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RESEARCH ARTICLE

Opiate Sensitivity in Fruit Flies

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Abstract

Substance use disorder is a debilitating clinical condition in which behavioral dependence results from biological, environmental, genetic, and psychosocial factors. An epidemic surrounding the use and abuse of opioids is ravaging the world. While considerable efforts have explored the social drivers of addiction, a deeper understanding of biological causes and genetic vulnerabilities, preventative interventions, and effective treatments, have all proven elusive. This perspective article aims to remind readers that addictive natural compounds such as cocaine, nicotine, cathinone, or morphine, evolved as defensive metabolites to deter insect herbivory. The molecular mechanisms underlying motivational seeking and learning/reward show remarkable conservation since their early emergence in bilateral metazoans. An extended coevolutionary arms race subsequently weaponized these compounds into disruptors of learning, motivation, and incentivized attention. When plant chemical defenses attack insect physiology, humans are rendered susceptible due to strong conservation in the underlying molecular machinery. This perspective addresses the paradox that opiates were shaped to target insect neuropharmacology, even though this taxon appears to lack the recognized opioid receptor clade of mammals. We argue that the link is to be found in the allatostatin receptor, a basal ortholog of opioid receptors. Moreover, preliminary evidence indicates that morphine reduces *Drosophila* feeding and locomotion, concordant with a purported role as a defensive compound reducing herbivory. An implementation via allatostatin-mediated mechanisms is likely. This research argues for a broader heuristic perspective of substance abuse and a recognition of the evolutionary constraints that have likely shaped the biological drivers of opioid sensitivity and of its behavioral targets.

Keywords: Substance use disorder, morphine, fentanyl, opioid addiction, plant-insect coevolution

Introduction

The present work demonstrates that fruit fly (*Drosophila*) neuromotor systems exhibit strong sensitivity to morphine, a dominant opiate in the latex of poppy seed heads (*Papaver somniferum*). The ability to suppress feeding and locomotion is consistent with opiates acting in a potent anti-herbivore defense. This is quite a remarkable result considering that frank opioid receptors are widely considered to be a unique molecular property of mammals. The work described here encourages us to critically review, or maybe even reconsider, long-held assumptions about the phylogenetic drivers of opioid signaling and the behavioral phenomena they control.

Substance Use Disorder (SUD) continues to exact a grim toll as opioid overdose deaths accelerate in a steep upwards trajectory. For 2021 in the USA alone, deaths have surpassed 100,000, a 29% increase over the previous year[1,2]. This societal burden has reached epidemic proportions, driven by the lethal prevalence of synthetic opioids, pandemic-related stressors, and problems in accessing care. It not only touches those with substance use disorder, but also cruelly impacts those who share with them their love, aspirations, and humanity. The Centers for Disease Control and Prevention (CDC) estimate the economic burden of prescription opioid misuse at $78.5 billion per year in the US. Almost 30% of patients prescribed opioids misuse them, over 10% developing an opioid use disorder (OUD), and at least 6% progress to heroin use within a few years. While some medications exist to treat components of SUD, relapse rates for those addicted to opioids remain exceptionally high at 40-90%[3,4]. The societal challenges in addressing the problem may benefit from the current perspective in which we consider the evolutionary context for addictive plant compounds, substances for which the label "human drugs of abuse" may be missing a significant part of the story, or may ultimately turn out to be a misnomer altogether.

Motivational models of drug-seeking and drug-taking behaviors are structured around neural circuits that are strengthened by repeated activation. Intentional, goal-directed behaviors progressively shift toward formed habits and instinctual responses[5,6]. Moreover, the underlying biological drivers combine with an avoidance of aversive consequences from drug withdrawal[7,8]. Reinforcement models, with an appetitive search for rewards and avoidance of aversive conditions, imply a high level of goal-directedness. As compulsive consumption emerges, drugs lose their rewarding value. Behavior becomes increasingly driven by the drug-related stimuli themselves and becomes self-regulating, stimulus bound, inflexible, and insensitive to considerations of outcomes[9,10]. Once established, affective processes exert powerful control[11] and are notoriously impervious to cognitive oversight.

Traditionally, SUD research has focused on close taxonomic associations between humans, primates, and other mammals. Generally considered an acquired disorder of altered cognition[12], the search for explanations has centered on psychological

explanations[13], and the presence of structures such as the nucleus accumbens or the mesolimbic dopaminergic (ML-DA) system are considered integral to its etiology[14]. In the present perspective, we aim to bring to the reader's attention a number of evolutionary considerations which permit us to reframe behavioral dependence on plant alkaloids as a chemical insult targeting conserved mechanisms in reward, learning, and memory[15]. Having emerged in a coevolutionary arms race between plants and their insect herbivores[16–18], addictive plant compounds represent a class of weaponized controllers for learned behaviors, driving motivation, perceptions of reward and incentive salience. It is important to note that the relevant molecular and neural mechanisms are shared far beyond humans and mammals[19,20], having arisen in a distant evolutionary past dating back to the initial emergence of the bilateral metazoan lineage[21–23]. Widely conserved owing to their critical function in organisms, they assist in negotiating an uncertain world[24–26]. Recognizing the deep phylogenetic roots of neurobehavioral integration, *Drosophila* offers a powerful experimental model system that can inform our understanding of the genetic, molecular, and neural complements of drug-reward, incentive salience, labeling of predictive cues, and their underlying motivational drivers.

The capacity to adapt behavioral responses with the coordinating actions of peptide signaling molecules emerged as an ancient property of neurons during early metazoan evolution. The targets are rhodopsin-like, G-protein-coupled receptors (GPCRs), that control changes in a wide range of downstream mechanisms. At least 30 such signaling systems have been traced to the common ancestor of bilateria, endowing them with the ability to cope with the vagaries of a complex world. Abilities to perform flexible learning[27,28], perceive aversive conditions[29,30], or show motivated engagement with the world[31,32], are shared across all animal phyla. In the current perspective we advance the argument that defensive plant alkaloids have evolved to target the fundamental neural mechanisms on which insects depend for coping with a complex world. When plants are able to interfere with such functions, they target an essential neurochemical achilles heel, and compromise the biological success of their predators.

Opiates, produced by species of plants in the genus *Papaver*, are potent modulators of a specialized subgroup of G-protein-coupled receptors (GPCRs) sensitive to binding allatostatin, galanin, somatostatin, and opioids. Exerting largely inhibitory, hyperpolarizing actions, they dampen sensitivity and responsiveness, and constrain mechanisms in metabolic, developmental, and behavioral functions. A receptor binding protostome allatostatin serves as a potent inhibitor of meal size and food intake. It emerged near the root of this clade, likely in Placozoans, prior to the emergence of bilateral metazoans, and dated to around 950 million years ago[33]. Although opioid receptors *per-se* have arisen only later in vertebrates, they share strong affinities to allatostatin GPCR[34].

Drosophila as a model to study opiate sensitivity

Flies, as an experimental system with high predictive validity, are suited to generate novel insights into the vulnerabilities to, and consequences of, addictive plant secondary metabolites (PSMs). Shared ancestry exists between 15,500 fly genes and 20,000 human ones, including the majority of genes linked to human disease that have close homologs in flies[35]. Strong molecular conservation exists in neural mechanisms, including biological clocks[36], learning[37], energy sensing[38], inflammation[39] and pain signaling[40]. Routine genetic engineering tools can be used in flies to generate stable inbred lines via molecular cloning (recombinant DNA), gene delivery (transformation, transfection, transduction), genome editing (TALEN, CRISPR), gene silencing (interference RNA, antisense DNA), gene disruption (transposable elements), and control of gene expression (optogenetics, GAL4/UAS). Moreover, crosses between stable genetic lines generate desired combinations of gene variants. Populations of recombinant inbred fly lines are available for mapping quantitative trait loci. The Drosophila Synthetic Population Resource (DSPR) contains 1700+ recombinant inbred lines, constructed from two highly recombined synthetic populations. With the genome of the founder lines and each recombinant inbred line known, researchers are able to query genetic determinants for behavior or other complex phenotypes of interest[41]. This approach has recently implicated loci that drive the consumption of cocaine and methamphetamine[42]. Flies also present advantageous life history traits with a short, simple reproduction cycle from egg to adult in 10 days, high fecundity, and a 60-day life span for aging studies. Small size allows the maintenance of large stocks, group housing on simple food, and a need for minimal care. This perspective argues that the fly model offers a unique vista into the neurobehavioral control of seeking, and that the ability to bridge disparate levels of organization from genes to complex behavior, offers a productive study system.

Drosophila exhibit behavioral sensitivities to PSMs and to alcohol, with distinct similarities to those observed in humans[43,44]. *Drosophila*'s dopamine system has been implicated in learning and plays a central role in the behavioral consequences of cocaine, nicotine, and ethanol[45]. A recent study found that the voluntary intake of psychoactive substances is regulated by the dopamine receptor Dop1R1[46]. Flies show compulsive ethanol use and relapse to high levels of consumption following ethanol deprivation[47]. Conditioned place preference (CPP) models demonstrate a time-dependent preference for ethanol and flies will endure aversive electrical shocks to acquire it[48]. Scabrous/Notch/Su(H) signaling induces long-lasting transcriptomic changes following alcohol consumption in *Drosophila*, changes that are believed to drive alcohol associative cue memory formation[49]. Moreover, genes regulating cocaine sensitivity and ethanol tolerance, initially discovered in *Drosophila*, exhibit distinct homologies to the behaviorally relevant genes in humans[50,51]. To date few studies in flies have examined behavioral sensitivity to

opiates or explored the possibility of opioid system homologs.

Deep phylogenetic roots of opioid signaling

Cleaved from larger precursor proteins, signaling peptides bind to rhodopsin-like, G-protein-coupled receptors (GPCRs) affecting a wide range of downstream mechanisms. More than 30 neuropeptide signaling systems have been traced to an emergence at the root of the bilateral Animalia. The chordate lineage then expanded the repertoire of neuropeptide signaling systems in vertebrates with two rounds of genome duplication, enabling the emergence of additional and subdivided functions[52]. Although invertebrates do not feature opioid receptors *per se*, the latter arose from a family of ancestral receptor genes with affinities to protostome allatostatin[53]. A scan of the *Drosophila* genome identified 44 different GPCRs sensitive to bioactive peptides[54,55]. At least 32 *Drosophila* receptors appear to form orthologs of 15 monophyletic vertebrate subgroups, while six pairs of receptors are paralogs, representing recent gene duplications. One evolutionary branch is of special interest as it contains four *Drosophila* receptors (AstC-R1, AstC-R2, AstA-R1 and AstA-R2) with strong sequence similarity to a group of vertebrate receptors for opioids, galanin, and somatostatin[52]. To examine the extent of this sequence similarity we queried these four allatostatin receptors with protein fold data on the recognition server PHYRE2[56]. The search reveals a high amino acid sequence identity/similarity of the two fly allatostatin C receptors AstC-R1 and AstC-R2 with the vertebrate opioid receptor clade. Relative differences in the degree of sequence overlap support an initial gene duplication in vertebrates that produced the split between MOR/DOR and KOR/ORL paralogs[57]. Total amino acid sequence of AstC-R2 was identical to 40% with kappa opioid receptor (OPRK1) and nociceptin (OPRL1), and 35% with mu (OPRM1) and delta opioid receptors (OPRD1). For AstC-R1 the total amino acid sequence was identical to 40% with kappa, 40% with nociceptin, 35% with mu, and 30% with delta opioid receptors. We used the fpocket software[58,59] to identify the amino acids constituting the ligand binding pockets of the two AstC receptors. Interestingly, the region exhibiting strong conservation between AstC and human opioid receptors are found in the receptor's pocket region, suggesting similar ligand-binding abilities.

Representing allatostatin binding, the ancestral members of the opioid receptor family play a key role in the regulation of metabolic homeostasis and the control of motivational drivers for hunger. Highly conserved energy sensing mechanisms utilize subunits of the 5'-AMP-activated protein kinase (AMPK - PRKAA2/PRKAB1/PRKAA1) to monitor cellular energy status. Activated by energy deficit, AMPK triggers a number of energy conserving mechanisms, upregulates internal defense mechanisms preventing starvation, and drives the search for food resources via the generation of 'hunger'[60]. When consumption has met alimentary goals, allatostatin acts as a potent inhibitor of meal

size, hunger, and food intake in a wide range of taxa[61–63], including flies[64,65]. As the organism is released from a need for food acquisition, allatostatin lights up natural reward circuitry and enhances learning for conditions and cues surrounding the satisfying encounter. This concept is supported by recent reports in which allatostatin acts as a satiety-signaling molecule with direct control over dopaminergic neurons in a fly's reward circuitry, i.e., the protocerebral anterior medial cell cluster (PAM). When activated en masse, this group drives reward perception and appetitive learning. A subset of PAM neurons (PAM-y3) is a key mediator of aversive learning, with activity strongly inhibited by allatostatin[66].

Behavioral sensitivity of *Drosophila* to opioids

Empirical evidence presented in this perspective demonstrates that *Drosophila* locomotion (Fig. 1) and feeding (Fig. 2) are highly sensitive to the presence of opioids in food. Berlin-K (Bloomington Drosophila Stock Center #8522) males (5-7 days old) were tested individually. Maintenance and tests were conducted in an environmental chamber at a temperature of 25°C, relative humidity of 70%, and a 12h light:12h dark cycle. To assess the effect of opioids on the activity of *Drosophila*, we individually tracked locomotion of 120 flies per treatment group in distinct cells with a layer of solid, standard Bloomington Drosophila Stock Center cornmeal food[67]. Treatment groups contained morphine or fentanyl added to the food at a concentration of 50 µg/mL and 4 µg/mL respectively (Fig. 1). Results reveal a strong diurnal pattern with dominant activity peaks during dawn and dusk with strong psychodepressant effects of both morphine and fentanyl. Locomotion was reduced during periods of high activity of controls, with robust psychomotor effects parallelling those described for human and other mammalian systems. Morphine effects on *Drosophila* feeding were quantified using high resolution measures of consumption recording with a modified CAFE assay [68]. Tested individually, the timing and volume of feeding events was tracked from capillaries containing liquid standard diet food. Results evidence significant diurnal variation, with increased feeding at dusk on the second day for the control group. Morphine treated groups show a consistent dose-dependent depression (Fig. 2).

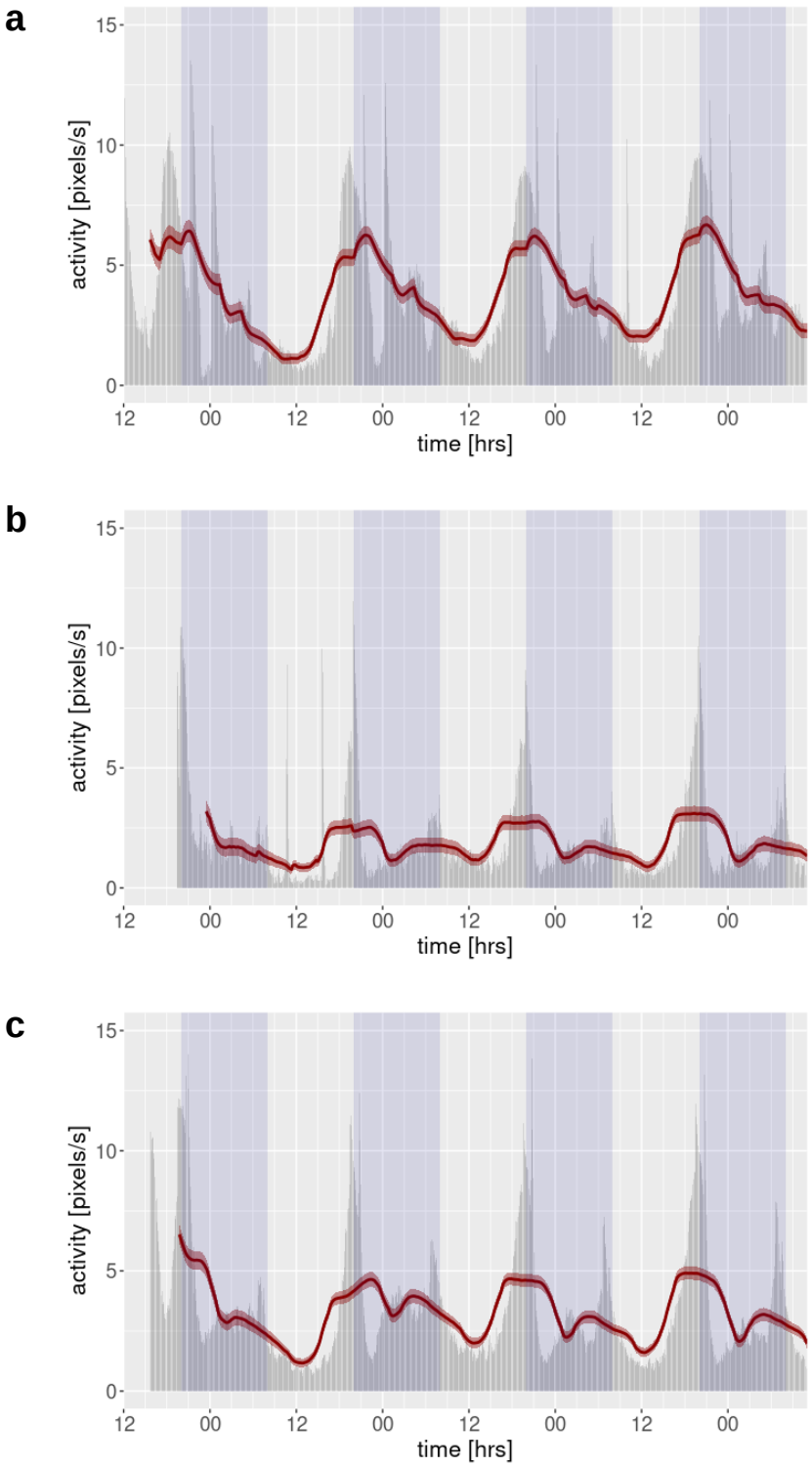

Figure 1. Diurnal activity is depressed by morphine and fentanyl. Actograms for 120 individually tracked fruit flies (mean distance traveled) were combined for animals maintained on (a) standard food, (b) food containing 50 µg/mL morphine, and (c) on 4 µg/mL fentanyl. Opiates/opioids, dissolved in the food for treatment groups, resulted in significant differences in psychomotor activity. Images from a stationary, overhead camera were analyzed with automated tracking at 1Hz and movement patterns were extracted

from these locations over time. Shaded areas mark the dark phase (8pm to 8am) of the diurnal rhythm. Plotted in lighter gray are individual bars representing the mean speed for each 2 min interval of the trial. Plotted in red is the smoothened average of activity across time. A nonlinear mixed-effects model (R package 'nmle', R Core Team, 2022) was used to perform a repeated measures, time series analysis with a complete model for diurnal patterns in motor activity and treatment with opioids. Results evidence a strong diurnal pattern with dominant activity peaks during dawn and dusk hours (Variable: Hour; $F_{(1,1128407)}$ = 30764.7, p<<0.001). Consistent psychodepressant effects of both morphine and fentanyl were evidenced in decreased levels of activity (Variable: Opioid Treatment; $F_{(1,1128407)}$ = 11677.6, p<<0.001). Moreover, locomotion was reduced in access during periods of normally high activity, mostly during dusk, night and dawn (Variable: Hour x Opioid Treatment; $F_{(1,1128407)}$ = 2515.5, p<<0.001). Psychodepressive effects strongly parallel those described in human and other mammalian systems.

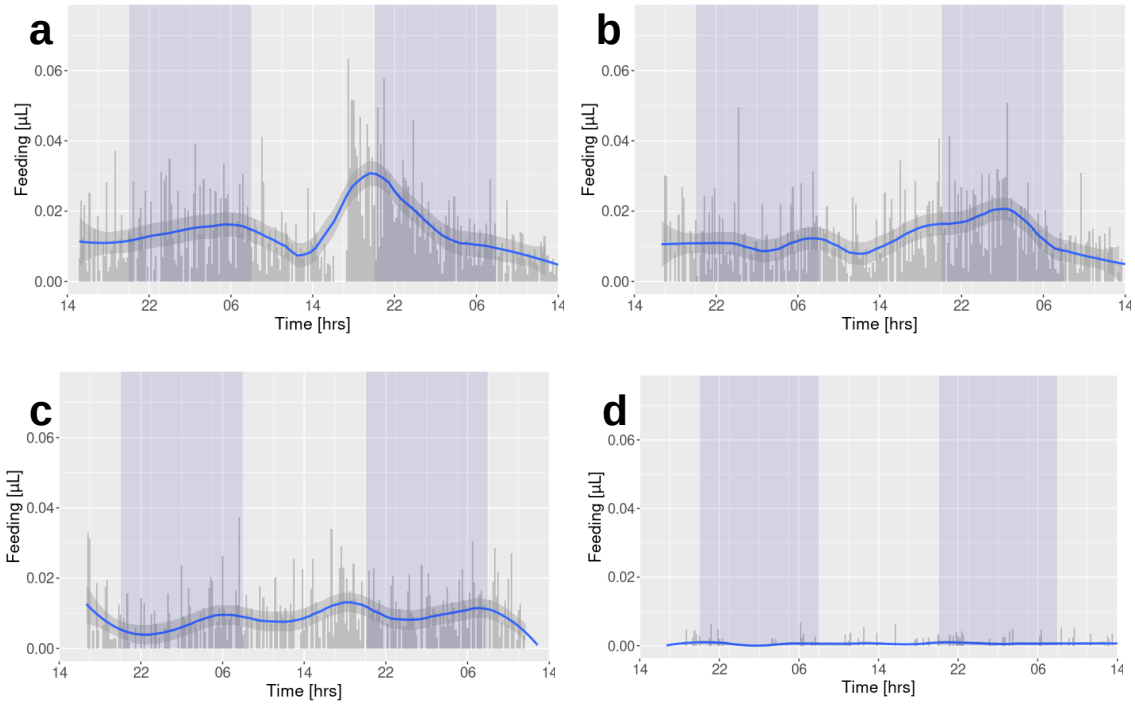

Figure 2. Morphine depresses *Drosophila* feeding in a dose-dependent manner. Food consumption is accumulated across groups for flies tracked individually, feeding on liquid diet using the standard ARC paradigm[68]. Flies in each group were given access to (a) liquid food (N=25), or to liquid food containing morphine at concentrations of (b) 25 µg/ml (N=25), (c) 250 µg/ml (N=22), and (d) 2500 µg/ml (N=24). Shaded areas mark the dark phase (8pm tot 8am) of the diurnal rhythm. Plotted in lighter gray are individual bars representing the average amount of consumed food for each 10 min interval of the trial. Plotted in red is the moving average of feeding metrics. A Nonlinear mixed-effects model (R package 'nmle', R Core Team, 2022) was used to perform a repeated measures time series analysis with a complete model for diurnal patterns in food consumption and treatment with opioids. Results evidence significant variation with time of the day (Variable: Hour; $F_{(1,1050599)}$ = 11.4, p<0.001). Consistent psychodepressant effects of different doses of morphine were evidenced in decreased levels of feeding (Variable: Morphine; $F_{(3,92)}$ = 32.1, p<0.001). Moreover, no significant changes in feeding were detected as a function of time of day (Variable: Hour x Opioid Treatment; $F_{(1,1128407)}$ = 2.4, p=0.065).

Discussion

Our data support the notion that the inclusion of an evolutionary perspective explains the origins and the role of defensive plant alkaloids. *Drosophila* presents both a powerful and promising experimental model organism that can help us unravel the cellular and circuit-level consequences of addictive compounds. Poppies have evolved a cocktail of opiates that appear to upregulate satiety mechanisms in its insect herbivores, restricting appetite in animals intent on consuming the plant's seed heads[69]. Shaped by an evolutionary arms race between plants and insects, morphine and its derivatives curtail the predator's perceptions of hunger and thereby limit food consumption. The evolution of opiates as defensive plant alkaloids is thus consistent with a role in limiting insect herbivory[16], explaining also a strong depression of alimentary functions and gastrointestinal and colonic motility in humans. While opioid receptors are yet to be characterized in *Drosophila,* sequence alignment indicates significant sequence similarities between mammalian opioid receptors and allatostatin GPCRs[54]. In addition to dopaminergic function driving search and anticipation of food reward, allatostatin plays a critical role in the regulation of food intake in a wide range of taxa[62,63,65]. When a critical and life-sustaining food resource has been acquired, allatostatin touches natural reward circuits, codes for a rewarding outcome, and enhances learning for the conditions and surrounding cues that gave rise to the satisfying encounter[70]. As a negative regulator of aversive signaling, it appears to directly connect satiety signaling with inherently rewarding properties. Ultimately, it may achieve this by enhanced coding for subjective perceptions of well-being and contentment[71].

Sequence homologies indicate that opioid analgesic properties arose from an ancestral allatostatin system, as a system that signals a release from bodily harm and tissue damage rather than from metabolic deficiencies. We thus view the way in which 'hunger' drives the search for food, and is counteracted by the allatostatin system, as fundamentally similar to when opioids signal a release from subjective perceptions of 'pain'. Strong support derives from recent reports of allatostatin's ability to modulate nociception and inflammatory pain[72]. The somatostatin/opioid GPCRs group thus appears to code for the positive valence associated with the escape from harmful conditions, such as hunger and pain[73]. Using morphine, opium poppies appear to pharmacologically drive insect GPCRs for allatostatin in order to suppress feeding and metabolic activity. Ties to reward circuitry may form the central core from which opioids derive their addictive properties.

Additional experimental work is needed to better understand the functional similarities between the opioid and allatostatin systems. By leveraging high-throughput behavioral studies and the richness of the available genetic techniques, *Drosophila* offers a unique model for the exploration of unknown molecular effectors and epistatic gene interactions that are at the basis of complex afflictions such as OUD. The predictive validity of this model is essential for our efforts to

combat the opioid epidemic. We therefore call for another level of analysis in OUD research: the inclusion and intersection of both ultimate and proximate causes of OUD in humans. As we see it, this explanation will remain unresolved until it includes the push and the pull between PSMs and insects.

Conclusions

The present work presents evidence that the opiate morphine is effective in reducing measures for both locomotion as well as feeding in *Drosophila*. The observation that potent opioid signaling drives psychodepressant effects in fruit flies may come as a surprise to those who accept that taxa lacking members of the widely recognized mammalian opioid receptors clade, are neither vulnerable to, nor the target of, addictive opiates. Our work is meant to serve as a reminder that the molecular targets of compounds driving compulsive drug taking in humans may actually be found in neuromodulatory systems of insects as the plant's primary predators. For a full explanation of any phenomenon, we would do well to consider ultimate perspectives and underlying phylogenetic pathways, alongside the more common, single-minded search for proximate mechanisms. Considerable empirical evidence supports the notion that poppies evolved opiates in a coevolutionary arms race as a defense against insect herbivory. With this front of mind, we hope to motivate a deeper, comprehensive discussion of substance use disorders, one that values a more inclusionary network approach (social and all -omics levels) in order to yield a better understanding of this important neurobehavioral phenomenon of major societal relevance.

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Conflict of Interest Statement

In addition to their academic positions, several authors are engaged in other research, governmental, and commercialization efforts. RH, MvS, and SH are co-founders of JuvaTech, Radmantis, and Veridat. JS is Director of Science and Research for the Ohio Attorney General at the Office of the Ohio Attorney General.

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Author Contributions

NK, BL, RH and MVS designed the study and wrote the article. NK and RH acquired and analyzed the data. JS, DJ and SH provided critical revisions to the article.

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