HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 2 (2022)

Sustained Anxiety-Like Behavior in Crayfish Exposed to Thermal Burn

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ABSTRACT

Both invertebrates and vertebrates make immediate simple reactions to noxious stimuli; a tail flip to escape is a typical example in crayfish. More complex sustained emotional responses, such as anxiety or fear, can be demonstrated in many vertebrates but until recently have not been seen in crustaceans. Several recent studies have found sustained anxiety-like behavior (ALB) in crayfish in response to electric shock and social bullying, and this behavior can be suppressed by anti-anxiety medications. In this study, crayfish were subjected to a thermal burn stress, and then observed for ALB in an established model (light-dark maze). Stressed animals spent almost all of their time in the dark zone, in contrast to unstressed crayfish, who demonstrated normal crayfish exploratory behavior. In this model, crayfish clearly demonstrated ALB in response to stressful burn. This research has implications for humane treatment of crustaceans.

Introduction

The central nervous system of the crayfish, as with other organisms within the decapod order, lacks the large central brain seen in vertebrates. This key difference has led some to conclude that crayfish, and other crustaceans, should not be able to experience complex emotions, such as pain or anxiety. However, in recent years, evidence has accumulated that crayfish can experience higher emotions, including anxiety and social bullying. (Fossat et al, 201; Fossat et al 2015; Bacque et al, 2017; Bacque et al, 2019) Furthermore, these emotions can be induced by serotonin and suppressed by benzodiazepines, chemicals usually associated with the vertebrate brain. (Fossat et al 2104)

There is little doubt that most animals, including crustaceans, do respond to painful stimuli. The response might be as simple as withdrawing from the stimulus, or it might be more sustained, affecting the psychological well-being of the individual. The question is whether crustaceans have lasting emotional and behavioral alterations in response to painful stimuli. Several previous studies have used a light-dark maze to assess behavioral alterations in crayfish. (Fossat et al, 2014; Fossat et al, 2015; Bacque et al, 2019) Crayfish are normally curious and when placed into a new environment, they typically explore the entire region (light and dark zones of a maze equally). These previous studies have shown that, in response to stressful stimuli, crayfish tend to abandon their curious nature, and instead hide in dark zones of the maze.

In this study, crayfish were exposed to a painful thermal stimulus and then placed in a light-dark maze. We propose that painful stimulation will cause crayfish to display anxiety-like behavior, manifested as increased time in the dark zone of the maze, along with decreased exploration of the environment.

Methods

Crayfish (Faxonius (formerly Orconectes) virilis, Ward Scientific, Rochester NY) were given 5 days to acclimate in a fresh water aquarium at room temperature. They were kept in groups of 3 in a 10 gallon tank, so as not to overcrowd the environment.

A maze was created in an aquarium tank with plexiglass walls, in the shape of a cross. (Figure 1) One limb of the cross was entirely masked with tape, making a dark space. For each tested condition, a crayfish was initially placed in the center of the maze, in the transitional zone. The crayfish was then free to travel throughout the maze, both light and dark zones, at its own will. The crayfish's location (light zone, dark zone, or transitional zone) was recorded at 10 second intervals for 10 minutes. The primary variables were total time spent in light and dark zones over the 10 minutes. Time at first entry into a light zone, as well as time at first entry into a dark zone were recorded. In addition, the number of transitions from light to dark zones was recorded as well. This last measurement may give a sense of the "curiosity" of the crayfish (which is inversely related to the level of stress), as each individual explores the environment.

For the test group, a crayfish was manually restrained by a gloved hand, and then a soldering iron heated to 150 degrees Fahrenheit was placed on a claw for 3 seconds, or until the crayfish actively withdrew. The crayfish was then immediately placed in the transitional zone of the maze. A second group of crayfish were manually restrained and touched with a room temperature soldering iron.

A control group of crayfish were placed in the maze without being restrained or touched with a soldering iron at all.

A two-way analysis of variance was performed, looking at the effects of treatment group and light-dark zone, and also their interactions.

This protocol was exempt from institutional animal committee review.

Results

In the control group, crayfish spent 55% of their time in the light zone of the maze, 36% in the dark zone, and 8% in the transitional zone. Half the crayfish went immediately into the light zone, and the other half went immediately into the dark zone, but all of the crayfish had multiple passes into the different zones. All of this reflects expected crayfish behavior, as individuals explore a new environment.

Crayfish who were restrained and touched with the room temperature soldering iron similarly spent time in both light and dark zones, and also were seen to emerge from one zone into another several times during the 10 minute test session. All 3 of these individuals initially entered the dark zone, but did not remain there for long.

In contrast, all the crayfish which were restrained and touched with a hot soldering iron spent most of their time in the dark zone. They immediately entered the dark zone and did not explore the environment during the test session. One individual did spend some time in the transitional zone, but none ever entered the light zone. Data is shown in Table 1.

The two-way analysis of variance showed that the marginal effects of zone (light or dark) and treatment group were not significant at the 5% level of significance ($p=.075$ and $p=.791$, respectively) but the interaction was highly significant ($p=.000$). The lack of significance of the marginal effect of zone means that, when averaged across the three groups, there was no significant difference between light and dark zones. The significance of the interaction means that the effect of zone was significantly different across the individual groups. Thus, for the unstressed group, more time was spent in the light compared to dark zone by an average of 46 seconds ($p=.562$); similarly for the group touched with a cold soldering iron, more time was spent by an average of 183 seconds ($p=.072$). However, for the group receiving the thermal stress, less time was spent in the light compared to dark zone by an average of 540 seconds ($p=.000$). There is a significant difference in these three effects, and this difference is attributable to the thermal stress group.

Post-hoc power analysis showed 100% power when comparing time spent in light zone and also time spent in dark zone, from the unstressed group to the thermal stress group.

Discussion

Painful or stressful stimuli induce simple, immediate responses. An organism may attempt to withdraw, or may become aggressive in response to such stressors. In animals with more complex central nervous systems, these stimuli may also cause lasting emotional changes, such as anxiety or anticipatory fear. Anxiety is not unique to humans; it can be demonstrated in most vertebrates. While simple responses to stressors are seen in invertebrates, it is not clear if invertebrates are capable of experiencing anxiety and lasting emotions. One might argue that popular human treatment of invertebrates assumes the contrary.

Based on neural mapping studies, it has been postulated that octopi, arguable one of the more complex invertebrates, are incapable of feeling pain (Key and Brown, 2018) and even vertebrate fish may lack the neural complexity needed to feel pain (Key 2015). However, the octopus does show affective changes related to painful stimuli (Crook 2021). Perception of pain, and emotional response to painful stimuli, appears to be more complex and perhaps widespread than previously thought. While there is universal agreement that mammals can have lasting emotional responses from pain, things appear less clear when it comes to fish and invertebrates.

When exposed to electric shock, shore crabs and hermit crabs both display changes in behavior, with increased movement and attempts to escape. (Elwood and Adams, 2015) Similarly, crayfish exposed to a thermal burn on a claw exhibit escape behaviors, such as tail-flipping. (Puri and Faulkes, 2015) Interestingly, crayfish exposed to cold or capsaician do not respond, suggesting these are not stressful stimuli. These studies confirm simple escape responses, but do not necessarily conclude that decapods can experience stress or anxiety beyond the stressful stimulus.

To further study anxiety-like behavior in crayfish, several studies have utilized a light-dark maze. (Fossat et al, 2014; Fossat et al, 2015; Bacque et al, 2017; Bacque et al, 2019) The maze is shaped like a 'plus,' with four arms. One or two of the arms are completely covered and dark. When normal crayfish are placed into the center of the maze and monitored over 10 minutes, the animals explore the maze, with more or less equal time in the dark and light zones, and frequent transitions from one arm to another. However, when crayfish are exposed to a stressful stimulus, they tend to hide in a dark arm of the maze, with markedly decreased time in the light zones, and less transitions from one zone to another. This lasting response to stress has been termed 'anxiety-like behavior.' (ALB)

In one study, crayfish exposed to an electric field demonstrated ALB. (Fossat et al, 2015) Similarly, crayfish who are victims of social harassment also show ALB. (Bacque et al, 2017) Crayfish who are about to molt (shed their outer shell) demonstrate ALB (presumably because they are vulnerable). (Bacque et al, 2019) In several of these studies, administration of anti-anxiety medications (benzodiazepines) can markedly decrease ALB. (Fossat et al, Bacque et al, 2019) Stressed crayfish were found to have higher levels of serotonin in their nervous system, and injection of serotonin into unstressed animals can induce ALB. (Fossat et al, 2014; Fossat et al. 2015) It is reasonable to conclude that crayfish, similar to vertebrates, demonstrate lasting emotional responses to stressors, and these responses can be ameliorated by anti-anxiety medications.

In this study, thermal stress lead to sustained ALB in crayfish. The animals immediately entered the dark zone once placed in the maze, then spent most of the time in the dark zone, rarely leaving to explore the light zones. All of this is in contrast to normal behavior seen in unstressed crayfish. The animals hid in the dark and lost their normal exploratory behavior in a new environment. Interestingly, animals restrained but not touched with heat did not demonstrate ALB, leading to the conclusion that burn is stressful to crayfish, whereas simple restraint is not.

The crayfish restrained but not touched with heat did initially consistently enter the dark zone, perhaps as a response to the stress of being restrained for 3 seconds. However, their time in the dark zone was brief and they explored the entire maze similarly to the control group. They did not demonstrate lasting ALB.

The light-dark maze protocol provides an estimation of an organism's stress/comfort level. A limitation of this research model is the inability to directly communicate with the crayfish. It is an assumption that decreased exploration of the maze, and taking refuge in a dark zone, represent abnormal or stressed behaviors. This assumption is logical and reasonable but is not proven. Further support comes from the demonstration that anti-anxiety medication can decrease ALB. In the end, it may not be possible to definitely define the crayfish emotional state, given challenges in communication across species.

Although the number of specimens in this study is low, the significance of the results is high. In keeping with good animal research principles, we chose to use the fewest number of animals required to support or refute our hypotheses. The initial research plan was to use more than twice as many animals per test group, but the initial results were striking. Post-hoc power analysis found the results to be reliable, even with small numbers of animals in each group, because of the extreme difference in time spent in light and dark zones between groups. Our institutional animal review committee does not require approval or specific oversight for research involving invertebrates, perhaps because it is not clear if invertebrates are capable of feeling pain or anxiety.

The results of this and other studies demonstrate that crayfish exhibit ALB in response to thermal stimulus (burn). It is reasonable to conclude that close relatives, such as lobster, may respond similarly when exposed to injurious heat.

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

Acknowledgments

I would like to thank my advisor for the valuable insight provided to me on this topic.

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