

Drugs, flies, and videotape: the effects of ethanol and cocaine on *Drosophila* locomotion

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Drosophila melanogaster has been introduced recently as a model organism in which to study the mechanisms by which drugs of abuse change behavior and by which the nervous system changes upon repeated drug exposure. Surprising similarities between flies and mammals have begun to emerge at the behavioral, neurochemical and molecular levels.

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Abbreviations

3IY	3-iodotyrosine
CNS	central nervous system
PKA	protein kinase A
TDC	tyrosine decarboxylase
VNC	ventral nerve cord

Introduction

Drugs of abuse have well-studied effects on locomotion in mammals. In general, low doses stimulate locomotion, whereas higher doses induce repetitive movements called ‘stereotypies’ and/or sedation. Repeated drug administration can lead to the development of behavioral sensitization, which is manifested as an increased response to the drug, or tolerance, which is characterized by a diminished response. Both the stimulant effects of drugs and their ability to induce behavioral sensitization have been proposed to model the positively reinforcing or rewarding properties of the drug [1,2].

Although not universally accepted, there is significant experimental evidence that supports this hypothesis: at least some of the molecules, neurochemicals and neuro-anatomical loci involved in positive reinforcement and reward are also involved in drug-induced locomotor stimulation and behavioral sensitization. For example, it is well known that mesolimbic dopamine systems are involved in drug reinforcement and reward [3]; these systems are also involved in the simpler motor responses induced by acute and repetitive drug administration [4,5]. It is therefore likely that insights into certain aspects of drug addiction can be obtained by studying these less complex, drug-induced changes in locomotion.

Here we review recent studies that aim to develop *D. melanogaster*, with its accessibility to genetic, molecular

and behavioral analysis, as a model system in which to study the mechanisms underlying drug-induced modulation of locomotor behavior [6,7].

Effects of ethanol on *Drosophila* locomotion

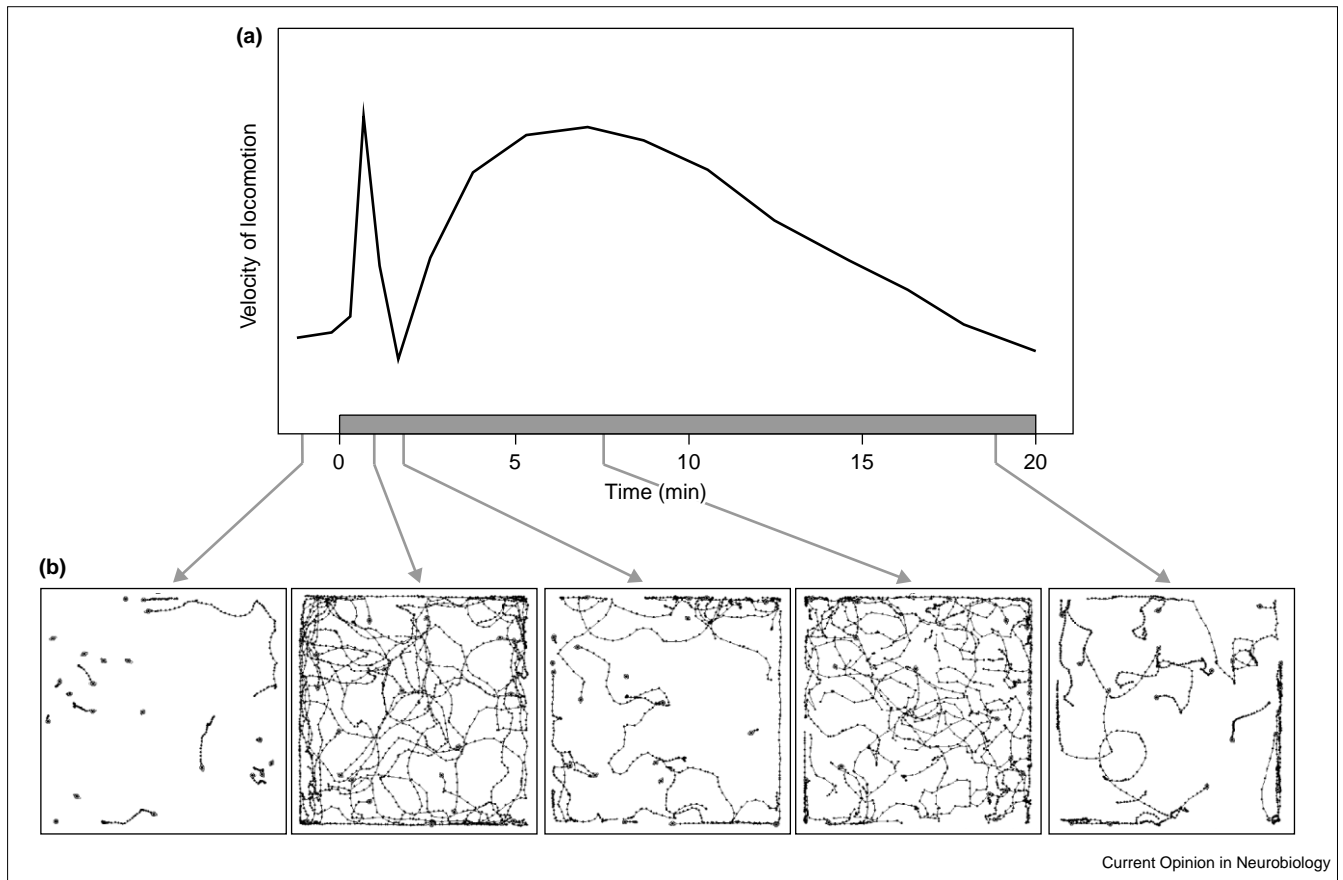
Exposing flies to ethanol vapor leads to immediate and marked changes in walking behavior, and several assays have been used recently to document these changes. These include a simple line-crossing assay [8•], an automated beam-breaking assay [9] and a video-tracking system that allows continuous monitoring of locomotion [10•,11]. Regardless of the assay used, the general conclusion is that, as in mammals, low doses of ethanol stimulate fly locomotion, whereas high doses depress it.

Intoxicated flies also change the direction of their movement more often than do sober flies [8•]. The full range of ethanol-induced behaviors is laid out temporally when flies are exposed to moderate doses of ethanol: as internal ethanol concentrations rise with time, the initial increase in locomotion is followed by incoordination, loss of postural control, and eventually sedation and immobility [8•,9,12•].

A more complex and multiphasic response to ethanol has been revealed with the video-tracking system, which can monitor fly position at 100-ms intervals. Immediately on initiation of the flow of ethanol vapor, flies respond with a pronounced and transient increase in walking speed (Figure 1). This transient hyperlocomotion is probably a response to the smell of ethanol, as it is abrogated by the removal of the third antennal segment [11], which contains most of the fly’s olfactory neurons [13]. As ethanol begins to accumulate, the flies enter a second more prolonged phase of hyperactivity that lasts several minutes (the kinetics depend on the ethanol dose used). Finally, flies gradually slow down, fall on their backs and become unable to right themselves. If ethanol exposure is discontinued at this stage, flies recover apparently normal behavior in 5–10 min.

Interestingly, the ethanol concentrations that stimulate (or disinhibit) and depress locomotion in flies — roughly 20 mM and 45 mM, respectively, in the flies — are very similar to those that have the same behavioral effects in mammals and in humans who do not drink [8•]. Flies have homologs of most genes whose products are affected by ethanol, including the NMDA (*N*-methyl-D-aspartate) and GABA_A (γ-aminobutyric acid type A) receptors, and various K⁺ and Ca²⁺ channels [14]. Whether the fly proteins are also sensitive to ethanol and, if so, mediate the behavioral effects of the drug, remains to be studied.

Figure 1



Effects of ethanol on *Drosophila* locomotion (a) Representation of the locomotor velocity of wild-type flies during an exposure to a moderate dose of ethanol (ethanol exposure period is shown by the grey horizontal

bar). (b) Computer-generated traces of the locomotor behavior of a group of 20 flies before and during exposure to ethanol vapor. Each panel corresponds to a 10-s time period recorded at the times indicated in (a).

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Role of dopamine in ethanol-induced locomotor stimulation

In the adult fly central nervous system (CNS), dopamine is synthesized by a small number of cells that are scattered throughout the brain and ventral nerve cord (VNC) [15]. Flies in which dopamine synthesis is reduced with the tyrosine hydroxylase inhibitor 3-iodotyrosine (3IY) [16] show a significant impairment in locomotor stimulation induced by ethanol [17].

Similarly, genetic ablation of neural activity in dopaminergic and serotonergic neurons (using targeted expression of tetanus toxin) reduces the level of ethanol-induced locomotor activity (K Woo and U Heberlein, unpublished data). Thus, as in mammals, dopaminergic neurotransmission contributes to ethanol-stimulated locomotor activity in *Drosophila*. It will be interesting to determine whether dopaminergic systems are also involved in reward-oriented behavior in flies, such as sucrose-rewarded learning, as they are in mammals [18].

Role of the cAMP pathway in ethanol-induced behaviors

A forward genetic screen for *Drosophila* mutants with altered ethanol sensitivity carried out by Moore *et al.* [19]

led to the discovery that *amnesiac*—a gene involved in olfactory learning and memory [20] that encodes a putative neuropeptide thought to activate the cAMP pathway [21]—regulates the effects of ethanol on postural control, as measured in the inebriometer. The inebriometer consists of a 4-foot long vertical column containing a series of sloping mesh baffles on which flies can stand. Flies are introduced into the top of the column; upon circulation of ethanol vapor, the flies lose postural control and begin to fall through the column. Sensitivity to intoxication correlates with the time required to elute the flies from the column at a particular concentration of ethanol [22, 23]

Mutations in the calcium/calmodulin-sensitive adenylate cyclase gene *rutabaga* and the main catalytic subunit of protein kinase A (PKA) also cause increased sensitivity to ethanol, as does pharmacological inhibition of PKA [19]. By contrast, flies with a mutation in PKA-R11, one of the regulatory subunits of PKA, are resistant to the effects of ethanol in a climbing assay [24]. Notably, mice with a targeted disruption in the homologous PKA-R11 β gene are resistant to the sedative effects of ethanol; these mice also voluntarily consume more ethanol [25].

Taken together, these studies clearly link the cAMP pathway to ethanol responsiveness in flies and rodents. The relationship between specific alterations in the pathway and various ethanol-induced behaviors is not simple, however, which probably reflects the complex regulation of the signal transduction pathway itself, as well as complex interactions among neuroanatomical loci where cAMP signaling regulates behavior.

Neuroanatomical loci regulating ethanol-induced behaviors

Rodan *et al.* [10•] have taken an unbiased approach to define regions of the brain and, eventually, the neural circuits where cAMP signaling may regulate ethanol-induced behaviors. By using the GAL4/UAS gene expression system [26], these researchers targeted the expression of a PKA inhibitor to different brain regions using a collection of GAL4 lines with diverse expression patterns in the CNS (see <http://www.fly-trap.org/>). Of nearly 70 GAL4 lines tested, only 3 showed a specific alteration of ethanol sensitivity in the inebriometer; two of these three lines also showed a delay in sedation when assayed in the locomotor tracking system. Thus, different behavioral effects of ethanol — loss of postural control and locomotor sedation — seem to be regulated by separable (yet overlapping) brain regions. Another important conclusion from this study is that disruption of PKA function in only a few brain regions alters the fly's sensitivity to ethanol.

Although clearly not the only cells involved in the regulation of ethanol-induced behaviors, a group of neurosecretory cells located in the pars intercerebralis are the most promising candidates defined by this study. These cells are known to express different neuropeptides in *Drosophila* and in other flies, and to project axon terminals to the ring gland — the endocrine gland of flies [27]. Interestingly, an enhancer trap insertion in the *amnesiac* gene, which presumably reports the activity of the *amnesiac* gene, is strongly expressed in the ring gland. It is therefore tempting to speculate that a functional connection exists between the pars intercerebralis neurosecretory cells and *amnesiac* in the regulation of ethanol sensitivity.

One of the GAL4 lines that caused a PKA inhibitor-dependent change in ethanol induced loss of postural control and sedation is strongly expressed in the mushroom bodies — prominent brain structures that are involved in olfactory conditioning [28]. This observation, together with the fact that several olfactory learning and memory mutants such as *amnesiac*, *rutabaga* and *fasciclin II* alter ethanol sensitivity [19,29], motivated an analysis of the role of mushroom bodies in ethanol sensitivity. Hydroxyurea-induced ablation of the mushroom bodies, a procedure that completely abolishes olfactory conditioning [30], did not affect ethanol sensitivity in the inebriometer [10•]. Thus, despite the overlap among the genes involved in regulating ethanol sensitivity and olfactory conditioning, the neural circuitry underlying these behaviors is distinct.

Ethanol tolerance

As shown by Scholz *et al.* [12•], even a single exposure to ethanol makes flies more resistant to a second challenge applied several hours later. This tolerance is manifested as a delay in ethanol-induced loss of postural control (in the inebriometer), or a delay in sedation (in the locomotor tracking system), and dissipates in about 24 hours. As the kinetics of ethanol accumulation during the first and second exposure are indistinguishable, this tolerance is, by definition, functional and probably relies on neuroadaptive mechanisms. Consistent with this hypothesis is the finding that tolerance development requires the structural and functional integrity of specific neurons of the central complex. Notably, these central complex neurons also regulate the patterns of spontaneous locomotion in flies [31].

The development of tolerance also relies on octopaminergic systems: flies mutant for tyramine- β -hydroxylase, an enzyme required for the synthesis of octopamine [32], display reduced tolerance. The residual tolerance must involve additional mechanisms, as tyramine- β -hydroxylase flies seem to be completely devoid of octopamine. In rodents, intact noradrenergic systems are required for tolerance development [33,34]. Because octopamine in invertebrates is thought to be the functional homologue of noradrenaline in vertebrates [35], these data highlight interesting similarities among the mechanisms of ethanol tolerance in flies and mammals.

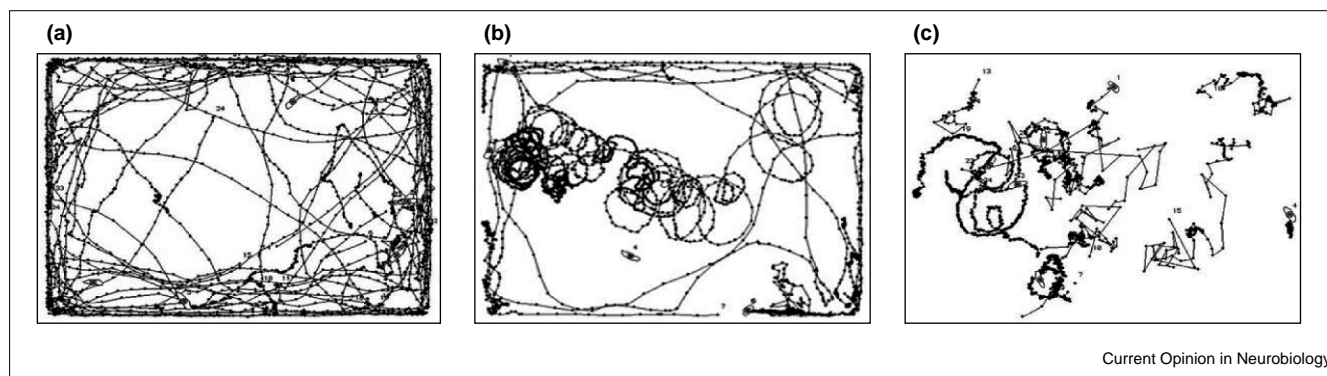
Cocaine-induced behaviors and the role of dopamine

McClung and Hirsh [36] showed that exposure to free-base cocaine, which is volatilized off a heated filament, induces a range of unusual behaviors in flies: low doses induce continuous grooming; intermediate doses lead to circling and other aberrant walking behavior; and high doses cause fast and uncontrolled movements, and eventually akinesia and even death (Figure 2). Continuous grooming and circling — behaviors referred to as 'stereotypies' — are also observed in rodents on administration of cocaine.

Bainton *et al.* [17•] showed that interference with dopamine synthesis through administration of the inhibitor 3IY reduces the effectiveness of cocaine in flies. In addition, the application of cocaine solution directly to the VNC of decapitated flies induces grooming and aberrant locomotion, an effect that is blocked by prior administration of a dopamine D1 receptor antagonist [37]. Similar behavioral effects are seen in the same decapitated fly preparation after the direct application of monoamines or dopamine receptor agonists [38].

The finding by Li *et al.* [39•] that inhibition of synaptic transmission in dopaminergic and serotonergic neurons leads to cocaine hypersensitivity is therefore surprising. As these neurons are silenced throughout development, compensatory adaptations such as hypersensitivity of the postsynaptic receptors may increase the behavioral effects

Figure 2



Computer-generated traces of the locomotor behavior of a group of five flies exposed to volatilized free-base cocaine. Each panel corresponds to a 1-min period starting 2 min after the end of the cocaine exposure. (a) Mock exposure; (b) exposure to 100 µg of cocaine; (c) exposure to 200 µg of cocaine.

of cocaine administration. The latter is supported by the finding that direct application of the dopamine receptor agonist quinpirole to the VNC of these flies induces an enhanced locomotor response [39•].

Taken together, these results indicate that dopaminergic systems mediate cocaine-induced behaviors in flies, probably through the direct inhibition of the monoamine transporters by cocaine [40–42]; the reuptake transporters for catecholamines are well-established targets of cocaine in mammals [43].

Cocaine sensitization

As shown by McClung and Hirsh [36], repeated exposure to cocaine makes flies increasingly sensitive to the behavioral effects of the drug. This behavioral sensitization takes time to develop (it is strongest roughly 6 hours after the first exposure) and is long lasting (dissipating about 2 days after a single exposure). The trace amine tyramine, which is synthesized from tyrosine by tyrosine decarboxylase (TDC), has been implicated in cocaine sensitization [44]. Flies carrying the *inactive* mutation have reduced TDC activity and concentrations of tyramine. Although these flies show normal initial sensitivity to cocaine, they fail to sensitize to subsequent drug exposures—a defect that can be rescued by tyramine, but not by tyrosine or octopamine feeding. The same study shows that after cocaine exposure TDC activity increases with the same kinetics as behavioral sensitization.

The recent cloning of mammalian G-coupled receptors that respond specifically to trace amines such as tyramine [45,46], suggests that tyramine may act as a neurotransmitter and/or modulator in its own right in mammals. Notably, one of these mammalian receptors also can be activated directly by amphetamine [46]. It will be interesting to determine whether this receptor is involved in cocaine sensitization in rodents.

Circadian rhythms and cocaine sensitization

Repeated cocaine exposure sensitizes not only the behavioral response to cocaine, but also the responsiveness of decapitated flies to application of the dopamine D2 receptor agonist quinpirole to the nerve cord. Andretic *et al.* [47] showed that these effects are abolished in flies with a mutation in the *period* gene—one of the central clock genes in *Drosophila* [48]. Two additional components of the central clock [49], *clock* and *cycle*, are also required for cocaine sensitization, which suggests that drug responses may be under circadian regulation.

Indeed, VNC responsiveness to quinpirole fluctuates with the time of day in wild-type flies kept in a 24-hour light/dark cycle [50•]. However, the story is not that simple. First, quinpirole responsiveness still cycles (albeit to a lesser degree) in clock-deficient *period* flies tested in a light/dark cycle, indicating that a functional clock is not essential for this cycling. Second, although light/dark-entrained wild-type flies still show robust circadian rhythms in constant darkness, their response to quinpirole changes very little under constant light conditions, indicating that a functional circadian rhythm is not sufficient for robust cycling of the nerve cord's response to quinpirole [50•]. Last, *timeless* mutant flies show normal behavioral sensitization [47]. Timeless is a molecular partner of Period and also an essential component of the circadian clock [49].

Taken together, these results indicate that behavioral responses to cocaine are not simply regulated by circadian rhythms, but that the two processes share some molecular components. Using microarrays, McDonald and Rosbash [51] recently identified 134 genes whose transcription is controlled by the circadian clock and 267 genes whose transcription is, directly or indirectly, regulated by the *clock* gene. Thus, some genes regulated by clock genes are not regulated by the circadian clock. Similar reasoning may apply to the regulation of drug responses by clock genes.

Inspired by the above-described experiments, Abarca *et al.* [52] analyzed the contribution of circadian rhythm and clock genes to cocaine sensitization in mice. They found that mice with targeted mutations in the *period* homologue *mPer1* (which have a short-period circadian rhythm in constant darkness [53]) fail to sensitize to the locomotor-activating effects of cocaine, whereas *mPer2* mutant mice (which have no rhythm in constant darkness [54]) hypersensitize. This study also showed that locomotor sensitization is under circadian control: mice show a higher behavioral sensitization if repeatedly injected in the early morning rather than at the end of the light period—findings that mimic those reported for amphetamine sensitization [55]. Earlier work showed that repeated methamphetamine exposure disrupts circadian locomotor and feeding rhythms of rats [56] and that repeated injections of the drug increase *mPer1* expression in the caudate putamen of mice [57]. Notably, both responses sensitize upon repeated drug administration.

Taken together, these results show that there is a functional link between circadian rhythms, clock genes and cocaine sensitization in both flies and mammals. Understanding how and where these clock genes regulate drug-induced behaviors should provide interesting new insight into this clock–drug connection.

Conclusions

The behaviors elicited by acute and chronic administration of ethanol and free-base cocaine in flies are remarkably similar, qualitatively and quantitatively, to those seen in mammals. In addition, some reassuring parallels have already emerged at the molecular and neurochemical levels. Although human addiction is an extremely complex condition that obviously cannot be recapitulated in a fruit fly, an understanding of the mechanisms underlying the acute and chronic responses to drugs can be achieved in this simple but powerful model system.

In humans, the degree of response to ethanol has a genetic component and is a good predictor of risk for alcoholism [58]. It is possible, therefore, that an understanding of the fairly simple behaviors induced by acute drug exposure may help us to gain insights into the more complex process of alcohol addiction. In the next few years, genes involved in the regulation of drug-induced behaviors will emerge from ongoing genetic screens in *Drosophila*. Their evaluation as potential candidate genes in mammalian systems, including humans, is most likely to follow. Exciting times lie ahead!

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