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Research report

Effects of chronic cocaine, morphine and methamphetamine on the mobility, immobility and stereotyped behaviors in crayfish

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ABSTRACT

The worth of crayfish as a model system for studies of addiction was not previously recognized because a drug-reward phenomenon had not been documented in this model system. In our previous experiments, we demonstrate that the crayfish natural reward pathways are sensitive to human drugs of abuse. This finding supports crayfish as a suitable model to characterize specific behaviors that are relevant in drug addiction research, and the current study builds on our previous findings. The aim of the present study was to investigate unconditioned neurobehavioral effects of repeated treatment regimens using cocaine, morphine, and methamphetamine for three consecutive days. We analyzed mobility, immobility and characterized stereotypic behaviors following intracardial infusions of 2.0 μg/g or 10.0 μg/g doses of cocaine, morphine, and methamphetamine for three days. The results showed that systemic cocaine, morphine, and methamphetamine increased mobility at a low dose of 2.0 μg/g more effectively than a high dose of 10.0 μg/g, while simultaneously showing that the high dose exerted a more prominent effect in increasing immobility. Moreover, systemic cocaine, morphine, and methamphetamine injections have discerning effects towards a group of defined unconditioned stereotyped behavioral patterns associated with each drug, rather than a shared universal behavioral effect. These findings provide insight into the behavioral and pharmacological basis responsible for the unconditioned effects of these drugs in crayfish.

1. Introduction

Model systems in drug addiction research have provided major breakthroughs in the understanding and characterization of the basic reward circuitry, including the development of behavioral measures to investigate the drug addiction phenomenon. The most common animal models include the roundworm *Caenorhabditis elegans* [1], the fruit fly *Drosophila melanogaster* [2], and the zebra fish *Danio rerio* [3,4]. Findings from these studies have been surprisingly consistent with analogous findings in higher-level organisms (i.e. the mouse *Mus musculus* and the rat *Rattus norvegicus* [5]). Model organisms have some characteristics in common including amenability for experimental manipulation and one or more special features, which qualify them to be outstanding subjects of research[6–8]. For instance, the fate of each of the constituent cells of *C. elegans* can be traced with exactness throughout its entire development, and in *D. melanogaster,* mutations are easily induced and the resulting phenotypes are easily observed.

Decapod crustaceans are among the most conspicuous aquatic invertebrates and are both ecologically and economically very important. Presently, there are more than 10000 species described, with 640 of them being freshwater crayfish [9]. The worth of crayfish as a model system for studies of addiction was not previously recognized because a drug-reward phenomenon had not been documented. However, in a series of behavioral and pharmacological studies, we demonstrated that the crayfish's natural reward system is sensitive to human drugs [10]. We showed that crayfish have the capability to show a conditioned place preference for environments in which they received cocaine, morphine and methamphetamine [11–15]. Moreover, we provided evidence of drug relapse, reinstatement, and drug seeking behaviors [16]. These findings indicate the usefulness of this model system in drug addiction research comparable to other invertebrate models.

Stereotypic behaviors, mobility and immobility parameters are behaviorally and pharmacologically important in drug addiction, because they measure the hypoactive or hyperactive effects particularly during

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drug-induced behavioral sensitization [17]. Crayfish, combines a complex behavioral gamut with detailed knowledge of neurochemical systems and a body size that supports in-vivo manipulations. Therefore, crayfish offer an excellent model system to characterize stereotypic behaviors associated with the differential effects of mammalian drugs of abuse (cocaine, morphine and methamphetamine). Our previous series of behavioral and pharmacological studies [10,14,16] in a place preference paradigm test revealed that cocaine and methamphetamine enhanced active exploratory locomotion responses in a dose dependent manner when crayfish were placed into a novel arena and drugs were infused systemically, into the pericardial system, or the head ganglion. These findings indicate that injected drugs have the capability to exert effects at a number of neural sites in crayfish, especially the stimulation of circuits for active locomotion behaviors. However, it is not clear whether there is the presence of selective and stereotypic behavioral effects associated with a specific drug, rather than more general effects. This study determined whether these drugs could differentially regulate subcomponents of locomotion, with a separate effect on each stereotypic behavior. The primary goal of this study is to explore the differential effects of cocaine, morphine, and methamphetamine in recruiting specific adaptive mobility responses in crayfish. For instance, if two or more drugs are injected into crayfish, is it possible that at the same dose, the effect of one drug might stimulate locomotion, and another drug could suppress locomotion, or have different effect altogether? Therefore, we determined whether repeated injections of cocaine, morphine and methamphetamine regime have distinct effects on multifarious locomotor activity of crayfish. We evaluated the unique mobility, immobility responses and stereotypic behaviors that match-up the specific pharmacological effects of cocaine, morphine and methamphetamine on crayfish. Our analyses identified the specific and differential effects of cocaine, morphine, and methamphetamine on locomotion and non-locomotion behavioral responses. The current study of characterizing crayfish's response to chronic cocaine, morphine and methamphetamine treatments further contributes to the wealth of theoretical and experiential contributions towards a broader understanding, and the characterization of the central workings of addiction at its key sites in crayfish.

2. Materials and methods

2.1. Animals

Intact intermolt male crayfish *(Orconectes rusticus;* weighing between 12.8–30.3 g) were used for all the experiments in this study. Animals were wild-caught in the local stream and transferred to the laboratory where they were kept in individual plastic containers on large flow-through holding trays filled to 2 cm depth with water, at a density of 20 animals per tank. The holding room was maintained on a 12 h light/dark cycle lights on from 0700 to 1900 h. The crayfish were fed small pieces of tuna every 3 days, and after feeding, the water was changed. Temperature of the holding and experimental rooms as well as the alley between them, was kept at 22 ± 1 °C.

Surgical protocol

In preparation for surgery, crayfish were cold-anesthetized by burial in crushed ice for approximately 20 min in an acrylic plastic box (4.0 m × 2.0 m × 2.0 m). During surgery, an incision was drilled in the caudal 1/3 of the dorsal carapace, lateral to the midline to avoid damaging the underlying heart. A 15 mm section of deactivated, fine-bore, fused silica capillary (Agilent, i.d. = 250 m) was implanted into the pericardial sinus, about 3 mm deep, and glued to the back of the animal. Following successful surgery, animals were returned to their plastic holding containers overnight for recovery.

2.2. Injection protocols

A deactivated, fine-bore, fused silica needle (Agilent, i.d. = 100 m) was connected to the implanted cannula with Tygon microbore tubing (Fisher Scientific, i.d. = 250 m). A microdialysis swivel (Intech, 375/25p) was utilized to prevent the cannula from becoming tangled. Drugs were administered to the pericardial system of crayfish using a microdialysis pump (CMA Model 102, CMA Microdialysis Inc., North Chelmsford, MA, USA). The void volume of the cannula was filled to enhance the immediate delivery of the drugs from the microdialysis pump (CMA Model 102, CMA Microdialysis Inc., North Chelmsford, MA, USA). Crayfish sizes were determined to accurately measure the correct amount of drug to administer for the size of each crayfish, and were then randomly assigned to an experimental group of cocaine, methamphetamine or morphine. We injected 2.0 μg/g or 10.0 μg/g doses of cocaine, methamphetamine or morphine (Sigma, St Louis USA) into the pericardial system of crayfish. 125 mM saline was injected for the control. The focus of the drug injections was into the pericardial system because, in crustaceans, it serves as a primary and effective neurochemical site for endogenous monoamine release [18], and any manipulation of amines at this site are translated to the nerve cord. Different drug dosages were selected based on a previous study [10] that revealed that low doses of mammalian drugs are effective in stimulating behavioral responses of crayfish [19]. We chose a low dose of 2.0 μg/g (dose) and a high dose (10.0 μg/g) to provide a wide range between a low and high range in determining the specific effects of these drugs on the activity of crayfish.

2.3. Behavioral analysis

We used a custom-designed video tracking system to measure the spatial activities of crayfish. The tracking system tracked 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 an Apple Power PC Macintosh (81001/100AV) computer. The location of crayfish was obtained using a freeware, Java-based application (available at http://iEthology.com/). Crayfish were randomly assigned to six experimental groups (n = 15): 2.0 μg/g cocaine, 2.0 μg/methamphetamine, 2.0 μg/g morphine, 10.0 μg/g cocaine, 10.0 μg/g methamphetamine, and 10.0 μg/g morphine. Each experimental group is controlled by vehicle injection (125 mM saline) which serve as control. Total injection volume was adjusted to 1/50 of the estimated hemolymph volume for each crayfish which was determined in previous experiments [20]. Drugs were injected ventrally into the second abdominal segment, lateral to the nerve cord. The syringe was held in place for approximately 20 s to prevent leakage from the injection site. Crayfish were injected with 2.0 μg/g or 10.0 μg/g of cocaine, morphine or methamphetamine over a five minute time span followed by continued tracking with no further injection for sixty minutes. This was repeated for three consecutive days. Activity tests were conducted three days after surgery, to allow ample time for a full recovery. Each crayfish was placed in an aquarium, and first injected with saline to establish baseline locomotion. Twenty minutes later, the crayfish were injected with 2.0 or 10.0 μg/g of cocaine, morphine or methamphetamine over five min followed by continuous tracking without infusion for another 60 min (Fig. 1).

2.4. Behavioral data analysis

Behavioral activities of each animal following each injection in the arena were recorded for sixty minutes. The different spatial activities of crayfish in the drug-treated animals were analyzed using a custom-designed video tracking system. The tracking system processes single video frames at 320/ms from a camera (Sony DCR-VX1000). The camera which was directly connected to the computer, was mounted above the tank to provide a general profile view of the spatial activities