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# The rewarding properties of methamphetamine in an invertebrate model of drug addiction

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## HIGHLIGHTS

- Methamphetamine is rewarding to crayfish when paired with a distinct stimulus.
- Crayfish is sensitive to different doses of METH.
- CPP offers a comparative method for addiction research in invertebrates.

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## ABSTRACT

The rewarding properties of drugs in the mammalian system depend on their ability to activate appetitive motivational states. The associated underlying mechanism is strongly conserved in evolution and invertebrates have recently emerged as a powerful new model in addiction research. The natural reward system in crayfish has surprisingly proven sensitive to human drugs of abuse, providing a new model for research into the basic biological mechanisms of drug addiction. In this study, we examined the presence of natural reward systems in crayfish, and then characterized its sensitivity to 2.5 μg/g, 5.0 μg/g and 10.0 μg/g doses of methamphetamine (METH). Using the conditioned place preference (CPP) paradigm, we demonstrated that irrespective of the number of doses of METH injected into the pericardial system, crayfish seek out a particular tactile environment that had previously been paired with the METH. This study demonstrates that crayfish offer a comparative and complementary approach in addiction research. It contributes an evolutionary context to our understanding of a key component in learning and of natural reward as an important life-sustaining process.

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## 1. Introduction

Functional and evolutionary conservation of neural circuits of reward is a feature of survival; it is found from insects to humans. In humans, a unique stimulus could elicit behavioral sensitization resulting in conditioned response despite abstinence from drugs for years [1]. Such effects caused by drugs can alter brain functions, and the resulting drug-associated behaviors can, in turn, be activated and maintained when a particular environmental cue is associated with the effect of the drug [2]. In the absence of the drug, the conditioned stimulus can sustain and even re-establish drug-seeking behavior [3]. In fact, the drug-related conditioned stimulus could maintain its efficiency for weeks after the initiation of withdrawal in rats [4]. Studies of the relationship between a behavioral response and a drug-related environmental stimulus in vertebrates [5–7] led to the inference that the attractiveness or the positive valence of the environmental cues can directly induce behavioral sensitization and promote drug-seeking behavior. Whether such generalizations can be extended to an invertebrate model of drug addiction is yet to be fully explored. The brain of crayfish has few neurons [8] when compared with the billions of neurons in the human brain.

What the brain of crayfish lacks in complexity, it makes this up in a way that makes crayfish an appealing animal in behavioral and addiction research. The crayfish model continues to play a unique role among invertebrate models in the study of neural mechanisms underlying a variety of behavioral phenomena. This is largely due to the presence of a nervous system that is uniquely amenable to a wide variety of neurophysiological, anatomical and biochemical approaches. Containing a reduced number of elements with neurons that can be repeatedly recognized across subjects, the crayfish is an excellent model to

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identify and characterize behaviors that are relevant in reward seeking [9]. With large 30–35 dopamine neurons that can repeatedly be recognized across subjects [8], the main strength of this model lies in the special experimental opportunities to first identify and then characterize the relevant neural circuits underlying a specific behavioral plasticity associated with reward. The simplicity of the crayfish system and the detailed knowledge about it enables us to explore activities of the well-known neuromodulators, and the brain neural network in more detail. In turn, this will help in development of crayfish into a new model for drug addiction research.

We have previously demonstrated that repeated intracirculatory infusions of morphine [2] and cocaine [10] serve as a reward when paired with a distinct visual or tactile environment. The current study represents an extension of these efforts, with the ultimate goal of developing crayfish into a new and robust model for drug addiction research. In the current study, we determined whether METH is rewarding to crayfish when paired with a distinct environment. We used a conditioned place preference procedure that paired METH, the unconditioned stimulus, with a distinct tactile environment to test the rewarding properties of METH in crayfish. We tested for the presence of CPP, and then explored the time course of the expression of the METH-induced CPP, as well as the movement of crayfish between the two compartments over the test session.

## 2. Materials and methods

### 2.1. Animals

Twenty-one male, intermolt male crayfish (*Orconectes rusticus*) with complete and intact appendages were collected from the local river. In the laboratory, the animals were maintained in a big tank of water (62″L × 29″W × 70″H; 400 gal) that is freshly aerated and flows through holding trays. Once in the laboratory, the animals were isolated in individual plastic containers (160 mm diameter, 95 mm depth) and maintained in flow-through holding trays that received freshly filtered/aerated water at 20 ± 1 °C. Crayfish were fed 1–2 times per week with tuna fish, earthworms or rabbit chow and housed under a 16:8 hour light/dark cycle.

### 2.2. Procedure

#### 2.2.1. Spatial activities and the initial unconditioned preferences of crayfish

Preliminary experiments were designed to explore the spatial activities of crayfish (body weights between 12.5 and 32.3 g), and the initial unconditioned preferences of crayfish in a drug-free condition inside the test aquarium. We conducted several preliminary trials during which most of the animals exhibited a population-level preference for the soft-textured compartments of the conditioning aquarium. We conducted the initial trials by placing individual crayfish in the aquarium for two consecutive days and monitored their spatial characteristics for 60 min. We used the amount of time spent in each compartment to assess the spatial activities and the initial unconditioned preferences. Analysis of the spatial activities of individual crayfish allowed us to use suitable controls in the CPP experiments. For instance, if an individual crayfish served as its own control condition in the METH conditioning phase of the experiments including determining the preference of each individual through an initial screening trial [11], the inter-trial reliability of such a presumed environmental preference would have been equivalent to a coin flip [12]. In the initial trials, we observed a stochastic preference for the soft-textured environment. This preference was expressed at the level of the whole experimental population and the preference shifted in the subsequent CPP experiments. Determination of the spatial activities and the initial unconditioned preferences of crayfish informed the decision to use an independent control group, rather than a within-subjects design in the METH-conditioning experiments. For the conditioning, crayfish were randomly assigned into three groups ($n = 7$ per group): (i) control, (ii) hard-texture/METH and (iii) soft-texture/METH groups.

#### 2.2.2. Designing of the place conditioning experiment to measure reward

The place-conditioning apparatus consisted of an opaque-white Plexiglas aquarium, measuring 220 mm × 90 mm × 75 mm (length, width and height). Water flowed in and out of the arena through tubes at each end of the aquarium. Four strip lamps, with 20 watt fluorescent bulbs mounted on the ceiling of the experimental room to provide lighting for the video recording of the behavioral activities of the animals. A digital camera (Sony DCR-VX1000-NTSC) was mounted above the tank and its image projected a view of the entire aquarium. Two distinctive cues that comprised of textural cues were used as the environmental stimuli in each aquarium. The aquarium was divided into equal compartments such that a distinct textured environment was always present in the opposite compartment. A removable, Plexiglas barrier separated the aquarium into four zones that comprised of two distinct tactile environments (soft and hard environments). The materials in the hard texture environment comprised of four white walls with a hard floor covered with crushed concrete gravel that was composed of unconsolidated rock fragments roughly between the class sizes of 5 and 10 mm.

The other zone comprised of four white walls and a floor covered with soft sand. The materials covered the entire floor of the aquarium so that the animals could not detect edges. The differences between the compartments consisted of soft and hard textural cues (Fig. 1). The first two compartments are represented by A and B, while the second two compartments are represented by C and D.

#### 2.2.3. Surgical procedure for implantation of cannula for drug injection

During surgery, the animals were anesthetized in crushed ice for about 20 min. Since the hematopoietic system of the crayfish is anteriorly located, we focused our surgery on the dorsal carapace, such that an incision was created in the caudal, 1/3 of the dorsal carapace, lateral of the midline. This was to avoid damaging the heart blood vessels and destroying the heart. Using our approach of focusing our surgery on the dorsal carapace, we achieved a 95% success rate in our surgeries without damaging the heart. A 15 mm section of deactivated, fine-bore, fused silica (Agilent, i.d. = 250 μm) was implanted into the pericardial sinus (allowing 3 mm to enter the sinus) and reinforced with superglue and bonding material. Following successful surgery, the animals were allowed to recover overnight.

#### 2.2.4. Drug injections

A microdialysis swivel (Intech, 375/25p,CMA Model 102, CMA Microdialysis Inc., North Chelmsford, MA, USA) was used to systematically inject METH (HCl, FW: 339.8; Sigma, St. Louis: C 5776) in different doses in the pericardial system of the crayfish. The different doses referred to the free-base concentrations and the METH was prepared in 125 mM saline. Different doses of METH were injected directly into the pericardial system which serves as a primary neurochemical site for endogenous monoamine release [8,12]. Injections of 125 mM saline served as the control. During injection protocol, we connected the deactivated, fine-bore, fused silica needle (Agilent, i.d. = 100 μm) to the implanted cannula with a short segment of Tygon microbore tubing (Fisher Scientific, i.d. = 250 μm). The injection was administered through the implanted cannula into the nerve cord as shown in Fig. 2. The syringe remained in place for approximately 15 s to avoid leakage from the point of injection. A successful cannula implant was confirmed via behavioral consequences of one bolus injection of 20 μg/g METH following the conclusion of the experiment. A strong reaction to the injection serves as a condition for inclusion of an animal in this study. The strong reaction was characterized by small muscle tremors in the walking legs, and a rapid upward movement of the whole body, but there was a no flipping of tail as was observed in our previous studies following a high dose of drug injection. We lost some animals during the course of the