dopaminergic neurons within the ventral tegmental area to the nucleus accumbens and prefrontal cortex, part of the mesocorticolimbic projections of the brain reward system (Joffe, Grueter, & Grueter, 2014). This system has evolved to promote evolutionary fitness of the organism, and is stimulated with primary rewards like food, sex, and social stimulation. The neurochemical changes which drugs induce in the brain reward system are much more potent than effects of primary rewards, and over time the repeated drug taking triggers long-lasting changes in neuronal functioning, such that the addictive drugs acquire, or even supersede, the attraction for the primary rewards (Robison & Nestler, 2011). Aside from drugs, several addictive behaviors, such as gambling or video games playing, can induce similar chemical changes in the brain reward system, emphasizing the importance of the reward processing in the development of addictions (Murch & Clark, 2015; Yip & Potenza, 2014).

Psychostimulants such as cocaine and methamphetamine are a particularly potent group of addictive drugs, because they strongly affect the balance of dopamine, serotonin, and noradrenaline. Their primary molecular targets are presynaptic plasma membrane monoamine transporters. Psychostimulants lead to inhibition and/or reversal of transporter action which results in prolonged activation of postsynaptic monoaminergic receptors and increased intracellular signaling. Molecular events triggered by the administration of a single dose of psychostimulant initiate a cascade of changes, and primes the brain for following administrations, which can be measured as a long-term change in behavioral response, such as sensitization.

In order to understand the long-term changes in the brain functioning, it is important to understand the changes in the regulation of gene expression, such as transcriptional activation induced by the initial drug taking (Nestler, 2012). Activation of some genes is transient, while others stay active for a long time due to epigenetic modulation of gene transcription (Kalda & Zharkovsky, 2015; Massart et al., 2015). An example of a gene that influences long-lasting molecular changes is a gene DeltaFosB. Levels of DeltaFosB cumulatively increase after each drug exposure, whereupon it acts as a chromatin regulator mediating epigenetic changes

(Robison & Nestler, 2011). This helps to explain how changes in gene expression can persist after drug-taking has ceased, leading to changes in the functioning and morphology of brain cells.

Circadian Genes and Addiction

Drosophila Melanogaster

Studies that used *Drosophila melanogaster* to investigate the genetics of cocaine and ethanol administration show that discoveries made in the fruit fly translate well into mammals, and can lead to important advances in the understanding of addiction. This is possible because of the genetic homology between flies and mammals, including humans, and similar principles of the nervous system functioning. After the sequencing of the fly genome in year 2000, the estimate was that about 75% of human disease genes have related sequences in *Drosophila*, suggesting that flies can be a good model to study function of a wide array of genes involved in human disease (Reiter, Potocki, Chien, Gribskov, & Bier, 2001). Thus, over the last several decades, flies are used not only to study fundamental principles of biological functioning, but increasingly to screen for genes involved in disease-related processes, and to study molecular mechanisms which lead to the development of disease (Pandey & Nichols, 2011). Addiction is only one of many pathological states where *Drosophila* is providing a significant contribution to the understanding of the basic cellular mechanisms (Kaun, Devineni, & Heberlein, 2012).

One important advantage that flies have over other traditional animal models, such as rodents, is that study of the genetic architecture of addiction using behavioral screening is much easier, cheaper and faster. Genetic screen is a method for identifying genes with a function in certain cellular process or behavior. It entails screening thousands of individuals with mutations randomly induced in their genomes in order to identify one or more individuals with significant changes in behavior. The animal is then studied molecularly to understand the causal link between mutation and behavior. This approach is often called forward genetics (from phenotype to gene), and it provides information about gene causation, instead of correlation, that is often gained from genomic studies. Because genetic screening can involve behavioral testing of thousands of individuals, the time and cost for a study in rodents is prohibitive, while it is manageable in flies.

In 1998 Jay Hirsh, and his collaborator Coleen McClung, showed that when a group of flies contained in a vial is exposed to volatilized form of cocaine (crack), it induces behaviors similar to those of mammals (McClung & Hirsh, 1998). Flies were exposed to cocaine, their behavior was video-recorded for 5 minutes, and then scored upon subsequent viewing. Administration of cocaine to drug-naive flies led to dose-dependent changes in behavior that ranged in intensity, from grooming, increased

locomotion, to stereotypic and unusual uncoordinated behaviors, spasms, and death at high doses. The response to a single dose was sex-dependent, and given the same dose, males were more affected. However, both sexes responded more strongly to the second dose of cocaine when given more than 6 hours, but less than 48 hours apart. Such increase in the intensity of behavioral response to the same dose is termed behavioral sensitization, and has previously been extensively studied in rodents.

Observation that cocaine induces behavioral sensitization in flies was important for several reasons. First, it showed that a relatively simple nervous system can produce similar type of behaviors as a much more complex nervous system of mammals. Second, it showed that, as in mammals, cocaine induces relatively long-lasting changes in the brain chemistry of the flies. Third, because of the advantages of fly genetics, this enabled genetic dissection of addictive behavior.

The unexpected finding was that three circadian genes - period (PER), Clock (CLK) and cycle (CYC), are necessary for development of behavioral sensitization to cocaine in *Drosophila*, as flies with mutations in those genes do not develop behavioral sensitization to repeated administration of cocaine (Andretic, Chaney, & Hirsh, 1999). Cocaine induces hyperactive behavior in circadian mutants, indicating that the circadian genes do not regulate behavioral responses to a single dose. Nevertheless, multiple doses do not induce behavioral sensitization, demonstrating that neuroplastic changes caused by multiple drug exposures depend on circadian genes. Circadian genes are transcriptional modulators and their presence is essential for circadian regulation of many physiological processes, such as metabolism and behavior. However, this was the first demonstration that they have a dedicated role in a non-circadian behavior.

Another unexpected finding was that behavioral sensitization to cocaine does not involve the presence of the circadian gene timeless (TIM) (Andretic et al., 1999). The circadian regulation of molecular timing system involves physical interaction through PAS interaction domain; between PER and TIM proteins acting as a transcriptional repressors, and CLK and CYC proteins acting as transcriptional activators. Since TIM flies develop normal behavioral sensitization, this suggests that the role that circadian genes play in the development of addiction is separate from their role in the regulation of circadian clock. Thus, in the regulation of drug-induced behaviors, circadian proteins PER, CLK and CYC likely interact with other proteins. This is not a uniqueness of fly behavior, as later studies in laboratory mammals verified the importance of PER, CLK, and CYC, but not TIM, in the regulation of several behaviors induced by different classes of addictive drugs (Rosenwasser, 2010). However, the identity of proteins that interact with circadian products remains elusive.

Current obstacle in our understanding of the molecular mechanisms underlying the development of behavioral sensitization lies in the lack of a high throughput test for behavioral sensitization in flies. The method which was used in the past is laborious and time–consuming, and does not allow for testing of large numbers of

flies in a genetic screen. An alternative method, based on the loss of the vertical climbing ability, was successfully used to identify new genes regulating sensitivity to a single dose of cocaine, but does not measure sensitization to repeated cocaine exposures (Heberlein, Tsai, Kapfhamer, & Lasek, 2009). The advancement in the understanding of the addiction-related behavior in part depends on the ability to design new tests to measure drug-related quantifiable phenotypes in model organisms. Behavioral tests for *Drosophila* have to satisfy similar requirements as human psychological tests. Ideal test should be objective (free of subjective judgment), reliable (results should be consistent between different trials), valid (it should measure the intended process), practical (allow for testing of large numbers of flies in behavioral screens), and it should be normative (distinguish the level of response between wild type and mutant flies).

Rodents

The report that certain circadian genes modulate behavioral sensitization in *Drosophila* sparked a series of studies in rodents to determine if circadian genes are similarly pleiotropic in mammals. Those studies indicated that: a) mice with mutations in circadian genes have changed behavioral responses to different drugs of abuse, b) exposure to addictive drugs changes the expression of circadian genes in different brain areas, and c) the interaction between circadian genes and addictive drugs involves dopaminergic system.

The first follow-up study that investigated the behavioral response of mPer1 and mPer2 mutant mice to cocaine injections showed that, as in flies, mPer genes modulate drug-induced behavior. mPer1 mutant mice, similar to PER mutant flies, do not sensitize to repeated cocaine injections, while surprisingly, Per2 mutants showed excessive sensitization (Abarca, Albrecht, & Spanagel, 2002). In a *Conditioned Place Preference test*, which measures reinforcing effects of cocaine, the study showed that mPer1 mutants do not perceive cocaine as rewarding, while mPer2 mice behave as wild-type controls. Overall, this indicated that PER proteins have conserved roles in the regulation of addiction-related behaviors in mammals. The diversity of phenotypes arising from mutations in different mammalian versions of mPer genes suggests the specificity of action acquired during duplications of the original PER gene. Follow-up studies showed that PER genes are involved in processing reward value of cocaine, a characteristic related to the addictive potential of the drug (Lynch, Girgenti, Breslin, Newton, & Taylor, 2008; McClung et al., 2005; Yuferov et al., 2003).

Probably no other circadian mouse mutant is better analyzed than mClock, where an array of aberrant responses to cocaine was linked to changed dopaminergic transmission. mClock mutants are hyperactive, show increased behavioral sensitization to cocaine, increased cocaine preference in *Conditioned Place Preference*, and an increase in the reward value of cocaine measured by intracranial

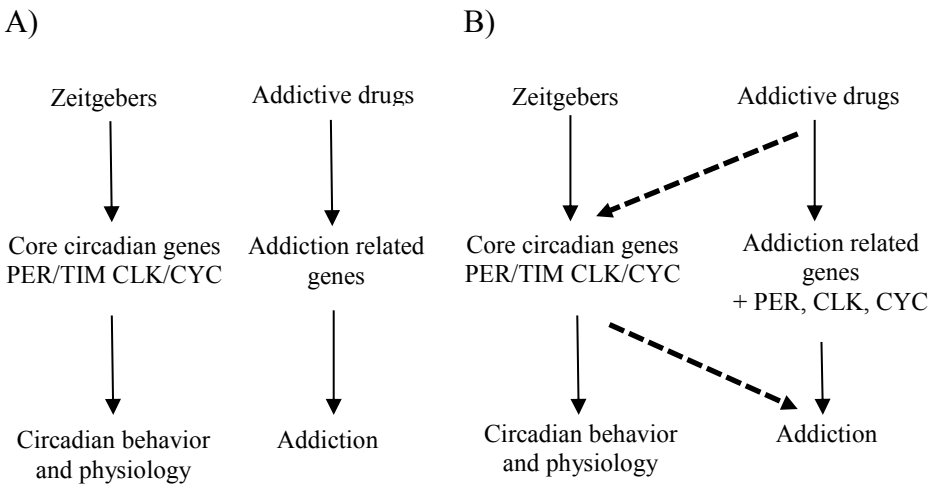
self-stimulation (McClung et al., 2005; Roybal et al., 2007). These behavioral phenotypes have been linked to several indicators of increased activity of dopaminergic system (McClung et al., 2005; Roybal et al., 2007). As the lack of a functional copy of CLK protein leads to changes in dopaminergic function, this suggests that mClk is involved in the regulation of dopaminergic system. Further support is the finding that activation of D1-type dopamine receptor leads to transcriptional activation of mPer, mClk, and mCyc genes, suggesting an important interplay between drugs, dopamine, and circadian genes (Imbesi et al., 2009; Uz et al., 2005). Thus, dopaminergic receptors are involved in the transcriptional response of circadian genes after drug administration, while circadian genes can modulate the activity of the dopaminergic system. These studies confirm that some circadian genes have both circadian function in modulating the activity of a dopaminergic system during 24 hours, and non-circadian in regulating the activity of dopaminergic system in response to drugs. Similar links between PER gene and dopaminergic system have also been observed in *Drosophila* (Andretic et al., 1999; Andretic & Hirsh, 2000). This further emphasizes the homology at the level of genes and function between invertebrates and mammals.

Circadian genes are induced not only by cocaine, but by other classes of drugs, suggesting a universal role in mediating effects of various drugs of abuse (Falcon, Ozburn, Mukherjee, Roybal, & McClung, 2013; Piechota et al., 2012; Uz et al., 2005; Wang et al., 2006; Yuferov et al., 2003). In particular, mPer genes have been linked with behavioral phenotypes related to alcohol consumption in rodents (Gamsby et al., 2013; Spanagel, Rosenwasser, Schumann, & Sarkar, 2005), while molecular studies have indicated the importance of mPer genes in the regulation of glutamatergic system in response to alcohol (Spanagel, Pendyala et al., 2005).

Humans

The involvement of circadian genes in the modulation of drug-induced behaviors in animals sparked a number of studies in humans, and they indicate that circadian system affects brain areas involved in addiction through two possible mechanisms (Figure 1). One is through indirect action of the circadian clock in the brain on neural or chemical components that regulate drug responses, resulting in the time-of-day variability. An example of such influence is circadian variability in several features of addiction, such as sensitivity to the drug, drug intake, and craving for the drug (Chandra, Scharf, & Shiffman, 2011; Sleipness, Jansen, Schenk, & Sorg, 2008; Sleipness, Sorg, & Jansen, 2007a, 2007b).

Figure 1. *Circadian Behavior and Addiction Share Common Genetic Elements*



Note: A) Circadian behavior and addiction used to be viewed as distinct behaviors regulated by distinct inputs, genes, and mechanisms. Zeitgebers, such as light and food, synchronize activity of the circadian genes resulting in synchronized circadian behavior and physiology. Consumption of addictive drugs can over time lead to the development of addiction by modulating the transcriptional activity of addiction-related genes involved in the neuromodulation of neuronal function. B) Recent discoveries suggest interdependency of addiction and circadian behavior through the effect that addictive drug consumption has on circadian genes. The circadian clock in the brain, and core circadian genes that regulate the clock, can indirectly affect neural or chemical components which regulate drug responses in order to result in time-of-day variability such as sensitivity to the drug, drug intake, and craving for the drug. Alternatively and/or concomitantly, consumption of addictive drugs affects circadian genes that function outside the circadian clock regulation. In this case, circadian genes together with other addiction-related genes regulate neuronal plasticity that leads to addiction-related behaviors. Disrupted circadian behavior increases the risk for developing addiction as do polymorphism in circadian genes.

Direct interaction between addiction and circadian system is through the action of circadian genes on non-circadian functions. In this way, circadian genes are a part of an orchestrated genetic response triggered by drug taking. In flies and mice, mutations in circadian genes disrupt drug-induced phenotypes, while association studies in humans point to changes in the gene sequence, or polymorphisms, and occurrence of particular behaviors. Polymorphisms, such as Single Nucleotide Polymorphism (SNP), can sometimes change the properties of a protein that the gene codes for, and consequently the phenotype that the protein influences. Humans have three variants of Period gene (hPer), and polymorphism in each one has been associated with alcohol addiction. SNP in the promoter region of hPer1 gene is associated with frequency of heavy drinking in adolescents under psychological stress and with alcohol dependence in adults (Dong et al., 2011). A different SNP in

the coding region of hPer2 gene is associated with stressful life events and drinking, and with sleep problems (Blomeyer et al., 2013; Comasco et al., 2010). A polymorphism in the promoter region of hPer3 gene regulates the abundance of hPer3 gene transcript in response to alcohol and stress (Wang et al., 2012), and a repeat polymorphism in the same gene is associated with insomnia problems in alcohol-dependent patients and heroin addiction (Brower, Wojnar, Sliwerska, Armitage, & Burmeister, 2012; Zou et al., 2008). In human studies, unlike some of those performed on laboratory animals, the causative role cannot be established, and the associations remain at the level of correlation.

Further evidence for the involvement of circadian system in addiction comes from studies showing that disruption of the circadian clock increases the risk for addictive behavior. Such is the case with people with "social jetlag", individuals who have significantly different sleeping schedule between workdays versus weekends. People suffering from "social jetlag" have increased risk for substance abuse and mood disorders (Foster et al., 2013; Kervran et al., 2015). Other disruptions of sleep/wake rhythm can also increase the risk of alcohol-related problems (Crum, Ford, Storr, & Chan, 2004; Crum, Storr, Chan, & Ford, 2004). In general, the risk of addiction is aggravated when circadian disruptions co-occur with mental disorders, commonly with bipolar disorder and depression, suggesting common neuronal mechanisms of those pathologies (Adan, 1994; Foster et al., 2013).

Circadian gene polymorphisms can influence lifestyle preferences, and this can increase the risk for other pathologies. Human chronotypes are associated with polymorphism in gene hClk and all three hPer genes, and refer to preference for activity in the morning hours (Early Chronotype), versus evening (Late Chronotype) (Carpen, Archer, Skene, Smits, & von Schantz, 2005; Carpen, von Schantz, Smits, Skene, & Archer, 2006; Johansson et al., 2003). In some cases, this leads to the inability to synchronize behavior with natural light/dark cycle, as in cases where time-at-work is significantly different from the chronotype. People with Late Chronotype often suffer from "social jetlag" because of the disparity between their preferred time of activity and the one set by society. A chronotype preference changes with aging, and adolescent population shows a natural shift toward Late Chronotype and higher risk for mood and addiction disorders (Hasler, Sitnick, Shaw, & Forbes, 2013). "Social jetlag" aside from leading to increased risk for addiction and mood disorder can increase the risk of certain health problems (Kantermann et al., 2013; Parsons et al., 2015).

The relationship between circadian clock and addiction is bidirectional, where disruptions of the circadian behavior predispose to addiction, while consumption of addictive drugs leads to circadian disruptions. Studies in laboratory animals have shown that different drugs induce expression of circadian genes in the SCN (a nucleus in the brain which regulates circadian behavior) (Brager, Stowie, Prosser, & Glass, 2013; Glass, Brager, Stowie, & Prosser, 2012), and outside the SCN, in areas involved in addiction (Falcon et al., 2013). Increased gene expression in the SCN can

lead to disrupted molecular cycling of circadian genes, resulting in disrupted circadian behavior, which further increases the risk for development of addiction. Induction of circadian genes in the reward regions (Webb et al., 2009; Wongchitrat, Mukda, Phansuwan-Pujito, & Govitrapong, 2013) can modulate motivation to consume drugs likely through the effect on dopaminergic signaling. Furthermore, circadian disruptions can sometimes persist after the drug consumption has ceased, thus contributing to relapse (Spanagel, Rosenwasser et al., 2005; Zhabenko, Wojnar, & Brower, 2012), or sometimes drug withdrawal can induce circadian disruptions, and in this way further decrease the probability of successful recovery (Li et al., 2009). Thus, a circadian gene polymorphism that a person is born with can increase or decrease the risk of becoming addicted, both in terms of circadian gene action in the SCN (chronotype), and in terms of action outside SCN (motivation for the drug and risk for mental disorder).

From current studies it is clear that circadian genes constitute an integral part of the brain response to drug taking; however, the relationship between the circadian molecular clock in the brain, circadian gene activation by drugs, and addiction, are only beginning to emerge. In cases of co-morbidity of addiction with mood disorders, a common genetic element that emerges are circadian genes as well. It remains to be determined in what degree do circadian genes affect addiction independently from the circadian clock, and how much does the circadian clock in the SCN, or more often its disruption, predispose a person to start, or continue, taking drugs.

The evidence that many risk factors for addiction in humans can be linked to genetic factors does not exclude the importance of the environment. The basic science research hopes to deliver an understanding of the basic genetic and neurobiological principles that contribute to the development of addiction. Such knowledge is then the basis for the development of specific and efficient pharmacological treatments for addiction. However, as the understanding of the role of circadian genes in addiction is growing, this points to a non-pharmacological approach that can be effective in preventing or treating addiction: modification of behavior. This refers to a number of behavioral interventions that can improve circadian rhythmicity and lead to improved synchronization of the circadian behavior to light/dark cycle, improved sleep hygiene and better sleep quality. As the co-morbidity of addiction with other mental disorders is high and is associated with disruption of circadian behavior, interventions aimed at improving circadian rhythmicity and synchronization might be helpful not only for addiction, but for improving the symptoms of mental disorders in general.

Conclusions

The understanding of the basic neural processes that govern complex human behaviors and disorders depends on the availability of the good model organisms for the basic research. This review presented an example of discoveries made in a model organism, *Drosophila melanogaster*, which influenced research in rodents and then in humans. The initial observation in flies that some circadian genes modulate behavioral responses to cocaine resulted in studies in mammals, showing that circadian genes are a part of a common pathway shared by many drugs of abuse. Discoveries that have been made give suggestions about potential modifications of behavior that can lower the risk of developing addiction and help in the treatment of addicted individuals.

The main points of this review are:

1. Use of model organisms, such as *Drosophila*, is essential in forward genetic approaches aimed at identifying genes that regulate particular behavior. Discovery that several circadian genes modulate behavioral sensitization to cocaine in *Drosophila* led to studies in mammals. Studies indicate that circadian genes are involved in the regulation of neuromodulation triggered by drug consumption which leads to the addicted brain.
2. Studies in rodents have indicated that circadian genes act as modulators of the dopaminergic system. There is a two-way interaction between circadian genes and dopaminergic system. Drugs induce transcriptional activation of circadian genes acting on dopaminergic receptors, and in turn, circadian genes can modulate dopaminergic system.
3. Association studies in humans further emphasize the role that circadian genes play in addiction. Epidemiological studies emphasize the importance of a well-adjusted circadian behavior in preventing addiction. Together, such studies suggest that behavioral modifications directed toward improving circadian functioning might help in prevention and treatment of addiction.
4. The mechanism of action of circadian genes in addiction remains unknown, as does the sequence of molecular events in which they participate to cause a long-term change in the nervous system functioning. Future studies will require the identification of new genes and their roles in this process. No other model organism possesses as many genetic advantages to do that as does *Drosophila melanogaster*. Its size is sufficiently small to allow for testing of large numbers of individuals in behavioral screens, and its brain is complex enough to endow flies with adaptive behavior. As the type of genetic answer we get in large part depends on the type of genetic test we use, it is important to design new tests for drug–induced behaviors. The promise of *Drosophila* as a model organism par excellence would certainly

be further promoted by devising a test to measure the rewarding potential of addictive drugs and demonstrating that psychostimulants act rewarding on *Drosophila*'s brain. Meantime, a high-throughput test for behavioral sensitization would allow for a behavioral screen to identify new genes in the circadian gene pathway.

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Od mušica do ljudi: Cirkadijalni geni u neurogenetici ovisnosti

Sažetak

Ovisnost je o drogama kronična bolest s teškim, nerijetko fatalnim posljedicama. Opojne droge izazivaju dugotrajne neuroadaptivne promjene u funkcioniranju živčanog sustava, a ne postoji učinkovit lijek koji bi ih suzbio ili ispravio. Ovisnici stoga pate od opetovanih recidiva, ponekad izazvanih okolinom, situacijom ili stresom koji je i bio povezan s uzimanjem droge.

Bihevioralna neuroznanost koristi modelne organizme za razumijevanje neurobioloških mehanizama koji uvjetuju ili koreliraju s razvojem ovisnosti, a odnedavno naglasak je na životinjama koje su genetski pogodne, kao miš (Mus musculus) ili vinska mušica (Drosophila melanogaster). Unatoč mnogim očitim razlikama između ljudi i Drosophile sličnosti na genetskoj razini i u području bazične neuronske fiziologije čine Drosophilu izvrsnim modelnim organizmom za izučavanje mnogih složenih ljudskih ponašanja, uključujući ovisnost. Otkriće da cirkadijalni geni kontroliraju razvoj bihevioralne senzitizacije na kokain kod Drosophile potaklo je niz istraživanja o ulozi koju cirkadijalni geni imaju u ponašanjima induciranim kokainom kod laboratorijskih životinja i ljudi. Rezultati pokazuju da cirkadijalni geni reguliraju ponašanja i molekularne odgovore na razne vrste opojnih droga. Štoviše, istraživanja na ljudima upućuju na međupovezanost između cirkadijalnog ponašanja, mentalnih oboljenja i ovisnosti, te sugeriraju da bihevioralni zahvati usmjereni na poboljšanje cirkadijalnog ponašanja mogu biti važni u prevenciji i liječenju ovisničkog ponašanja.

Ključne riječi: ovisnost, cirkadijalnost, modelni organizmi, vinska mušica (Drosophila melanogaster), geni

De moscas a humanos: Genes circadianos en la neurogenética de la adicción

Resumen

La drogadicción es una de las enfermedades crónicas con consecuencias graves, a veces fatales. Las drogas adictivas causan cambios neuroadaptivos a largo plazo en el funcionamiento del sistema nervioso y de momento no existe un medicamento eficiente que lo reprimiría o corregiría. Por eso los drogadictos sufren repetidas recaídas, a veces causadas por el entorno, situación o estrés previamente relacionado con el consumo de drogas.

Neurociencia conductual usa organismos modelos para comprender mecanismos neurobiológicos que condicionan o se correlacionan con el desarrollo de la adicción, y recientemente el enfoque está en los animales genéticamente convenientes, como ratón (Musmusculus) o mosca del vinagre (Drosophila melanogaster). A pesar de las numerosas diferencias obvias entre los humanos y las moscas del vinagre, la semejanza al nivel genético y aquel de fisiología neuronal básica convierten dicha mosca del vinagre en un excelente organismo modelo para estudiar muchas conductas humanas complejas, incluida la adicción. Al descubrir que los genes circadianos controlan el desarrollo de la sensibilización de comportamiento de la mosca del vinagre en cuanto a la cocaína, se provocó toda una serie de investigaciones sobre el papel que tienen los genes circadianos para los comportamientos inducidos por la cocaína tanto en los animales de laboratorio, como en los humanos. Los resultados indican que los genes circadianos regulan comportamientos y respuestas moleculares a diferentes tipos de drogas adictivas. Es más, las investigaciones en los humanos muestran la interconexión entre el comportamiento circadiano, enfermedades mentales y adicción, y proponen que las intervenciones en el comportamiento enfocadas en la mejora del comportamiento circadiano pueden ser importantes en la prevención y tratamiento de conductas adictivas.

Palabras claves: adicción, circadiano, organismos modelos, mosca del vinagre, genes

Primljeno: 16.05.2016.