Physiology & Behavior 153 (2016) 40–46

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

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

Adebobola Imeh-Nathaniel [d], Adekunle Adedeji [b], Robert Huber [c], Thomas I. Nathaniel [a,*]

[a] University of South Carolina, School of Medicine-Greenville, 607 Grove Road, Greenville, SC 29605, USA
[b] Department of Health Sciences, East Tennessee State University, Johnson City, TN 37614, USA
[c] Department of Biological Sciences, Bowling Green State University, Life Sciences Bldg., Bowling Green, OH 43403, USA
[d] Department of Biology, North Greenville University, Tigerville, SC 29688, USA

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.

ARTICLE INFO

Article history:
Received 9 November 2014
Received in revised form 11 September 2015
Accepted 14 October 2015
Available online 22 October 2015

Keywords:
METH
CPP
Crayfish
Drug
Addiction
Reward
Invertebrate

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

* Corresponding author at: Department of Biomedical Sciences, University of South Carolina, School of Medicine-Greenville, 607 Grove Road, Greenville, SC 29605, USA.
E-mail address: nathanit@greenvillemed.sc.edu (T.I. Nathaniel).

http://dx.doi.org/10.1016/j.physbeh.2015.10.017
0031-9384/Published by Elsevier Inc.

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

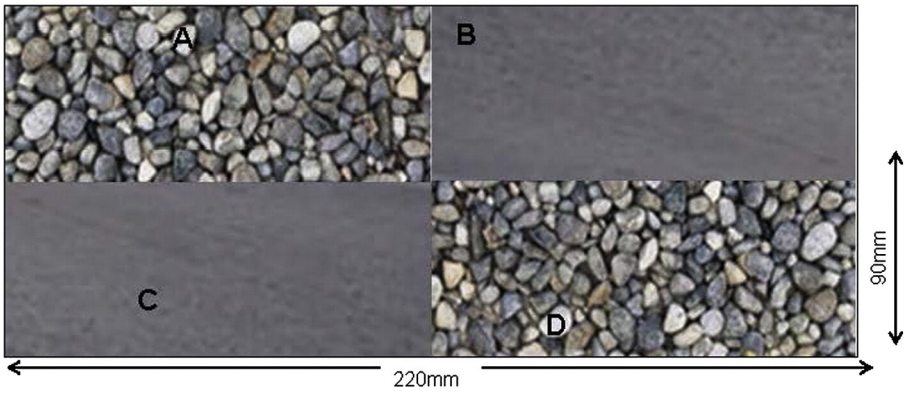

Fig. 1. The experimental aquarium was divided into two zones. The first zone comprised of four white walls with a hard floor covered with crushed concrete gravel that composed of unconsolidated rock fragments of size classes roughly between 5 and 10 mm. The second zone comprised of four white walls and a floor covered with soft sand. The sand covered the whole floor of the aquarium, such that the animals could not detect edges. The differences between the two compartments consisted of soft and hard textural cues.

the experiments. The number of loss ranged between 4 and 6% for the 2.5 μg/g and 5.0 μg/g and less than 2% for the 10.0 μg/g dose. This might not be attributed to the toxicity of METH especially as we did not record many losses in the 10.0 μg/g dose. It could be associated with the positioning of the cannula probably affecting the pericardial system. In general, we recorded over 90% success in the implantation of the cannula and METH injections. This is similar to what we had in our previous studies on cocaine or morphine. Our previous studies provided the basis for investigating the context specificity of the 2.5 μg/g, 5.0 μg/g and 10.0 μg/g doses of the METH-conditioned novelty effect in crayfish. Similar doses were rewarding for cocaine [10] and morphine [13].

2.2.5. Behavioral analysis

We used a video-tracking system to analyze spatial activities of crayfish. We set up the system to extract the spatial coordinates of crayfish from a single video frame at a temporal resolution of 1/3 Hz. The digital camera (Sony DCR-VX1000) that was mounted on the ceiling recorded the behavioral activities of crayfish. The signal from the camcorder was streamed to a video digitizer on a powered Macintosh (81001/100AV) computer. Video tracking was performed using a freeware Java framework for the analysis of the behavioral data (available on the Internet at http://caspar.bgsu.edu/software/Java/).

2.2.6. Testing of drug (METH-induced) CPP

We previously demonstrated that repeated intracirculatory infusions of 2.5 μg/g, 5.0 μg/g and 10.0 μg/g doses of morphine over five days served as a reward when paired with a distinct visual or tactile environment [13] and thus stimulated unconditional behavioral responses in crayfish [2]. Similar doses of cocaine were rewarding to crayfish when paired with a distinct visual stimulus [12]. The aforementioned studies provided the basis for investigating the context specificity of the 2.5 μg/g, 5.0 μg/g and 10.0 μg/g doses of the METH-conditioned novelty

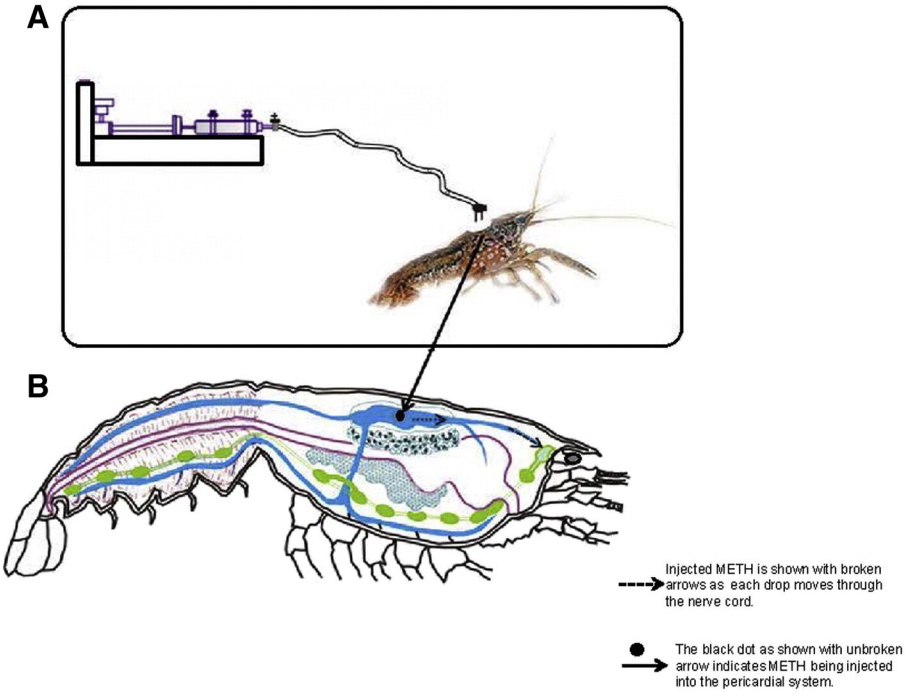

Fig. 2. Method for delivering METH into the pericardial system of crayfish. During the METH injection, 0.5 m of deactivated, fine-bore, fused silica needle (Agilent, i.d. = 100 m) was coupled to a crayfish with implanted cannula in the pericardial system at the caudal, 1/3 of the dorsal carapace (A), directly into the pericardial sinus magnified in (B). The coupling was done using Tygon microbore tubing (Fisher Scientific, i.d. = 250 m). The tubing was connected to a microdialysis swivel (Intech, 375/25p).

effect in crayfish. Similar doses were rewarding for cocaine [10] and morphine [13] in our previous studies.

The place-conditioning paradigm used in this study has been described previously [13]. Briefly, crayfish were randomly assigned into three groups ($n = 7$ per group) including controlled, hard-texture/METH and soft-texture/METH groups, such that the hard-texture/METH or soft-texture/METH group received different doses of METH during conditioning. Each group of animals received the same dose of METH (2.5 μg/g, 5.0 μg/g and 10.0 μg/g) each day. The control group received a saline injection during conditioning. For 5 successive days, each animal received two conditioning sessions per day (morning and afternoon), one in each environment (i.e., METH-treated crayfish received 1 drug and 1 vehicle infusion/day in the first two compartments (represented by A and B), while crayfish in the control group received 2 vehicle infusions/day in the second two compartment (represented by C and D). Our experimental design consisted of three phases (Fig. 3), pre-exposures that explore the spatial activities of crayfish, the conditioning and the CPP test. In the pre-exposure test, we measured spatial characteristics of crayfish locomotion within the test aquarium by placing individual crayfish ($n = 7$) in the test aquarium for two consecutive days for 60 min.

We measured their movement and spatial activities in the aquarium using the video-tracking system. A detailed description of the experimental design used in the current study is presented in Fig. 3. During the conditioning trials, we attached the injection cannula to the tubing and directly connected it to the crayfish. The animal was gently placed in the experimental aquarium followed by a METH injection for the first five minutes of the 30 min session. Conditioning sessions were conducted twice per day. Each animal was restricted to one side of the CPP apparatus for 30 min during a morning session and confined to the opposite side of the apparatus for 30 min during the afternoon session. We used the biased-CPP design by pairing the unconditioned stimulus (US) with the initially non-preferred side of the apparatus. We adapted this approach because previous studies [14] revealed that though reward-CPP is established regardless of whether a biased or unbiased design is used, the biased design approach has an advantage of greater sensitivity in detecting varying degrees of preference shifts [15]. All possible pairwise combinations of the environment and drugs were tested during conditioning. For instance, the starting side for the first conditioning session was the hard-texture/METH group that was exposed to their initially hard-texture, non-preferred side immediately following drug injection. The control group of crayfish was exposed to their initially soft-texture, preferred side immediately following saline injections. Animals received the opposite of these conditions during the afternoon session. Likewise, the soft-texture/METH group was first exposed to the soft-texture, preferred environment following METH injections followed by saline injections. The control group was exposed to the hard-texture, non-preferred environment. Subsequently, the animals received the opposite of these conditions during the afternoon session. The control group consisted of crayfish that received vehicle infusions in both the hard-texture and soft-texture environments. A vehicle-treated animal received two saline injections each day. Conditioning sessions were conducted at the same time each day. In summary, for five successive days, each animal received two conditioning sessions per day (morning and afternoon), one in each environment (i.e., METH-treated crayfish received 1 drug and 1 vehicle infusion/day in the first two compartments, while crayfish in the control group received 2 vehicle infusions/day in the second two compartments. Morning and afternoon sessions were separated by 9 h (8.00 a.m. and 6:00 p.m.) to allow for sufficient METH clearance from the hemolymph.

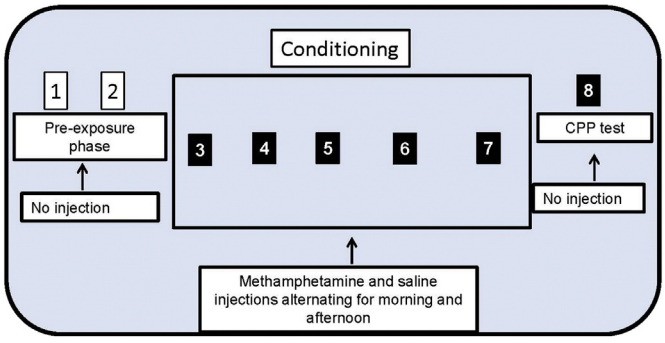

Fig. 3. Schematic illustration of the experimental design used in the current study. Our experimental design comprised of three phases: pre-exposures that explore the spatial activities of crayfish, the conditioning and the CPP test. The conditioning consisted of five alternate days (3–7) of drug and saline injections. For the conditioning experiment, crayfish received METH injections in both the hard-texture and the soft-texture environment in a biased approach experimental design (i.e., drug was paired with the initially non-preferred side). Different doses of METH were injected alternately for five consecutive days. Following conditioning, the animals were confined to the conditioning compartment for 30 min. The partition separating the compartments was removed, and on day eight crayfish were placed at the center and allowed to move freely for 60 min. No injections were given on day eight of the preference test, thus maintaining the same procedure as that used during the preliminary baseline test of exploring the spatial activities of crayfish. Crayfish was allowed free access to the entire aquarium for 60 min. The amount of time spent in each compartment was recorded to assess individual unconditioned preferences.

2.2.7. Measuring METH-induced CPP

For the CPP test, the Plexiglas barrier was gently removed to prevent the textural cues, particularly the sand, from drifting and mixing. Each crayfish was placed at the center of the aquarium. Animals were allowed to move in both the hard and soft-texture compartments for 60 min. By allowing the animal free access to both environments, we were able to maintain the same protocol that we used when measuring the spatial activities of crayfish in an unconditioned environment. The amount of time spent in each environment was measured to determine individual, unconditioned preferences. The percentage of time spent in each compartment was expressed for the 60 min duration of CPP test. We used an increased time spent in the paired environment as a measure of preference for the specific stimulus. It is important to point out that we used the conditioned place-preference procedure approach to measure the rewarding effects of METH in crayfish by pairing METH as the unconditioned stimulus with two contrasting tactile environments. Each animal had the opportunity to explore either environment and the time spent in either environment was considered as an index of the reinforcing value of METH. The animal's preference to spend more time in either the soft or hard compartment was considered an expression of the positive-reinforcing experience within that compartment. In this context, our CPP test associated METH consumption and the learned environment; that was, in turn, used to measure the rewarding properties of METH in crayfish. The CPP variable was % time spent in the drug-paired environment on the test day (% time on drug-paired side = (minutes on drug-paired side / 60 min) × 100%).

2.3. Statistical analysis

We used the pre-conditioning and CPP test outcomes to determine the amount of time spent in each compartment. A direct comparison of time spent between the soft or hard was analyzed using the Student's *t*-test. We used ANOVA to determine the significant effect of different doses of METH (2.5 μg/g, 5.0 μg/g and 10.0 μg/g) in CPP-induced rewarding effect of METH. Statistically significant effects were followed by Bonferroni post hoc comparisons. In using ANOVA, we considered the independence of the groups being compared. We used Mauchly's test to test for sphericity to meet the assumption that the relationships between pairs are equal in the parametric test. The normal distribution of all data was tested with the exploratory data analysis (EDA) before the use of parametric test.

3. Results

In assessing the spatial activities of crayfish in the drug-free unconditioned environment, our hypothesis was that crayfish will spend an equal amount of time in each of the tactile (soft or hard texture) environments. However, it turns out that crayfish preferred the soft environment following repeated measures of the spatial activities for one hour each day. During the first day, crayfish spent 57.02% ± 2.14 (S.E.M.) of time in the soft compartment and 39.01 ± 2.13% (S.E.M.) in the hard, rocky compartment. The preference was significant (*t*-test (μ = 50.0%); $t[6] = 3.56$, $P = < 0.05$). During the second day, crayfish maintained the preference for the soft compartment (61.01% ± 4.67) while 42.05% ± 4.24 (S.E.M.) of its time was spent in the hard compartment (Fig. 4). The preference for the soft compartment shifted to the hard environment during the CPP test.

3.1. Systemic injection of METH is rewarding to crayfish after five days of CPP conditioning and test

For the hard-texture/METH group, the crayfish showed a significant environmental preference following treatment with METH when conditioned in a textured compartment for five days. METH-induced CPP was observed such that 2.5 μg/g of METH produces 52.9% ± 1.9 (S.E.M.), while 5.0 μg/g and 10.0 μg/g promote spatial activity of 56.8% ± 2.8 (SEM), and 64.49% ± 3.3 (S.E.M.), respective of time preference for the hard-textured compartment (Fig. 5). The METH-conditioning effects were shown when crayfish that were treated with METH were paired with the naturally unpreferred hard environment. ANOVA ($F[4,30] = 21.13$; $P < 0.001$) reveals a significant effect, indicating a larger amount of time being spent in the METH-paired, hard-textured compartment when compared to saline conditioning, such that a conditioned place preference was established. Eta-squared, indicating a measure of effect size, is large (0.88), suggesting that METH-induced place preference for the hard-texture compartment produced 88% of the overall (effect + error) variance. The ANOVA factor indicated the METH conditioning effect on crayfish was high (statistical power; $1 - \beta = 1.00$) suggesting that METH-induced CPP can be consistently replicated with a high degree of reliability.

For the soft-texture/METH group, crayfish spent a greater amount of time in the saline-paired compartment (2.5 μg/g; 58.1% ± 2.3 (S.E.M.), 5.0 μg/g; 59.4% ± 3.1 (S.E.M.), and 10.0 μg/g; 61.2% ± 3.0 (S.E.M.) than the METH-paired compartment (2.5 μg/g; 41.9 ± 1.8 S.E.M.), 5.0 μg/g; 40.6 ± 1.3 (S.E.M.) and 10.0 μg/g; 38.8 ± 1.2 (S.E.M.). This result indicates that vehicle-treated crayfish showed a natural preference for the soft-textured compartment (Fig. 6).

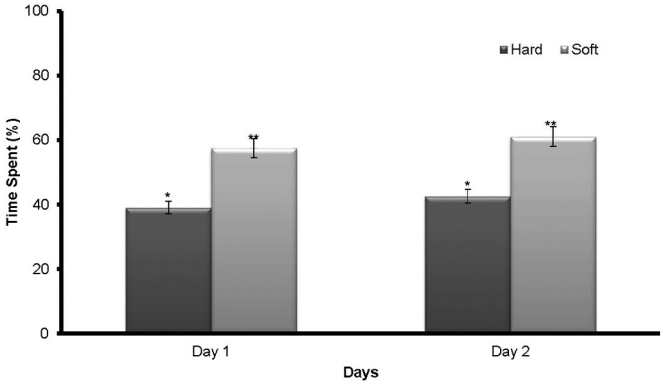

Fig. 4. The crayfish seemed to prefer the soft-texture compartment the following two days of repeated measures. The preference for the soft-texture compartment was significant in the first day (*t*-test (μ = 50.0%); $t[6] = 3.56$, $^{**}P = < 0.05$). During the second day, crayfish maintained a significant (*t*-test (μ = 50.0%); $t[6] = 3.53$, $^{**}P = < 0.05$) preference for the soft-texture compartment, and the preference for the soft compartment shifted to the hard-texture environment during the CPP test.

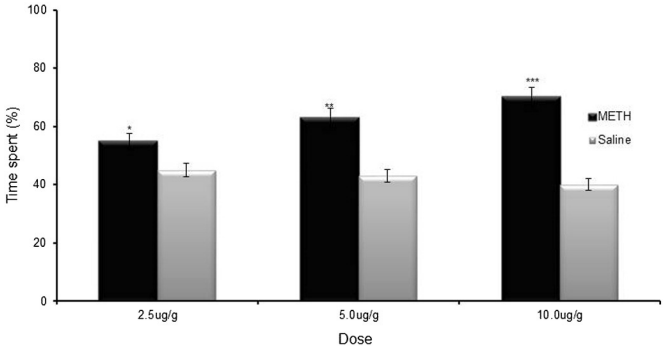

Fig. 5. Repeated infusions of METH induced CPP in crayfish in the hard-textured environment. Crayfish showed a significant preference for the hard-textured compartment following five days of injections with 2.5 μg/g, 5.0 μg/g and 10.0 μg/g doses of METH, such that a conditioned place preference was established. Post hoc test comparison indicates that crayfish treated with 5.0 μg/g and 10.0 μg/g ($^{***}P < 0.05$ and $^{**}P < 0.01$) were higher and different from the crayfish treated with 2.5 μg/g of METH ($^{*}P < 0.001$) when compared with the saline-paired crayfish.

In summary, repeated infusions of different doses of METH induced CPP in crayfish in the hard-textured environment such that a conditioned place preference was established, when compared with the control, i.e. saline-paired crayfish. Therefore, crayfish only formed a reward association when the METH treatment was paired with the hard-textured environment, but not when it was conditioned with the soft-textured environment. On the other hand, repeated injections of different doses of METH did not produce a CPP in the soft-texture, such that crayfish spent a greater amount of time in the saline-paired compartment than in the METH-paired, soft-texture.

4. Discussion

We previously demonstrated that repeated intracirculatory infusions of 2.5 μg/g, 5.0 μg/g and 10.0 μg/g doses of morphine over five days served as a reward when paired with a distinct visual or tactile environment [13,16] and stimulated unconditional behavioral responses in crayfish [2]. A previous study [12] reveals that crayfish is sensitive to incentive properties of the conditioned stimuli when paired with lower doses of cocaine. The aforementioned studies encouraged us to examine context-specificity of the METH-conditioned novelty effect in crayfish.

Our finding that METH-induced CPP was established in crayfish in a specific environmental textural cue (hard-texture stimulus) reveals that METH targets neural pathways in crayfish that serve as powerful rewards. This finding indicates that crayfish may represent an efficient model for studying the primary sites of METH to explore the proximate

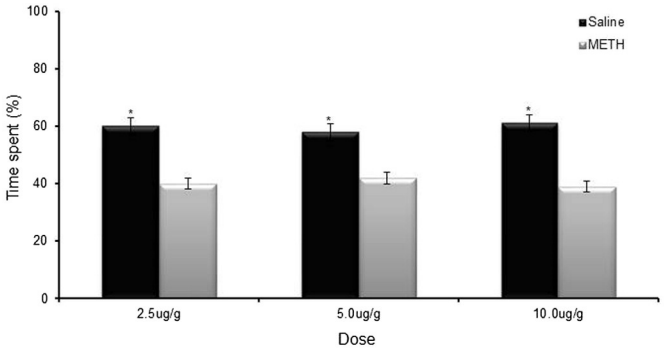

Fig. 6. Repeated infusions of METH in crayfish ($n = 7$) for the soft-texture environment during CPP test. Paired, repeated injections of 2.5 μg/g, 5.0 μg/g and 10.0 μg/g doses of METH did not produce a CPP in the soft-texture, such that crayfish spent a greater amount of time in the saline-paired compartment than in the METH-paired, soft-texture compartment ($F[4,30] = 6.86$, $^{*}P < 0.005$).

mechanisms and fundamental neurobiological alterations that underlie drug addiction in an invertebrate model. Irrespective of the dose, METH was perceived as rewarding by the crayfish when paired with the hard-texture compartment. Indeed, a METH-induced CPP was established in all doses of METH injections when compared with saline injections. The established CPP was significantly lower at 2.5 μg/g when compared with 5.0 μg/g and 10.0 μg/g doses of METH. Although there was no statistical significant difference between CPP induced at 5.0 μg/g and 10.0 μg/g doses, a METH-induced CPP was established at all METH doses because there was propensity for a greater preference of the METH-paired compartment at higher doses (see Fig. 3). Our finding that CPP was established only in the hard-texture compartment and not in the soft-texture compartment, suggests that crayfish might have found the hard texture environment to be novel when compared to the soft-texture environment.

An important question relevant to our result is; how is the hard-texture more novel particularly since they live in the hard environments in the wild where they were taken from? Clearly, crayfish live under rocky structures and hide under them for protection [17]. In this context, a successful adaptive behavior in a complex environment such as the one that crayfish lives, including learning and decision making about food, shelter or conspecifics requires accurate assessment of actions and choices, and contribute to a particular life style. The ability of crayfish's brain to integrate and control such adaptive responses may enhance the search for life-supporting environmental conditions including identifying protective shelters under the rock- an abode for the crayfish. For this reason, the specificity for the preference of the hard-texture environment may be related to the inherent ability to use tactile cues to find a hard rock for shelter especially when they need to withdraw under a rock, waiting for dark, at which time they come out to forage for food. The structural characteristics of a preferred environment or object by crayfish are modulated by the neural mechanism that is associated with tactile response. It is possible that crayfish might have used such ability to distinguish between the two contrasting environments provided during the CPP experiments. Indeed, the hard-textured rocks may have been perceived as relatively novel compared to the soft nature of the soft sand environment in the aquarium. In this context, novelty is conceivably implicated in the susceptibility of stimulus to a conditioning phenomenon in crayfish. The formation of reward in crayfish can be linked to the pleasurable effects of METH to the hard-textured stimulus as the environmental facilitator. That crayfish only formed a reward association when the METH treatment was paired with the hard-textured environment, but not when it was conditioned with the soft-textured environment indicates that such explicitness may be linked to the inherent sensitivity to the saliency of the hard-textured environment. A change in a potential affective state of crayfish and associative learning possibly facilitate the formation of a conditioned response to the METH-paired hard-texture environment. Taken together, crayfish as a model of drug addiction illustrates the conceptual reliability of the sensitivity and rewarding properties of mammalian drugs of abuse to an invertebrate system, which unlike mammals exhibits simple neuronal organization.

In mammals, psychostimulants are generally known to interfere with the monoamine chemistry to induce reward when exposed to a distinct visual environment [12]. Our finding that METH is rewarding to an invertebrate system with simple neuronal organization indicates that mammalian drugs of addiction are likely to initiate reward beyond those peculiar to humans. Since behavioral sensitization to drugs is a consequence of repeated drug administration that results in augmentation of the behavioral effects upon re-administration [18–20], behavioral responses of crayfish could be attributed not only to a direct pharmacological effect of the METH but also to learned associations of the distinct hard-textured stimulus with the drug-rewarding experience. Although mammalian drugs of abuse vary in their molecular mechanisms of action [21–25], they generally have the ability to induce many behavioral and neurobiological adaptations. For this reason, addictive drugs can take over and control a behavior when the brain fails to distinguish whether specific reward circuits were activated by adaptive natural rewards or falsely triggered by a class of addictive psychostimulant substances such as METH, cocaine [18,26–28] or morphine [29]. Indeed, activation of major substrates or circuits of reward by addictive drugs can be more consistent and powerful than activation triggered by natural contexts. These adaptations represent important concepts for study in drug addiction research because subsets of the behavioral and neurobiological adaptations form the core processes that mediate addiction. The ability of the crayfish's brain to integrate and control such adaptive responses may enhance the search for life-supporting environmental conditions including identifying protective shelters under the rock. In the current study, we report that the environmental context influences the ability of METH to engage the brain circuits of crayfish potentially involved in drug reward-dependent behavioral plasticity. Future studies are necessary to identify specific brain targets and circuits of METH-associated reward in the brain of crayfish.

We are particularly excited about the potential role of the crayfish model in behavioral neuroscience research, especially in contributing to an evolutionary, comparative context to our understanding of natural reward as an important life-sustaining process. Our current ability to characterize i) experimental conditions of natural and agonistic-responsive reward mechanisms in crayfish, ii) precise pharmacological and behavioral conditions under which METH-associated reward fosters place conditioning, presence and extent of sensitization, iii) extinction and reinstatement with dose–response relationships [13], and iv) molecular mechanism of cue-elicited rewarding [10] indicates towards developing crayfish into a new model of reward that is amenable to electrophysiological and molecular analyses in well-defined neurocircuitries. Crayfish is a new model that will make available a novel, multi-faceted, robust model system with a demonstrated complement of positive-reinforcing properties for reward and will be followed by detailed knowledge of the neurocircuitries of reward seeking, which will be investigated in our future studies.

Acknowledgments

This study was funded by USCOMG Start-fund.

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