Crayfish Learning: Addiction and the Ganglionic Brain

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Abstract

Recognizing addiction as a phenomenon with deep evolutionary roots grants valuable new perspectives into understanding its behavioral features, as well as its underlying neural mechanisms and genetic architecture. Although now generally misbranded as “human drugs of abuse,” addictive plant alkaloids originally arose as potent chemical defenses against insect herbivory. The products of this evolutionary arms race, compounds such as nicotine, cathinone, or morphine, target essential biological mechanisms for motivation and learning and act as weaponized disruptors. Human vulnerabilities to these addictive drugs may thus represent little more than collateral damage arising from deep homology, i.e., shared biological implementation of behavioral functions with taxa that trace back to the early divergence of bilateral metazoans. Consistent with such a view, invertebrate preparations exhibit a rich spectrum of behavioral and neural consequences in response to drug exposure. Although there is certainly evidence for addiction-like phenomena in many invertebrate lineages, the present review focuses attention primarily on our recent work in crayfish. Using this decapod crustacean model, we have characterized a range of amphetamines, cathinones, and opioids for evidence of unconditioned intoxication, sympathomimetic properties, psychostimulant sensitization, conditioned cue learning, and operant self-administration. Overall, our findings on drug-sensitive reward in crayfish bear striking similarities to equivalent phenomena illustrated in mammals. Experimentally tractable invertebrate models may thus provide fundamental insights into the homo- and paralogous mechanisms mediating responses to addictive drugs, while illuminating the limits of such contrasts.

Keywords *Crustacea* · Conditioned cue preference · Operant self-administration · Psychostimulant sensitization · Drug reward · Amphetamine · Opioid · Cathinone

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Introduction

The public, policy makers, and those tasked with prevention and treatment of addiction commonly view drug dependence as a moral failing, a lifestyle choice with ruinous personal consequences, or a habit in serious need of cognitive control (Hester, Lubman, & Yücel, 2010). Such perspectives, though arguably applicable in the assessment of ultimate outcomes, have fallen short in generating effective solutions to the problem. Not only does it fail to help those suffering from substance abuse cope with the damage, it also provides little support to those within the addict's immediate social circle, or guidance in addressing the significant and growing societal burden of substance abuse (Florence, Zhou, Luo, & Xu, 2016). For the past 20 years the U.S. National Institute of Drug Abuse has advocated for a brain disease model of addiction (BDMA) and empirical findings in behavioral neuroscience have advanced promising avenues for reframing this phenomenon into a broader, and more synthetic, perspective (Volkow & Koob, 2015). Chief among these is a view that considers addiction to be the result of a disordered learning process, manifested as a single-minded focus on, compulsive search for, and use of drugs despite experiencing incontrovertibly harmful consequences (Keiflin & Janak, 2015). In essence, all evidence points to addiction as a neural disorder in which drugs are able to commandeer neuronal circuitries for nonassociative and associative learning (Alcaro, Huber, & Panksepp, 2007), fundamental neural functions that are responsive to direct cognitive control to only a limited extent.

The search for understanding and solutions, whether individual therapeutics or societal level interventions, has benefited greatly by approaching the problem from a disease perspective (Volkow & Koob, 2015). The current consensus among behavioral neuroscientists is that drug addiction is less a deliberate choice of harmful outcomes, but rather one in which the brain circuits responsible for decision making (or exerting “free will”) have been eroded and functionally compromised. Thus, the resulting behavioral dysregulation arises from the actions of drugs exerting their effects on the circuitry underlying learning processes. The ultimate goal of learning is to optimize behavioral choices—balancing the perception of current needs, informed by memories of past experiences, to arrive at beneficial decisions. First, needs or goals are implemented as the primary drivers of behavior through motivated search (Panksepp, 2014). A prediction of outcomes for available choices can then be formulated based on memories of past outcomes and experienced consequences. It is clear that the ability to recognize, remember, and respond to patterns in surrounding conditions is widely conserved across taxa (Shettleworth, 2010). The most efficient learning processes require tradeoffs to be made between generalization and specificity, and between computational speed and flexibility (Redish & Mizumori, 2015). This system creates an essential Achilles heel of animals, one that renders them vulnerable to a class of potent weaponized learning disruptors, generated by plants as a defense against herbivore predation. These addictive alkaloids represent another, more covert, line of plant defense beyond bitter taste and toxicity (Wink, 2018; Wink & Schimmer, 2018). By targeting fundamental machinery for motivation and learning, the evolution of countermeasures is much restricted. Any mutation that lessens sensitivity to addictive alkaloids inevitably generates unfortunate side effects, such as reduced initiative, dysregulation of reward perceptions, and impaired judgement.

Learning is an essential mental process that directly interfaces with the mechanisms for perception, attention, thinking, emotions, and, especially, decision making (Berridge, Robinson, & Aldridge, 2009). The ability to structure behavior that is both flexible and responsive to past events, requires the successful execution of a number of distinct functions (Figure 1). “Appetitive motivational states” are the key drivers of behavior, facilitating an organism’s pursuit of goals and acquisition of the resources it needs in a competitive and uncertain world. Items aligned with a physiological or biological need (e.g., food, water, and mates) are predisposed to capture an animal’s attention. Moreover, natural interest readily extends to any paired cues via Pavlovian labeling (Fanselow & Wassum, 2015). Acting as natural rewards, the resources and their environmental predictors are inherently endowed with “incentive salience,” a property that both focuses the animal’s interest and biases its behavior in order to encounter the particular resource more frequently (Panksepp & Wilson, 2016). Incentive salience, and the pursuit it generates, are also readily acquired by previously neutral items when paired with an unexpected favorable outcome. Beneficial scenarios energize an animal’s internal valuation function, encoded as a perception of “reward.” Because reward magnitude is amplified by the degree to which the experience exceeds expectations, the same situation may confer either a minor or a major reward, depending on what was anticipated (Fletcher et al., 2001). As a result, items encountered in unfamiliar circumstances (and therefore lacking a prior expectational framework) are inherently more likely to generate enhanced reward function, acquire stronger motivational salience, and be pursued more vigorously. Expectations, representing feed-forward weights of the reward circuit, are continuously being updated in response to current events, such that incoming experiences are recalibrated through its motivational- and value-system filters.

Drugs of abuse critically interfere with central features of this carefully calibrated and situationally balanced learning system (Robbins, Ersche, & Everitt, 2008). In

Fig. 1 Schematic illustrating a balanced feedback model (a) of functional reward elements in the pursuit of natural rewards, and their dysregulation (b) when exposed to addictive alkaloids. **a** In profitable situations, activation of reward circuitry tags the resource gained and surrounding cues with special significance (i.e., incentive salience). As subsequent needs dictate, this generates directed searching for the item and associated cues. **b** Exposure to addictive plant alkaloids enhances perceptions of benefit and imbues surrounding cues with incentive salience. Repeated drug encounters exert effects on this sensitized reward circuitry, assigning ever greater reward and significance to the drug experience, and driving compulsive search. Once established, this addictive cycle is exceedingly recalcitrant to change, whether by reducing (“devaluing”) or extinguishing (“unlearning”) the inappropriate cue pairing

particular, they affect motivation through potent sympathomimetic and psychostimulant effects, focus attention on coincidental cues and conditions, and generate perceptions of reward where none are earned. As a consequence, key inputs to the decision-making framework are fundamentally compromised, jeopardizing the animal's ability to identify and execute a profitable strategy. The ultimate target of addictive drugs appear to be key sites within the core of reward circuitry. By artificially exaggerating perceptions of inherent value, and understating associated risks, the animal anticipates outcomes with vastly inflated benefits and is prevented from learning via injurious actions and repeated past mistakes. A further consequence of this drug-energized reward illusion selects coincident cues and artificially imbues them with a high degree of incentive salience, conferring attractive and stimulant properties (Robbins et al., 2008). Repeated drug use amplifies these impacts as drug-pairing generates escalating levels of attention and perceived value. Appetitive behaviors directed at drug-rewarded items then soar as a result of this "psychostimulant sensitization," commonly recognized as the onset of craving (Robinson, Robinson, & Berridge, 2013). Drug-induced distortions of reward thus bring about an enduring state of desire, or "wanting," rather than one of enhanced pleasure, or "liking," for the drug.

Overwhelming evidence points to fundamental mechanisms of learning and reward as the essential target of addictive drugs. These mechanisms are reflected in exceedingly deep homologies of neural mechanisms, including signaling (Pandey, Mersha, & Dhillon, 2013), chemical modulation (Blenau & Thamm, 2011), receptor elements (Katz & Lillvis, 2014), as well as neural functioning (Kravitz, 2000; Egnor & Branson, 2016), and likely trace back at least to the early evolution of bilateral metazoans (Ginsburg & Jablonka, 2010). Here we explore the extent of this functional homology in a decapod crustacean model that combines an experimentally tractable nervous system and sophisticated behavior for cue recognition and learning. Such capacities in crayfish include associative learning of chemical cues related to potential food prey (Weisbord, Callaghan, & Pyle, 2012) and predation risk (Hazlett, Acquistapace, & Gherardi, 2002), as well as visual cues in facial and social recognition (Van der Velden, Zheng, Patullo, & Macmillan, 2008; Jiménez-Morales et al., 2018). Although not highly regarded for their cognitive complexity, crayfish are known to make value-based decisions by weighing the costs and benefits of different behavioral options, and selecting adaptive behavioral output based on the activation patterns of identifiable neural circuits (Liden, Phillips, & Herberholz, 2010). Below we outline crayfish susceptibility to mammalian drugs of abuse, progressing through unconditioned effects, nonassociative learning, and leading to several associative conditioning processes (Huber et al., 2018).

Intoxication and Psychostimulation

Psychoactive substances generate disturbances in a wide range of neural functions including perception, affect, cognition, and behavior. Ethanol, one of the most commonly used psychoactives, has an initial period of psychostimulation and disinhibition, followed by a loss of motor and postural control, an effect demonstrated in a broad range of taxa (Heinz, Beck, Meyer-Lindenberg, Sterzer, & Heinz, 2011; Moore et al., 1998; Vonghia et al., 2008). Crayfish placed into water containing alcohol display

distinct motor deficits, followed by an inability to right themselves (Swierzbinski, Lazarchik, & Herberholz, 2017). Upon prolonged exposure, individuals develop tolerance and recover much of their motor control (Strawn & Cooper, 2002). Several plant families produce psychoactive alkaloids with potent, sympathomimetic effects. For instance, cocaine, nicotine, and cathinone, are plant derivatives with varying targets and modes of action, but all act via adrenergic activation and the modulation of monoamine neurotransmitter systems (Eshleman et al., 2013). Crayfish which received doses of 1–10 $\mu\text{g/g}$ cocaine showed highly exaggerated claw waving, accompanied by rapid backwards walking and escape behaviors. Then followed the adoption of a rigid posture with outstretched legs, a flexed abdomen, and claws extended forward and angled downwards before individuals lost the ability to right themselves for a dose dependent period of time (Panksepp & Huber, 2004). Similar locomotor depression was also observed on the first of several days of repeated cocaine administration (Nathaniel, Panksepp, & Huber, 2012). On subsequent days cocaine had a distinctly stimulant effect, with increased travel distance and speed interrupted by fewer and shorter stationary periods (Nathaniel et al., 2012). Individuals treated with an equivalent dose of amphetamine (1–10 $\mu\text{g/g}$) show markedly different behavioral effects. They circled the tank in close wall contact for extended periods of time, vigorously waved their antennae, and interacted with any objects they encounter (Panksepp & Huber, 2004). These patterns are reminiscent of exploratory behavior that occurs when a crayfish enters novel surroundings, albeit greatly exaggerated and accompanied by tremors in walking legs and bouts of grooming. Individuals injected with 3,4-Methylenedioxymethamphetamine (MDMA) and 4-Methylmethamphetamine (4-MMA) or their cathinone analogs methylone and mephedrone, exhibited prolonged periods of repetitive motor patterns, interspersed with brief bouts of upward extension in rigid immobility (Gore, van Staaden, Sprague, & Huber, *in review*). Repetitive behaviors included horizontal waving of claws, eyestalk and carapace grooming, and stereotyped movements of walking legs and antennae. At higher concentrations, Methylone triggered what appeared to be evasive behaviors, including backward walking and tail flips, accompanied by tremors, and uncoordinated leg movements. In all instances, repeated administration of these compounds further enhanced the frequency, intensity, and duration of stereotyped movements (Gore et al., *in review*).

Behavioral stimulation and the generation of reward signals depend fundamentally on a drug's capacity to activate dopamine-mediated, appetitive search. This motivational effect maps onto the SEEKING network (Panksepp, 2014), indicating that this condition extends beyond mammals, for which the concept had originally been proposed. Psychostimulant effects closely parallel those seen in mammals, and appear to be a critical requirement for the development of rewarding functions in crayfish. Addictive drugs, which show psychostimulant effects, at least at lower concentrations, include several substituted amphetamines and cathinones (Gore et al., *in review*) and opioids such as morphine and heroin (Imeh-Nathaniel, Okon, Huber, & Nathaniel, 2014). Consistent with this hypothesis, depression of morphine function produces a corresponding decrease in exploratory motor patterns (Imeh-Nathaniel et al., 2014).

In an attempt to identify the neural substrates underlying these psychostimulant effects, we administered amphetamine at different anatomical sites, and compared the time course and dose response curves of the resulting behavioral effects (Alcaro, Panksepp, & Huber, 2011). Three different measures of exploratory behavior were

used viz., locomotion, antennal movements, and rearing up along the arena sides. Amphetamine triggered increases in exploratory behaviors with both systemic (via the pericardial cavity) and targeted infusions. However, effects occurred more rapidly and were considerably larger when the drug was applied directly above the supraesophageal ganglion (see Figure 2). All indications are then that the critical neural sites responsive to phenylethylamine stimulation are located in the crayfish brain. This comprises several distinct and functionally specialized neural structures, most of which are primarily devoted to odor processing. The most likely source for the psychostimulant effects noted are the accessory lobes, a large paired region of dense neuropil, surrounded by clusters of neurons, and located on either side of the midline (Sandeman, Beltz, & Sandeman, 1995). This structure is thought to function as a multimodal processing center, responsive to visual, tactile, and olfactory inputs, despite lacking primary afferents. Interneurons from the deutocerebral commissure provide inputs to the accessory lobe, whereas efferent projections from a dorsal cluster of cell somata project back into the deutocerebral commissure neuropil. Forming connections with the dorsal giant aminergic neurons in accessory lobe glomeruli, these interneurons project to proto- and deutocerebrum, as well as to the central body.

Psychostimulant Sensitization

A single administration of psychostimulant activates exploration in a treated individual. This response amplifies with repeated administration, persists into withdrawal, and is considered an expression of the onset of drug craving (Robinson & Berridge, 1993; Robinson et al., 2013). In crayfish, repeated systemic infusions of morphine produced persistent locomotor sensitization, with even a single morphine dose sufficient to induce long-term behavioral sensitization (Nathaniel, Panksepp, & Huber, 2010). Recent work has extended this research to include a range of methyl-substituted phenylethylamines, including mephedrone, methylone, 4-MMA and MDMA (i.e., ecstasy). Administration of a single dose of these substances likewise stimulated increased locomotion and enhanced behavioral interactions with the surrounding. That single doses of morphine and other stimulant drugs induce long-term behavioral sensitization in crayfish demonstrates the fundamental sensitivity of their neural substrates to this type of chemical insult (Panksepp & Huber, 2004). It also offers a

Fig. 2 Schematic of the crayfish nervous system overlaying Thomas Henry Huxley's original drawing [Public domain, via Wikimedia Commons] Ventral ganglia and longitudinal connectives indicated in red with subesophageal ganglion “brain” (SEG), first thoracic ganglion (T1), and first abdominal ganglion (A1)

powerful, evolutionary, comparative context for understanding natural variation of psychostimulant effects and the determinants of drug vulnerability (Nathaniel et al., 2010). In mammals, the degree of sensitization serves as a potent predictor for vulnerability to enter an addiction cycle (Berridge & Robinson, 2016). Whether such measures are equally predictive of addictive potential in crayfish awaits a population level study.

Cue Learning and Reward

All organisms with the capacity to learn readily associate a cue when it cooccurs with a significant consequence. In this way, cues assume a predictive role in signaling; outcomes perceived as rewarding generate appetitive responses with increased preference for the associated cues, whereas those perceived as punishing lead to avoidance of the paired cue. We explored the reinforcing properties of psychostimulants in crayfish using a series of conditioned place preference (CPP) experiments in which a particular visual or tactile environment was paired with psychostimulant infusions over multiple days. A robust preference for the associated environments emerged as crayfish strongly sought out cues that had previously been paired with the administration of amphetamine or cocaine (Panksepp & Huber, 2004), opioid (Nathaniel et al., 2010), methamphetamine (Imeh-Nathaniel, Adediji, Huber, & Nathaniel, 2016), or synthetic cathinones (Gore, 2017). When systemic infusion was paired with a distinct visual environment, amphetamine elicited stronger drug-paired conditioning than did cocaine (Panksepp & Huber, 2004). Although multiple instances of paired training were necessary to develop the full extent of substrate conditioning, a single drug application was sufficient to enhance cue preference. Crayfish reward is similarly sensitive to methamphetamine (2.5, 5.0, and 10.0 $\mu\text{g/g}$ doses), with animals exhibiting a strong preference for the paired tactile environment following conditioning trials (Imeh-Nathaniel et al., 2016). More recently, we compared the behavioral effects resulting from treatment with select, novel, synthetic cathinones (methyldone, mephedrone), to their better known substituted methylmethamphetamine analogs (Gore et al., [in review](#)). Our work confirmed the potent ability of methylmethamphetamines to bias an individual's preference towards cues experienced in their presence. Synthetic cathinone analogs produced a similar preference for drug-paired substrates, but required higher doses to do so. A ketone in the side chain of cathinones renders them less lipophilic than their amphetamine counterparts, perhaps accounting for the lower potency relative to their structural amphetamine equivalents.

Extinction and Reinstatement

Once formed, learned associations between drugs and the cues or events with which they are paired, are highly resistant to attempts at weakening through behavioral intervention. Extinction trials present the cue but withhold the drug reward to which the animal had been accustomed. With varying degrees of effort, a conditioned response (preference) to drug cues can eventually be reduced through repeated extinction trials. Extinction of drug-paired cues may share a number of behavioral, neural,

and conceptual parallels with the extinction of conditioned fear (Peters, Kalivas, & Quirk, 2009). We examined the changes occurring in crayfish behavior when the established drug-cue pairing was disrupted by pairing the cue with saline. Such extinction trials resulted in a range of atypical crayfish behaviors (including hyperlocomotion), suggestive of drug withdrawal. Following extinction of morphine-induced CPP, morphine-experienced crayfish readily reinstated CPP when challenged with a small, priming dose of morphine (Nathaniel et al., 2009, 2010). This association appears likewise unrelenting in crayfish as it is in mammals (Stewart & Wise, 1992), highlighted by the fact that CPP for morphine could not be disrupted by drug free periods lasting as long as five days (Nathaniel et al., 2009, 2010).

Operant Learning

The need to assess and remember contingencies has shaped behavioral responses since the mechanisms for learning and memory organization emerged in a long-distant evolutionary past. The extent to which crayfish can adjust their choices based on consequences was explored in a spatially explicit paradigm, where environmental cues (e.g., tactile and visual) in an arena were paired with either mild electric shock (punishment; Bhimani & Huber, 2015) or psychostimulant administration (reward; Datta, van Staaden, & Huber, 2018). Heat maps summarizing space utilization in the punishment experiments (see Figure 3) reveal that crayfish quickly learned to avoid shock-paired substrates, and confined themselves to the “safe” substrate (see Figures 3b and c). Yoked controls show a spike in locomotor activity following each (noncontingent) shock, but did not learn to avoid these quadrants (see Figure 3d), eliminating unconditioned responses as an explanation (Bhimani & Huber, 2015). A second set of experiments replaced the mild electric shock with the delivery of a drug bolus into either the pericardial

Fig. 3 Heatmaps for accumulated population coordinates of crayfish receiving mild electric shock on a particular substrate quadrants are arranged diagonally with soft substrate (upper left, lower right) and hard substrate (vice versa). Coordinates are plotted for controls receiving no shock (a), “master” animals receiving shock paired with either soft substrate (b), or hard substrate (c), and yoked controls of the master groups (d). Treatment groups displayed distinct qualitative and quantitative differences in locomotion and space utilization. Untreated controls spent the majority of time along the arena wall, or exploring the substrate at the quadrant borders. Crayfish in the master groups, earning repeated electric shocks on a particular substrate, learned to avoid these quadrants and settled onto the safe substrate. Yoked controls, receiving most shocks while in their preferred peripheral location, moved further into the center of the arena (Data replotted from Bhimani & Huber, 2015)

cavity, or directly above the supraesophageal ganglion. In this spatially contingent version of a self-administration paradigm, enhanced operant responding indicates that the drug acts as an effective reinforcer of behavioral choices. Furthermore, crayfish more readily learned to self-administer amphetamine when drug was delivered directly to the brain, rather than systemically (Datta et al., 2018).

Conclusions

The crayfish system has generated insights into a broad spectrum of addiction components, including the fundamental biological mechanisms of drug effects, how the seeking tendency is implemented in a “simple” nervous system, and how motivation and reward dispositions are affected by drugs of abuse. This complements data from a variety of other invertebrate systems (Søvik & Barron, 2013), and validates the approaches of Nesse (1994) and Panksepp, Knutson, and Burgdorf (2002), who have long promoted the notion that an integrative evolutionary framework might provide a more nuanced understanding of addictive processes to inform prevention, treatment, and social policies.

Despite lacking both a “brain” as conventionally defined, and the formal structures believed essential to human addiction, learned drug responses in crayfish bear a striking resemblance to those of the dominant vertebrate models. Combined with their distinctly modular and experimentally tractable nervous system, crayfish provide a unique opportunity to probe reward circuits at the level of the individual neuron. Doing so will deliver at least two benefits; first, enabling prediction of key features to be sought in the more complex vertebrate systems, and second, identifying boundaries (if any) of the in-/vertebrate comparison. Together these would reveal any unique functional aspects of learning (and by extension addiction) arising later in evolution, which would be legitimate treatment targets for human addiction.

Individual differences in human vulnerability to addiction derives from complex interactions of the individual’s biological make-up, environment, and age (Sagheddu & Melis, 2015; Becker & Koob, 2016). It remains to be seen whether, and to what extent, similar variations in drug vulnerability exist in invertebrate taxa. Individual differences among invertebrates have been amply demonstrated in multiple other contexts (Mather & Logue, 2013; Fisher, Rodríguez-Muñoz, & Tregenza, 2015; Walton & Toth, 2016), so there is no reason to think that addiction should be any different. In humans, family and twin studies suggest that approximately 50% of the variation in susceptibility to addiction is attributable to genetic causes (Bierut, 2011). Although the specific proportion is arguable, it is clear that the underlying mechanisms regulating these persistent behavioral abnormalities involve changes in gene expression throughout the brain’s reward circuitry, and that epigenetics may play a significant role (Walker & Nestler, 2018). The marbled crayfish (*Procambarus virginalis*), with its easy maintenance, parthenogenetic reproduction (Vogt, 2018), and population status as a single global clone (Gutekunst et al., 2018), has great potential for addressing many of the outstanding questions, such as the relative contributions of quantitative, qualitative, population, and mechanistic variation.

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Compliance with ethical standards

Conflict of interest Authors van Staaden and Huber declare that they have no conflict of interest.

Ethical Standards The research protocols described in this paper are exempt from review by the Institutional Animal Care & Use Committee of Bowling Green State University.

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