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Research report

Repeated cocaine treatments induce distinct locomotor effects in Crayfish

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ABSTRACT

A repeated injection of cocaine regime is known to induce complex locomotion alterations in both vertebrate and invertebrate models of drug addiction. However, the specific effect of cocaine on behaviorally distinct locomotion and non locomotion parameters is not well known. The present experiments determined whether cocaine has distinct effect on multifarious locomotor activity of crayfish (*Orconectes rusticus*). Following repeated injections of 2.5 μg/g or 10.0 μg/g dose of cocaine for three days, videotaped recordings of locomotion were analyzed to determine whether repeated injections of cocaine produced distinct effect on multifarious locomotor activity of crayfish. Cocaine decreased immobility in day 1 when compared with saline. Thereafter, cocaine increased immobility in days 2 and 3. Repeated injections of cocaine increased distance traveled, average speed, mobility and decreased lingering episodes. These findings indicate that cocaine has distinct action on movement and non-movement behavioral activities, suggesting that locomotion as a unitary phenomenon comprised of assemblage of multifarious components, which can be manipulated and separated by cocaine in crayfish.

1. Introduction

Monoamine systems are known to represent prominent candidates that control locomotion. The stimulant effect of monoamines can transform neural functions at various levels to facilitate coordinated motoric responses to environmental perturbations [21,29]. Dopamine is a monoamine neurotransmitter that works in conjunction with other neuromodulating systems to selectively stimulate locomotion and sensitize animals toward conditioned or unconditioned states [5,6,9,19,50,54]. Instead of orchestrating behavior 'in itself', amines seem to fine-tune continuing activity, and facilitate the emergence of a specific behavior [27], such as locomotion that can be used to adjust to another type of behavior or situation. It has been shown that cocaine functions by inhibiting biogenic amine reuptake transporters in mammals [26,52], and invertebrates [4,43,55] to stimulate the dopamine and activate psychomotoric activities during exploratory behavior [1,10,53].

Cocaine is a plant alkaloid, which is obtained from coca plant (*Erythroxylum* spp.) leaves. It is a neurotoxin that protects the coca plant from herbivory by significantly interfering with motor control in many arthropod pests [37]. Acute or chronic administration of cocaine is known to alter locomotor activity during grooming, feeding, and uncontrolled repetitive behaviors in both vertebrates and invertebrates [15]. Cocaine is also known to be rewarding to many animal models of drug addiction [26,47]. The neurobiological models of drug abuse proposed that drug use is initiated and maintained by being rewarding [47]. Nevertheless, cocaine is one of the most commonly used drugs, and a plant neurotoxin that is thought to rebuke, not reward, during consumption by herbivores. Thus, human susceptibility to consume plant neurotoxins is contemplated to be a paradox with far-reaching implications for current drug-reward theory. An important argument to resolve the paradox includes the possibility that humans may have evolved specific abilities to counter-exploit plant neurotoxins, or that cocaine evolved to deter insect and not mammals. Another line of argument is that there are fundamental differences that exist in the responses of mammals to cocaine compared with those of arthropods [37]. A previous report by Wolf and Heberlein [55] suggests that cocaine is not rewarding to insects or other arthropods or even invertebrates in general. However, a study by Panksepp and Huber [41] supports the rewarding properties of cocaine to crayfish, whereas studies by McClung and Hirsh [16,31] canvassed for the role of cocaine in stimulating unconditioned motor responses in invertebrates. The emergent picture that arises from the existing studies is that cocaine like any other drug is capable of activating the motivational seeking system to allow animals to pursue and search for materials that are needed for survival, and also promote unconditional locomotion responses to various environmental challenges.

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A considerable number of studies revealed that cocaine typically induced excitatory influence on the dopamine system to promote diverse locomotor responses in vertebrates [2,7,11,14,20,22,25,30,42,44,49,52], and invertebrates [13,16,46]. The sophisticated genetics combined with relatively simple neuroanatomy of invertebrates make them an elegant resource for characterizing cocaine-induced locomotion behavioral changes.

The crayfish model is particularly unique because it contains a reduced number of elements of experimentally accessible nervous system with neurons that can commonly be recognized across individuals. Thus, crayfish offer an excellent preparation in which we used to characterize specific locomotion substrates, such as lingering episodes, distance traveled, average speed, and mobility and immobility parameters. These substrates are behaviorally and pharmacologically relevant in drug addiction, especially during drug-induced behavioral sensitization. A previous series of experiments [41] that examined the presence of natural reward systems in crayfish revealed that crayfish search for those environments that had previously been paired with psychostimulants (cocaine and amphetamine) in a place preference paradigm test. These drugs were shown to stimulate diverse exploratory behaviors that included active locomotion responses in a dose dependent manner when crayfish were placed into a novel arena and drugs were infused systemically or directly into pericardial system or the head ganglion [1]. This finding indicates that the injected psychostimulants exerted their effects at a number of neural sites, including the stimulation of circuits for active locomotion behaviors, suggesting the presence of selective effects towards specific behavioral patterns associated with the drug, rather than a more general active state. It thus, implies that cocaine could regulate subcomponents of locomotion, probably with a separate effect in each case, modulated by the dopamine system. The primary goal of our current research is to explore the proximate effects of cocaine in recruiting specific adaptive locomotion responses in crayfish. For instance, if two or more different patterns of locomotion are to be expressed following cocaine injections, is there any possibility that the effect of cocaine might stimulate one act and suppress another or the same act at different time point? Therefore, we determined whether repeated injections of cocaine regime have distinct effects on multifarious locomotor activity of crayfish. We evaluated the unique locomotion responses that match-up the specific pharmacological effects of cocaine on crayfish. Our analyses identified the specific effects of cocaine on locomotion parameters and non locomotion behavioral responses.

2. Materials and methods

2.1. Animals

Intact, intermolt male Crayfish (*Orconectes rusticus*) were used for all the experiments in this study (body weights 15.5–26.8 g). Animals were wild-caught from the river. Individuals were maintained in the home aquarium with a large flow-through holding trays. Water was pumped up from a large home aquarium container where it was continuously filtered and aerated. Temperature was maintained at $20 \pm 1\,^{\circ}C$. The animals were housed in the home aquarium under 16:8 light/dark cycles and were fed twice a week with small pieces of tuna.

2.2. Apparatus

We designed an open field test by constructing a rectangular aquarium made from Plexiglas (2.2 m × 0.9 m × 0.75 m; Fig. 1) with four translucent walls. The tank received slow 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. We mounted a digital a Carl zeiss Sony DCR-VX1000-NTSC camera with 40× optical zooming on the ceiling above the aquarium, and it covered the aquarium providing area profile view.

2.3. Surgical protocol for the implantation of cannula into the pericardial system

Prior to surgery, we anesthetized the animals by burying them in crushed ice for about 20 min in preparation for surgery. The effectiveness of our anesthesia approach was confirmed when the appendages were not moving in the crushed ice. Using the tip of the injection needle, an incision was created in the caudal 1/3 of the dorsal carapace, lateral of the midline to avoid damaging the heart blood vessels, and destroying the heart. After each successful surgery, we implanted a 15 mm section of deactivated, fine-bore, fused silica capillary (Agilent, i.d. = 250 μm) into the pericardial sinus, about 2.5 mm deep, and stiffened it to the carapace with super glue. Thereafter, each animal was returned to a plastic holding container overnight for recovery.

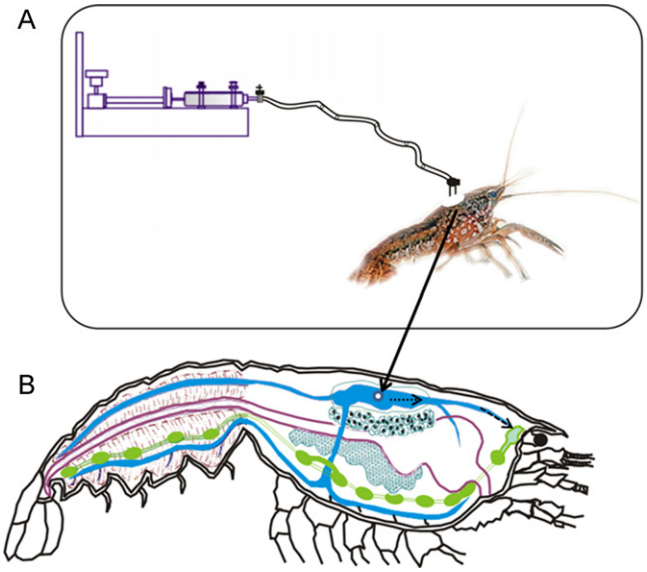

Fig. 1. Different doses of cocaine were injected directly into the pericardial system which serves as a primary neurochemical site for endogenous monoamine release. During injection protocol, we connected the deactivated, fine-bore, fused silica needle (A; Agilent, i.d. = 100 μm) to the implanted cannula with a short segment of Tygon microbore tubing (Fisher Scientific, i.d. = 250 μm). We injected directly into the pericardial system (see long black arrow). This method of drug injection is mainly useful in crustaceans, because the pericardial organs are primary sites of releasable monoamines and amine alteration in the blood eventually reach the brain (B; broken arrows indicate the movement of the drug into the brain).

2.4. Drug injections

During drug injections, the deactivated, fine-bore, fused silica needle (Agilent, i.d. = 100 μm) was connected to the implanted cannula with a short segment of Tygon microbore tubing (Fisher Scientific, i.d. = 250 μm). We injected the animals with 2.5 μg/g or 10.0 μg/g of the animal body weight of cocaine (Sigma, St Louis USA) using a microdialysis swivel (intech, 375/25p,CMA Model 102, CMA Microdialysis Inc., North Chelmsford, MA, USA) into the pericardial system of crayfish which is known to serve as a primary neurochemical site for endogenous monoamine release [40], and any manipulations of amines at that site are transported to the nerve cord. Considering that a low dose of cocaine is known to stimulate locomotion while a high dose suppresses locomotion [47], we chose two doses of 2.5 μg/g (low dose) and 10.0 μg/g (high dose) to determine the specific of cocaine on the diverse locomotion patterns.

Crayfish were randomly distributed into three treatment groups ($n = 9$ per group). The first two groups received 2.5 μg/g or 10.0 μg/g dose of cocaine (refer to free base concentrations), while the third group represent a vehicle-injected (125 mM saline) group serving as control. Total injection volumes were adjusted to 1/50 of the estimated hemolymph volume for each crayfish which was determined in previous experiments [41] and were delivered ventrally into the second abdominal segment, lateral to the nerve cord. The syringe was held in place for approximately 15 s to prevent leakage from the injection site. Thus, each crayfish was injected with 2.5 μg/g or cocaine 10.0 μg/g dose of cocaine for three consecutive days. The crayfish was placed into the aquarium and injected with cocaine over 5 min followed by continued tracking without injection for another 60 min. Multifactorial locomotion tests were conducted 3 days after surgery, to allow time for full recovery after the surgery. Each crayfish was placed in aquarium, and first injected with saline to establish baseline locomotion. Twenty minutes later, the animal was injected with one of doses of cocaine (2.5 μg/g or 10.0 μg/g) over 5 min followed by continuous tracking without infusion for another 60 min.

2.5. Behavioral analysis

Trials consisted simply of making videotape recordings of the locomotion behavior shown by the test animal alone in the arena for 60 min, and later analyzing

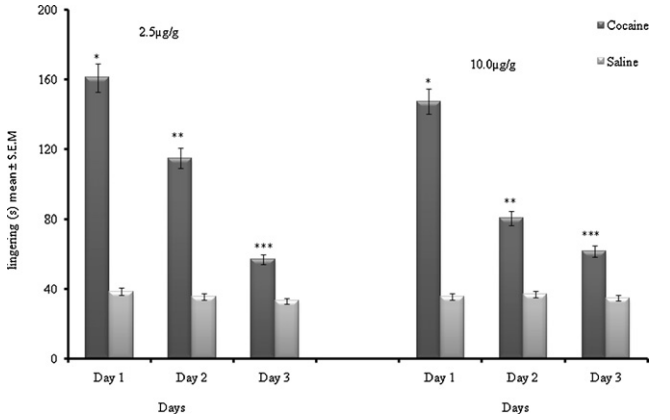

Fig. 2. Effects of cocaine on lingering episodes of crayfish following repeated administrations of 2.5 μg/g ($n = 9$) or 10.0 μg/g ($n = 9$) doses of cocaine. Graphs illustrate the average time for the lingering episodes during the 60 min of behavioral testing following three days of repeated intrapericardial injection. Data for lingering episodes are given as mean ± S.E.M. The average time of lingering for (a) 2.5 μg/g and (b) 10.0 μg/g cocaine-treated groups are shown in comparison with the saline-treated group. ANOVA with repeated measures found a significant difference between the different doses of cocaine [$F(1,20) = 93.87$, $P < 0.001$], a significant effect of the different days of treatments on lingering locomotion parameter [$F(5,100) = 10.09$, $P < 0.001$]. There was a non-significant interaction between drug and the different days of treatments [$F(5,100) = 0.24$, $P = 0.95$]. All cocaine-treated groups differed from saline in the post hoc pair-wise comparisons analysis ($P < 0.05$). Post hoc test reveals the differences for the 3 days of cocaine injections (*, **, ***, $P < 0.05$).

them. We analyzed different aspects of locomotion in cocaine treated animals using a custom-designed video tracking system. Our tracking system processes a single video frames at 320 ms from a camera (Sony DCR-VX1000) that we mounted above the tank to provide a general profile view of the spatial activities of the animals. We streamed the videos to a computer. The spatial activities of crayfish were analyzed using the Any-maze (Stoelting Co. USA), that uses as input the automatically digitized time-series of the animal's location for the visualization, analysis, capturing, tracking and quantification of each specific motion pattern.

The variables that were analyzed in this study are: (1) average speed; the average speed of the animals movement cm/sec. (2), Lingering; the locomotor behavior that is restricted to a small area. (3) Distance traveled; the total distance traveled during the 60 min of the behavioral recording. (4) Average duration of immobility; defined as the animal remaining completely motionless, showing no movement of any part of the body or a decrease in the frequency of active, non-locomotor activities such as rearing and exploration. (5) Average time of mobility; this is the time when the animal is moving in space or an increase in the frequency of locomotor activities such as rearing and exploration. We selected locomotion and non locomotion parameters that were salient for the 3 days of data collection.

2.6. Statistical analysis

All statistical analyses were done using the SPSS version 15.0 (Prentice Hall, USA). We considered the repeated measures ANOVA with a between-subjects factor to determine the existence of significant differences between the doses of cocaine (2.5 μg/g and 10.0 μg/g), and to analyze the effect of the three days of cocaine treatments on the multifarious locomotion parameters. Statistically significant effects were followed by post hoc pair-wise comparisons. In using repeated measures 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 parametric test. The normal distribution of all data was tested with the exploratory data analysis (EDA) before use of parametric test. One-way ANOVA was used to analyze differences in drug treated animals and saline treated animals for the three days of each dose of cocaine injections. A direct comparison of means of the multifarious locomotion parameters between the 2.5 μg/g and 10.0 μg/g doses of cocaine was done using the Students *t*-test. Analyses specific to each experiment are outlined in the appropriate section. All analyses were declared statistically significant when $P < 0.05$.

3. Results

Regardless of dose, intra-circulatory injections of cocaine resulted in enduring changes in the locomotion parameters of crayfish when compared with saline injections (Fig. 2). Following three days of repeated cocaine treatments, ANOVA with repeated measures found a significant effect of doses [$F(1,20) = 93.87$, $P < 0.001$], and days [$F(5,100) = 10.09$, $P < 0.001$] on the lingering episodes of crayfish. However, there was no significant interaction between doses of cocaine and the different days of treatments [$F(5,100) = 0.24$, $P = 0.95$]. All cocaine-treated groups differed from saline in the post hoc pair-wise comparisons analysis.

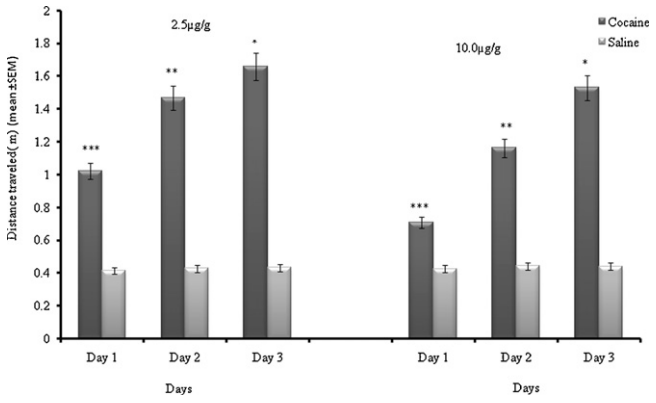

Fig. 3. Effects of 2.5 μg/g or 10.0 μg/g of cocaine on the locomotion (distance traveled) over a 3 day period of repeated injections. Mean total distance (±SEM) for crayfish ($n = 9$) for 60-min exposures. A 2.5 μg/g dose of cocaine significantly stimulates distance traveled ($F[5,60] = 4.32$, $P = 0.001$). The effect of 10.0 μg/g dose of cocaine was also significant ($F[5,60] = 6.74$, $P < 0.001$) when compared with saline treated animals, and post hoc test separates all days from each other (*, **, ***, $P < 0.05$).

Observation of our videotaped recordings revealed that lingering episodes seemed to coincide very well with the subjective notion of stops. Lingering episodes were spatially localized, covering distances of rarely more than two times the length of a crayfish. In other words, although there is no a priori reason preventing a crayfish from covering a long distance in the lingering mode, it in fact hardly ever does so especially when exploring the corners of the aquarium, where most of the lingering episodes occurred.

Fig. 3 illustrates the effects of 2.5 μg/g or 10.0 μg/g of cocaine on the locomotion (distance traveled). A 2 by 3 mixed ANOVA for the between-groups variable, dose of cocaine (2.5 μg/g and 10.0 μg/g), and the three days of cocaine treatments revealed a significant effect of doses of cocaine on distance traveled by crayfish[$F(1,20) = 228.5$, $P < 0.001$]. There was a significant effect of the different days of cocaine injections on the distance traveled [$F(5,100) = 9.32$, $P < 0.001$], and a non-significant interaction between doses and days of treatments [$F(5,100) = 0.22$, $P = 0.95$]. Post hoc pair-wise comparisons analysis revealed no significant difference ($P > 0.05$) between means of the saline-treated animals.

Since 2.5 μg/g and 10.0 μg/g doses of cocaine demonstrated a consistent increase in locomotion, we examined how this increase changed with time by examining the average speed (m/s) during the 60 min testing time. Average speed markedly increased with an increase in the days of cocaine injections (Fig. 4). ANOVA revealed a significant effect of doses [$F(1,20) = 118.32$, $P < 0.001$], a significant effect of days [$F(5,100) = 18.58$, $P < 0.001$], and a significant interaction between doses of cocaine and days of treatments [$F(5,100) = 9.83$, $P < 0.001$]. Post hoc pair-wise comparisons analysis revealed no significant difference ($P > 0.05$) between the means of average speed in the saline-paired conditions. A low dose of cocaine (2.5 μg/g) appeared to increase the speed consistently with each day of cocaine injection. However, the effect of 10.0 μg/g seemed to significantly reduce the speed with a no clear-cut relationship between the days of cocaine injections.

Mobility increased with an increase in the number of days of cocaine injections (2.5 μg/g and 10.0 μg/g) compared with saline injections (Fig. 5). This was confirmed by a significant effect of doses [$F(1,20) = 114.14$, $P = 0.001$], a significant effect of days [$F(5,100) = 18.80$, $P < 0.001$], and a significant interaction between

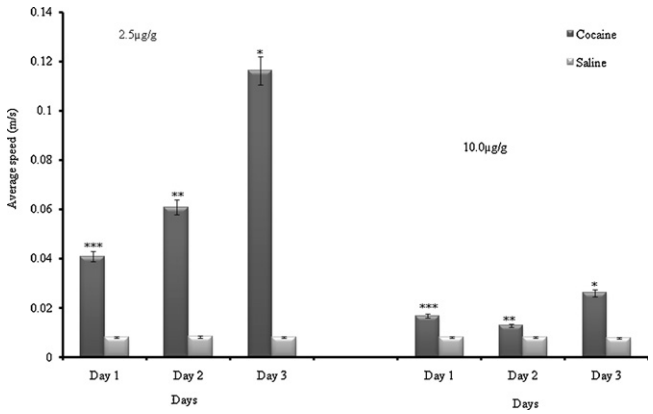

Fig. 4. Effects of cocaine injections on average speed of crayfish. A low dose of cocaine (2.5 μg/g) produced a significant increase in average speed of crayfish ($F[5,60] = 232.31$, $P < 0.001$). The effect of a high dose (10.0 μg/g) was also significant ($F[5,60] = 5.99$, $P < 0.001$) when compared with the effect of saline injections. Post hoc tests differentiates all three days of cocaine treatments from each other (*, **, ***, $P < 0.05$). *t*-Test paired sample analysis revealed that average speed (m/s) at 2.5 μg/g was significantly (*t*-test; $t\,[10] = 5.69$, $P < 0.001$) higher than the average speed for the 10.0 μg/g dose of cocaine.

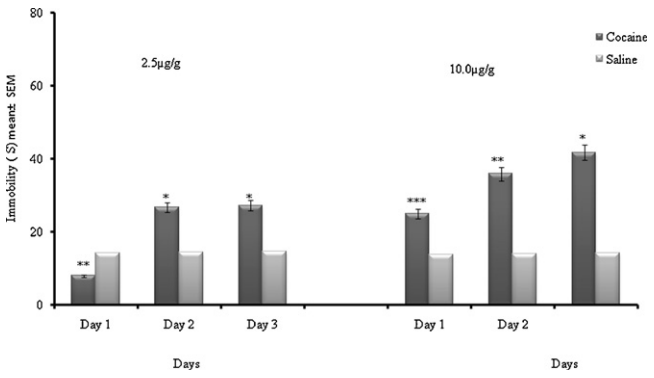

Fig. 6. Effects of cocaine on immobility activity of crayfish. ANOVA revealed a significant effect of 2.5 μg/g dose of cocaine in increasing immobility in crayfish $F[5,60] = 3.61$ *P*, $P = 0.0016$, when compared with the effect of saline. The effect of 10.0 μg/g was also significant ($F[5,60] = 5.18$, $P < 0.001$), when compared with saline infusions, and post hoc test separates all days from each other (*, **, ***, $P < 0.05$). The means of time of immobility for the 10.0 μg/g treated animals (102.57 ± 6.7) were significantly (*t*-test; $t\,[10] = 2.87$, $P = 0.003$) higher than 2.5 μg/g (62.02 ± 4.9) treated animals.

doses and days of treatments [$F(5,100) = 3.39$, $P = 0.007$]. This result suggests that cocaine specifically increased responsiveness by a general sensitization of motor responses to stimulation in crayfish.

Immobility increased with an increase in the number of days of cocaine injections (Fig. 6). There was a significant effect of doses [$F(1,20) = 202.62$, $P = 0.001$], and days [$F(5,100) = 8.01$, $P = 0.001$] on immobility. The interaction between doses and days of treatments on immobility of crayfish was not significant [$F(5,100) = 1.45$, $P = 0.21$]. In the 2.5 μg/g treated animals, cocaine decreased immobility, such that immobility was higher for saline treated animals in the first day when compared with cocaine treated animals. However, the effect was reversed (higher in cocaine treated animals in the second and third days) for the 2.5 μg/g of cocaine treatment when compared with saline-treated animals. In the 10.0 μg/g treated animals, cocaine significantly increased immobility following an increase in the number of days of injections.

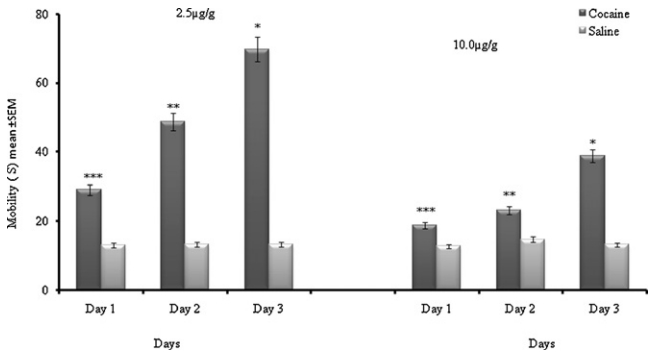

Fig. 5. Effects of 2.5 μg/g or 10.0 μg/g dose of cocaine on mobility (mean ± SEM) of crayfish. Cocaine increased mobility in the open field test for all days of drug testing. ANOVA revealed a significant effect of 2.5 μg/g dose of cocaine in increasing locomotion crayfish ($F[5,60] = 12.28$, $P < 0.001$), when compared with the effect of saline. The effect of 10.0 μg/g was also significant ($F[5,60] = 7.97$, $P < 0.001$), when compared with saline infusions. Mobility in 2.5 μg/g treated animals (147.55 ± 8.7) was significantly (*t*-test; $t\,[10] = 5.64$, $P < 0.001$) higher than animals treated with 10.0 μg/g (80.79 ± 5.6). Asterisks (*, **, ***, $P < 0.05$) indicate results of post hoc pairwise comparison evaluations differentiating all days of mobility measurement.

4. Discussion

Three major findings arose from the experiments in this study. First, we found that cocaine increased distance traveled, average speed, mobility and immobility, while repeated injections of cocaine decreased lingering episodes in crayfish. This result suggests that cocaine has a distinct effect on each sub-component of locomotion. Second, we observed that lingering episodes were spatially localized; covering distances of rarely more than two times the length of a crayfish. Lingering occurrences seem to reflect the decision of crayfish to stay in a place and or progress. This reflects resistance or will to change in preparation for an activity that can be modulated by cocaine treatment.

Finally, we observed that cocaine decreased immobility in day 1 when compared with saline. However, immobility was increased in day 2 and 3 time points respectively. The observed effect in days 2 and 3 tempts us to speculate that the increased effect of cocaine on immobility in days 2 and 3 may be due to desensitization of the involved receptors. This might occur due to potential understimulation or overstimulation of dopamine receptors. The role of cocaine in inducing immobility has also been shown in rodents [34]. Generally, desensitization in mammals can occur after only a single exposure to a drug, which can take place after only a single exposure to a drug and can develop within minutes of drug injections [48].

We observed that cocaine-treated crayfish still traveled effectively within the experimental aquarium in animals treated with 2.5 μg/g or 10 μg/g dose, such that cocaine increased the distance traveled when compared with saline injections. Thus, it appears that 2.5 μg/g or 10 μg/g dose of cocaine did not disrupt motor control in crayfish; instead it induced behavioral sensitization. This is contrary to the effect on immobility in the second and third day of injections, which further indicates the distinct effect of cocaine on locomotion and non movement parameters. Our finding that repeated injection with low or high dose of cocaine stimulated distance traveled behavior in crayfish is consistent with the effect of cocaine increasing responsiveness to motoric activities in invertebrate systems [16,31,37,3].

In vertebrates, repeated exposure to psychostimulants result in behavioral sensitization, defined as an amplified response in locomotor activity which is used to model aspects of drug addiction [42,45]. Behavioral sensitization in crayfish is reflected in the progressive augmentation of locomotion responses to cocaine that develops during the repeated injections of cocaine. The effects of

cocaine on locomotion did not appear to be simply an indirect result of the suppression of other behaviors, because the average speed of locomotion showed significant increase at both low (2.5 μg/g) and high doses (10.0 μg/g) of cocaine when compared with saline injections. The significant increase in the average speed was more pronounced at a low dose of 2.5 μg/g, when compared with the high dose (10.0 μg/g) of cocaine. The result indicates that the effect of dose-dependent and repeated cocaine administration on the behavior of crayfish paralleled with many effects commonly reported with rodents (i.e., increased locomotion) following repeated cocaine treatment [24,32] or evoking changes in *Caenorhabditis elegans* locomotor activity [51]. According to our current results, cocaine is capable of producing distinct effects on movement and non-movement activities in crayfish. It therefore becomes obvious that these distinct responses are not just a mere reflection of the unconditioned effects of cocaine, but that locomotion as a unitary phenomenon comprised of assemblage of multifarious components that can be manipulated and separated by cocaine in crayfish. Given that locomotion responses are important to study the neurobiology of drug abuse, our data suggest that distinct locomotion responses to cocaine during unconditioning test could serve as models for characterizing drug-induced behavioral sensitization.

Cocaine is a powerful addictive substance that inhibits monoamine transporters, including dopamine, serotonin and norepinephrine transporters in both vertebrate and invertebrate models of drug addiction. Although we did not identify the specific neurotransmitter(s) mediating the different effects of cocaine in crayfish, a growing body of evidence [18,33] suggests that in addition to dopamine, serotonin plays an important role in regulating locomotion behavioral effects of cocaine. Crayfish possess serotoninergic, dopaminergic and tyraminergic neurons, suggesting that cocaine may evoke a serotonin or and dopamine mediated response through inhibition of neurotransmission to modulate unique locomotion effect to cocaine.

The advancement that originated from crustaceans research over the past several years contributed significantly to the understanding of fundamental behavioral processes. For instance, the lead way studies of transmission at the neuromuscular junction [12], the role of glutamate and GABA as excitatory and inhibitory neurotransmitters [23,28,39], the neural coordination of escape [17], the complex modulation of stomatogastric networks [38] and the biasing effects in behaviors, such as aggression [27]. These studies suggest that crustaceans have instinctive behaviors that are quite complex and amendable by learning and experience, and represent a novel system for studying the mechanism of drug-induced behavioral sensitization. The efficacy of the crayfish model system for drug addiction research was not previously known because a drug-induced phenomenon had not been characterized. Our studies [8,35,36,41] that crayfish are able of exhibit conditioned place preference for environments in which they received cocaine or morphine remedied this deficiency. Overall, these antecedents of drug addiction research in crayfish together with the current study further strengthen the idea that the crayfish model offers a comparative and complementary approach in drug addiction research. Neuronal simplicity combined with the potential for elegant neuroanatomical and behavioral analyses further support the notion that the crayfish model is highly suited for comprehensive, experimental analyses of specific locomotion responses that characterized drug properties at the behavioral level.

The brain networks that facilitate exploratory behaviors in crayfish control the sustaining mechanism that promotes locomotion approach to diverse niches for survival during reward seeking [1]. The ability of crayfish's brain to synergize and regulate such adaptive locomotion responses is thought to facilitate the animals' ability to search for promising and surviving environmental niches. The importance of our current analysis is the identification that cocaine has a role in differentially influencing each component of locomotion, such as lingering episodes, distance traveled, average speed, mobility and immobility parameters. These are specific behavioral patterns associated with exploration, which is behaviorally and pharmacologically germane in drug addiction research.

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