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Research report

Effects of a single and repeated morphine treatment on conditioned and unconditioned behavioral sensitization in Crayfish

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ABSTRACT

Recent neuroethological work suggests that drug-sensitive reward in Crayfish represents a useful new model system for the study of drug dependence. Monoamine re-uptake mechanisms, which are conserved across vertebrate and invertebrate taxa, offer sites of action for testing drug-induced behavioral sensitization. The present study explored drug-associated behavioral sensitization in Crayfish by concurrently mapping measures of locomotion and rewarding properties of morphine. Behavioral effects of mammalian drugs of abuse are thought to depend on the patterns of drug regimens, and are similar across vertebrates. In this study, we determined whether behavioral sensitization induced by single and repeated morphine treatments extend to invertebrates. The first set of experiments indicated that intracirculatory infusions of single or repeated doses of morphine (2.5 μg/g, 5.0 μg/g and 10.0 μg/g) result in persistent and comparable locomotory sensitization even 5 days following the infusion. In the second experiment, we explored the short and long-term rewarding effects of a single or repeated morphine drug regimen using the conditioned place preference (CPP) experiment. Morphine-induced CPP also persisted for a drug free period of 5 days, indicating that this amount of time was not sufficient to disrupt the established CPP between morphine and context-dependent cues in Crayfish. Results from our study indicate that a single dose of morphine was sufficient to induce long-term behavioral sensitization in Crayfish, and that such effect is comparable to the effect of repeated morphine regimes. Behavioral sensitization studies in Crayfish thus contribute an evolutionary, comparative context to our understanding of the natural variation of reward as an important life-sustaining process.

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1. Introduction

Several lines of evidence indicate that drug-induced sensitization is associated with a continued and enduring amplification of positive reinforcement effects following drug intake [1–4]. The degree of drug-induced behavioral sensitization depends on the precise patterns of drug regimes [1]. For instance, repeated drug intake separated by long intervals is thought to be more effective in inducing sensitization, when compared to a chronic dosage regime involving either high and/or escalating dosage with short intervals [2–4]. Repeated, intermittent, or chronic exposure to amphetamine cause unrelenting sensitization by enhancing locomotion in rats, with marginal intensification after 3 days of treatment and profound effects after one week of treatment [5,6]. Repeated treatments of rats with morphine [7] and cocaine [8] also induce long-lasting behavioral sensitization. Even a rat's single exposure to cocaine [2,9–12], amphetamine [3] and morphine [13] produces enduring sensitization. The aforementioned studies in mammals provide a wealth of information about the consequences of a repeated and a single drug exposure regime on behavioral sensitization. Taken together, the findings across vertebrates indicate that repeated and single drug treatments can comparably induce behavioral sensitization. Whether the comparable effects of single and repeated drug regimes can be extended to an invertebrate model of drug addiction had yet to be explored. We tested this issue in the current study in wild caught population of Crayfish exposed to repeated or single morphine regime.

Aiming to unravel major scientific issues using a simpler system approach can be traced back to the genetic analysis of yeast, bacteria or fruitflies in a search for fundamental mechanisms controlling gene expression and growth in multicellular systems. There is no doubt, there are many scientific issues that are yet to be fully resolved, and most of them lie within the field of neuroscience. Precisely, how neurons and neural circuit give rise to behavior, and how experience and the external environment affect these interactions are central issues in drug addiction research. Crayfish with

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relatively highly modular, neural and neuromodulatory systems offer an intriguing model system to explore the neurobiological and behavioral mechanisms that are involved in drug-induced behavioral sensitization. This work may effectively complement studies in mammals.

It is well known that the compulsive components of addiction hinge on motivational subcortical neural circuits, with anatomical, neurochemical and motivational similarities shared across all vertebrates and even extends to invertebrates [14,15], such as crustaceans. For instance, the central nervous system of crustaceans contains neuromodulatory systems, which contain the same monoamines that in vertebrates are targets of drugs such as cocaine and morphine. The aminergic system in Crayfish is mapped on less than 1000 large and accessible neurons [16–18] that contain about 30–35 dopamine neurons located in the brain and nerve cord of Crayfish [19]. To date, all integral elements underlying addictive behaviors, are mapped on the dopamine neurcohemical system that promotes drug-associated reinforcement [37]. The dopamine is a neurochemical signal that is conserved and shared across all mammals and non mammalian species especially in invertebrates such as Bees [38], arthropods [39] and in Crayfish [18]. Crayfish with highly stereotype behavioral patterns offer an opportunity to characterize proximate neurochemical mechanisms and fundamental neurobiological changes that underlie reward to amphetamine, cocaine [20] and morphine [37] in our previous studies.

Using Crayfish in the current study, we determined whether behavioral sensitization evoked by a single and repeated drug pretreatment regimes, which are thought to represent the same neurobiochemical behavioral sensitization effect in vertebrates [21] can be observed in an invertebrate model of drug addiction. First, we characterized the effects of a single and repeated morphine exposure on locomotion behavior in Crayfish using an open field test. We evaluated locomotion performances to determine the effect of immediate morphine treatment. Five days later, we re-assessed locomotory performance to determine the presence of long-lasting effect of morphine on behavioral sensitization. In the second experiment, we used a place preference conditioning procedure that paired a bolus of morphine with the unconditioned stimulus (UCS) of a textured background environment to explore the rewarding effect of single and repeated morphine regimes in Crayfish. This allowed us to measure the strength of the association of the textured environmental cues with morphine. This article presents the immediate as well as the long-term conditioned and unconditioned behavioral changes in Crayfish that accompanied single and repeated treatments with morphine.

2. Materials and methods

2.1. Animals

Intact, intermolt male Crayfish (*Orconectes rusticus*) were used for all the experiments in this study (body weights 12.5–28.6 g). Animals were wild-caught from the Portage River near Bowling Green, OH. Individuals were maintained in the laboratory in individual plastic containers on large flow-through holding trays. Water was pumped up from a large container where it was continuously filtered and aerated. Temperature was maintained at $20 \pm 1\,^{\circ}C$. The animals were housed under 16:8 light/dark cycle and were fed twice a week with small pieces of tuna.

2.2. Apparatus

For the unconditioning experiment, we constructed a rectangular aquarium made from Plexiglas ($2.2\,m \times 0.9\,m \times 0.75\,m$). The walls of the aquarium were translucent. The tank received continuous flow of aerated water. Lighting for video recording was provided by four strip lamps with 20 W florescent bulbs at the sides of the aquarium. A digital camera (Sony DCR-VX1000) was mounted above and provided viewing angle sufficient to cover the entire aquarium. For the conditioning tests, we used the same aquarium ($2.2\,m \times 0.9\,m \times 0.75\,m$), and divided it into two compartments with the floor covered by a hard and a soft-texture respectively. The hard-textured environment consisted of thick and smooth tiles, while the soft-texture environment was created by lining the floor of the aquarium compartment with soft felt material.

2.3. Surgical protocol

Crayfish were buried in crushed ice for about 20 min in preparation for surgery. During surgery, an incision was drilled in the caudal 1/3 of the dorsal carapace, lateral of the midline to avoid damaging the underlying heart. A 15 mm section of deactivated, fine-bore, fused silica capillary (Agilent, i.d. = 250 μm) was implanted into the pericardial sinus, about 3 mm deep, and stiffened with cyanoacrylate glue. Following successful surgery, animals were returned to their plastic holding containers overnight for recovery.

2.4. Injection protocols

Deactivated, fine-bore, fused silica needle (Agilent, i.d. = 100 μm) was connected to the implanted cannula with a short section of Tygon microbore tubing (Fisher Scientific, i.d. = 250 μm). A microdialysis swivel (intech, 375/25p) prevented the canula from becoming tangled. The void volume of the cannula was filled to assure immediate delivery when the microdialysis pump (CMA Model 102, CMA Microdialysis Inc., North Chelmsford, MA, USA) was used to deliver different doses (2.5 μg 5.0 μg and 10.0 μg/g of the animal body weight) of morphine sulphate (Sigma, St Louis USA) into the pericardial system of Crayfish. 125 mM saline was used as a control. We administered drug delivered directly into the pericardial system which in crustaceans, also serves as a primary and effective neurochemical site for endogenous monoamine release [35].

2.5. Behavioral analysis

A custom-designed video tracking system was used to provide a detailed analysis of the spatial activities of Crayfish. The tracking system obtained single video frames ever 300 ms from a camera (Sony DCR-VX1000) mounted above the tank. The video signal was streamed to a video digitizer board on a Apple Power PC Macintosh (81001/100AV) computer. The location of Crayfish was obtained using a freeware, Java-based application (available at http://iEthology.com/).

2.6. Statistical analysis

We determined the pre-conditioning and CPP test outcomes by analyzing the time spent in each compartment. A direct comparison of time spent between the soft or hard texture was analyzed using the Student's *t*-test. To characterize morphine-induced unconditioned behavioral sensitization, locomotor performances were obtained for each 15 min interval within the 60 min test session. A 3×4 mixed-model ANOVA compared between-group variance between different doses of morphine (2.5 μg/g, 5.0 μg/g and 10.0 μg/g), and individual time intervals to assess pre- and 5 day post-treatment of CPP-induced rewarding effect of morphine. A statistically significant effect was followed by post-hoc pair-wise comparisons. All our analyses were done using the SPSS version 15.0 (Prentice Hall, USA). Analyses specific to each experiment are outlined in the appropriate result section. In addition, specific behavior patterns of Crayfish following the drug-induced behavioral sensitization are described.

2.7. Experimental design

2.7.1. Experiment I: unconditioned locomotion test

Unconditioned tests were conducted a day after surgery. Each Crayfish was injected with one of several doses of morphine (2.5 μg/g 5.0 μg/g and 10.0 μg/g). The Crayfish was placed into the aquarium and injected with morphine over 5 min followed by continued tracking without infusion for another 60 min.

2.7.2. Unconditioned spatial movement patterns and surface preference

Spatial activities of Crayfish were assessed inside the test aquarium. We placed individual Crayfish in the aquarium for 2 consecutive days and their spatial characteristics were monitored for 60 min each day between 10.00 and 11.00 am. Four strip lamps with 20 W florescent bulbs were mounted at the sides of the aquarium to provide illumination. The amount of time spent in each compartment was monitored and used to measure the Crayfish's natural preference for soft- or hard-textured surfaces.

2.7.3. Experiment II: Morphine-induced CPP

During the place conditioning test, 28 Crayfish were randomly assigned to one of 4 groups ($n = 7$ per group) using a two by two factorial design for all combinations of soft vs. texture and control vs. morphine (2.5 μg/g 5.0 μg/g and 10.0 μg/g doses). Crayfish received random injections of morphine in the hard and soft-texture compartments. For the control group, Crayfish received 125 mM saline injections in both the hard and soft-texture compartments. The conditioning session commenced when a Crayfish was connected to the infusion cannula and placed in the separated hard or soft-texture compartment. The separation was done using a removable Plexiglas divider. After placing the Crayfish into the aquarium, morphine injection lasted for 5 min for the session. Thereafter, Crayfish were allowed to move freely for another 25 min. For the single drug regime, prior to drug administration, Crayfish were injected with saline followed by 20 min of saline to establish baseline locomotor activity. The animals were left to move freely and not injected again