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Visual Learning and Discrimination of Abstract Visual Shapes by Crayfish

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Abstract

In nature, animals commonly experience multiple combinations of stimuli at various points in time. When two or more seemingly unrelated stimuli are detected at the same time using different sensory systems, there is the potential for an association to form between the stimuli. The animal may learn and show a response originally associated with one stimulus when it now detects the second stimulus. These stimuli are the unconditioned and conditioned stimulus of classical and operant conditioning. Many species of animals are known to learn via operant conditioning and a variety of responses can become associated with formerly neutral stimuli. Crayfish for example can learn new danger signals by association with the unconditioned cue of alarm odor, but can also learn to recognize stimuli associated with food as indicators of a feeding opportunity, and even learn social status through visual and chemical signals. Based on the results of our study, we will be able to make a definitive statement about the capabilities of crayfish to associate abstract visual shapes with food rewards. The classical conditioning trials for many days involve giving the crayfish a five-minute acclimation period in the testing tank and then releasing them for twenty minutes to explore and view the visual symbol in the presence of food each day. Eventually the food reward is removed after repeated exposure and if a learned association between the visual symbol and food reward occurs, we would expect crayfish spending a larger amount of time in the section of the tank with the reward symbol even when food is absent. Preliminary indications are that they are in fact capable of learning abstract visual symbols.

INTRODUCTION

Virtually all species of animals are known to learn by classical and operant conditioning and a variety of responses can become associated with formerly neutral stimuli (Pavlov & Anrep, 1927; Dukas, 1998; Wisenden & Millard, 2001; Domjan, 2005). Operant conditioning uses the consequences of these stimuli to modify their behavior to form a new behavior. In particular, reinforcement is a consequence that causes a behavior to occur with greater frequency. Pigeons and bees for example are capable of such operant learning, where they learn to associate food rewards with novel stimuli (Giurfa et al., 1997; Peissig et al., 2006). Crayfish are capable of similar learning as well. When they have no experience with the odor of goldfish, they do not show any response to the odor. However, if crayfish detect goldfish odor and a crayfish alarm (warning) odor at the same time, they will treat goldfish odor as a danger signal in the future (Hazlett, 2003). This type of learned association is common for important aspects like predator avoidance, foraging behavior and even learned social status through association of visual and chemical signals (Hazlett, 1994; Daws et al., 2002; Bergman et al., 2003; Zulandt et al., 2008).

There have been several studies on learning in the crayfish species *Orconectes rusticus* and *Orconectes virilis*. Individuals of both species can learn to treat a formerly neutral stimulus as a danger signal, but individuals of *Orconectes rusticus* remember this association longer (Hazlett et al., 2002; Acquistapace et al., 2003). Crayfish also perform visually-mediated behaviors less frequently when light levels are reduced suggests that they rely heavily on visual information when its available and will even alter their behavior in the absence of visual input (Bruski & Dunham, 1987; Mitchell & Hazlett, 1996). Hence we designed a study that tested a species of crayfish, *Orconectes propinquus*, in the same genus of the two crayfish listed above shown to be capable of operant conditioning. *Orconectes propinquus* has an exceptional visual system that when tested may be able to view visual symbols (conditioned stimulus) and learn to associate these shapes with food rewards (unconditioned stimulus; Fig. 1). Based on the results of our study, we will be able to make a definitive statement about the capabilities of crayfish to associate abstract shapes with food rewards.

Figure 1: Head of a crayfish with very well developed compound eyes.

METHODS

Adult male *Orconectes propinquus* crayfish (Fig. 2) were used for operant conditioning, as well as one *Orconectes virilis* (Fig. 3) to examine species learning ability. Additional trials of *Orconectes propinquus* are currently in progress and will continue throughout 2010-2011 academic year. *Orconectes propinquus* were selected because of their exceptional visual system and because they were in the genus as previously tested crayfish in other learning studies (Zulandt et al., 2008). Crayfish tested were isolated in individual tanks (Fig. 4) before conditioning started and for the duration of the testing period to remove prior social influences (Bergman et al, 2003). Crayfish were not fed for three days to increase the effectiveness and motivation to seek the food reward.

Figure 2: Adult male *Orconectes propinquus* were used for testing.

Figure 3: Adult male *Orconectes virilis* were used in conditioning.

Figure 4: Isolation tanks used for removal of social influences.

Initial testing of the crayfish involved training them to recognize the syringe with pureed perch as the food reward. Then testing began by moving the syringe around the small tank at random positions to avoid side preference as a confounding variable. It was then decided that the original tank design was too small (15cm x 30cm) and a larger tank (25cm x 50cm) was used to allow for more exploratory behavior and for easier quantification of behavioral choices that may indicate a learned stimulus (i.e. visual symbol). The new testing tank was divided into three zones; a reward zone, a neutral zone in the center portion of the tank, and a non-reward zone opposite the reward zone. Following this tank alteration, the visual symbol (plus sign) and blank card were used at either end of the tank (Fig. 5).

Figure 5: Reward symbol and blank card used for conditioning trials

It was later determined that the syringe that contained the food reward seemed to become the conditioned variable more so than the visual symbol that was observed in the presence of the syringe. It was also observed that disturbances on the surface of the water alerted subjects to the reward side instead of the presence of the visual symbol, thus we constructed disturbance dampeners from PVC pipe at either end of the tank and brought the water level above the bottom so to negate surface disturbances. Food rewards were then administered through the dampener to reduced disturbances (Fig. 6). Other observations indicated that movements outside the transparent tank walls influenced crayfish behavior within the tank during testing. Walls were then made opaque to remove the potential use outside visual stimuli by crayfish. It should also be noted that the removal of outside visual stimuli was important due to the fact that crayfish demonstrate thigmotaxis (i.e. move while

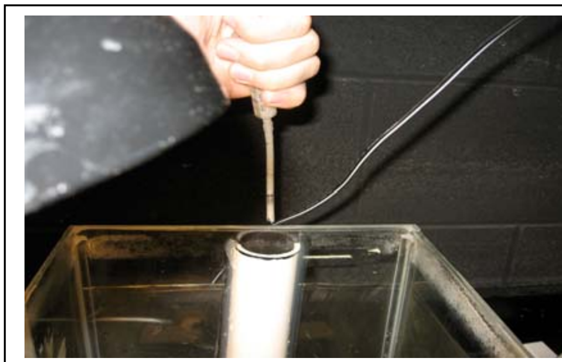


Figure 6: Food being administered through disturbance dampeners.

attempting to keep one claw in contact with a tank wall). Since the subjects spent such a great amount of time at the walls of the testing tank and could see outside stimuli more often, making the walls opaque was a necessary change. Outside illumination was reduced and one stationary light was mounted above the tank and used to illuminate the experimental setup. Lastly, transportation from the isolation tanks to the test tank agitated the subjects and consequently they ignored the food reward and/or the reward symbol. We added an isolation cage to the experimental tank within the neutral zone at the center of the tank to allow for five minutes of acclimation to the testing tank (Bergman et al., 2003). With these alterations in place, confounding variables and outside stimuli have been reduced and the resulting procedure allows for clear and consistent testing.

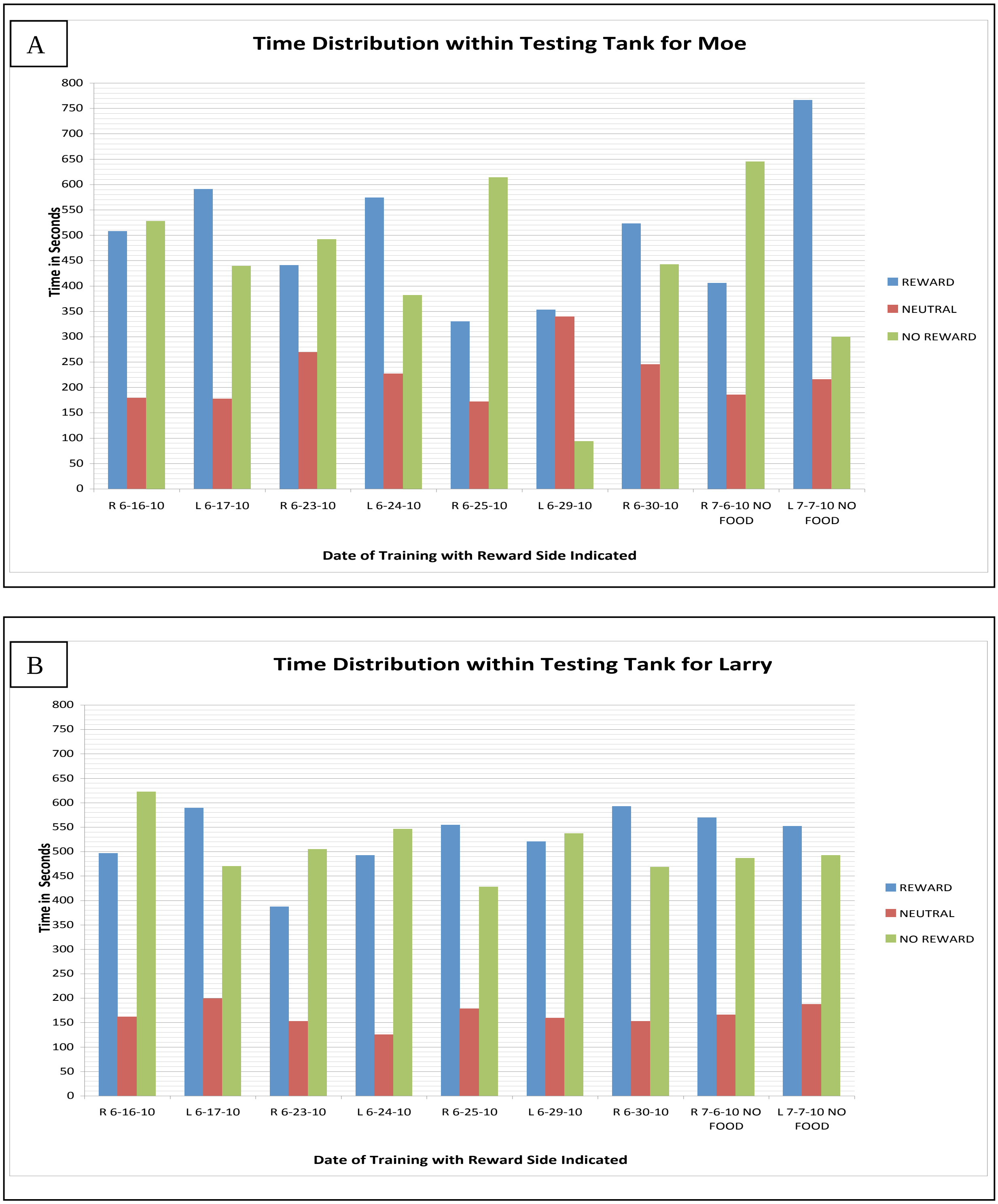
The final procedure that we developed is as follows. Large males (with plans to use small males and female crayfish in place) were chosen to isolate for approximately one week to remove prior social influences that could potentially influence behavior. The first step of a daily fourteen-day training period was to place a test subject in isolation within the testing tank for five minutes. Following this each crayfish was allowed to explore the testing tank for twenty minutes. Three individual food rewards were administered during the twenty minutes, but only when the test subject left the reward side and then returned to the reward side (i.e. visual symbol side). After the twenty minutes elapsed the test subjects were replaced in their individual isolation tanks. At the end of each experimental day, the orientation of the testing tank was reversed so that a side would not become associated with the food reward. After the fourteen days of operant conditioning the subjects were submitted to two days of testing, each twenty minutes, without food rewards to test see if there was a significant response. It was determined that the best way to detect a response to the visual reward symbol was to record the total time spent in the reward, neutral, and non-reward zones for the final tests. The total average time spent for all subjects was then compared to see if there was a significant difference between the time spent in the reward and non-reward zones. A student T test was used to analyze the resulting data with significant being $P<0.05$.

RESULTS

Figures A, B, C and D show individual results for each crayfish tested using classical conditioning with visual symbols. Shown is the time spent on the “reward”, “neutral”, and “no reward” areas of the tank. Reward conditions consisted of a food pairing with a constant visual symbol. No reward was the absence of food and a visual symbol. The center portion of the tank was considered a neutral zone.

The following graphs show the typical responses during the training procedure. Graph A shows a crayfish that alternates between spending more time in the reward zone and then switches to spending more time in the non-reward zone later in the training, and this trend holds true even in the final days of testing. Graph B shows a crayfish that spent nearly 50% of its time in the reward and non-reward zones, however toward the end of training there is a bias towards the reward side. Graph C shows a crayfish with a clear preference for the reward zone throughout the entire training period, but during the final tests that clear preference diminishes to a point where the times are not different enough to be significant. Graph D shows a crayfish’s behavior similar to graph A, in that the time spent in each zone switches nearly every day. The difference from Graph A is that during the final days of testing, while the subject from graph A spent more time in the non-reward zone in day one and the reward zone in day two, whereas the subject from Graph D does the exact opposite.

On the horizontal axis notes can be seen showing which side of the testing tank the reward symbol was on, the date, and the presence of a food reward during the test. Only the final two days testing the association were done without a food reward present.


A
Time Distribution within Testing Tank for Moe
Time in Seconds
0
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100
150
200
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400
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600
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700
750
800
R 6-16-10
L 6-17-10
R 6-23-10
L 6-24-10
R 6-25-10
L 6-29-10
R 6-30-10
R 7-6-10 NO FOOD
L 7-7-10 NO FOOD
Date of Training with Reward Side Indicated
REWARD
NEUTRAL
NO REWARD
B
Time Distribution within Testing Tank for Larry
Time in Seconds
0
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550
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650
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750
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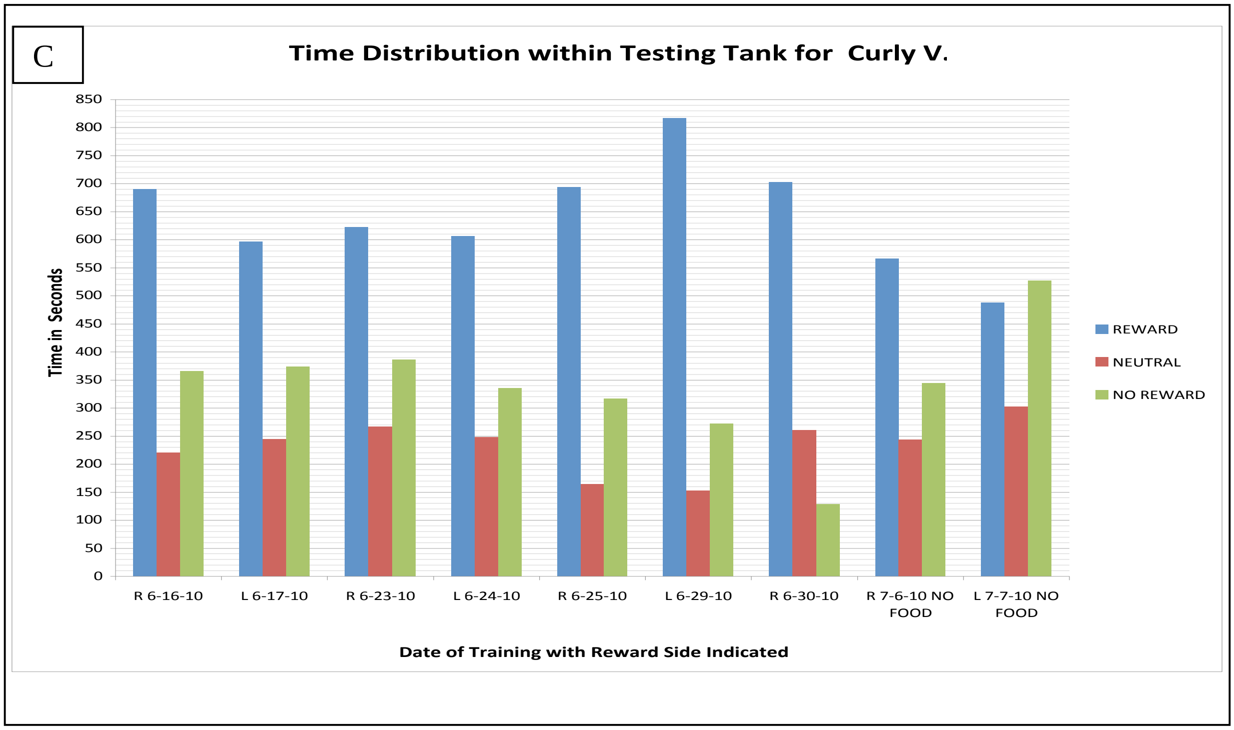
R 6-16-10
L 6-17-10
R 6-23-10
L 6-24-10
R 6-25-10
L 6-29-10
R 6-30-10
R 7-6-10 NO FOOD
L 7-7-10 NO FOOD
Date of Training with Reward Side Indicated
REWARD
NEUTRAL
NO REWARD


C
Time Distribution within Testing Tank for Curly V.
Time in Seconds
850
800
750
700
650
600
550
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450
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100
50
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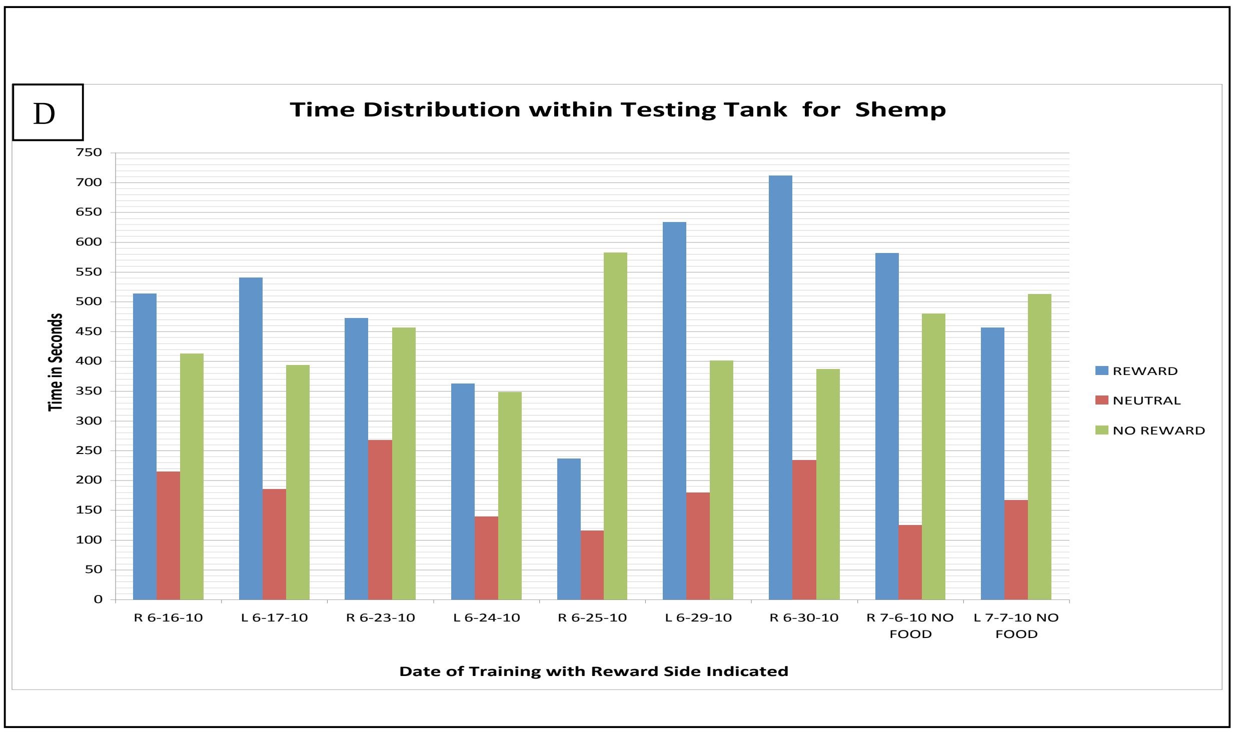
R 6-16-10
L 6-17-10
R 6-23-10
L 6-24-10
R 6-25-10
L 6-29-10
R 6-30-10
R 7-6-10 NO FOOD
L 7-7-10 NO FOOD
Date of Training with Reward Side Indicated
REWARD
NEUTRAL
NO REWARD


D
Time Distribution within Testing Tank for Shemp
Time in Seconds
750
700
650
600
550
500
450
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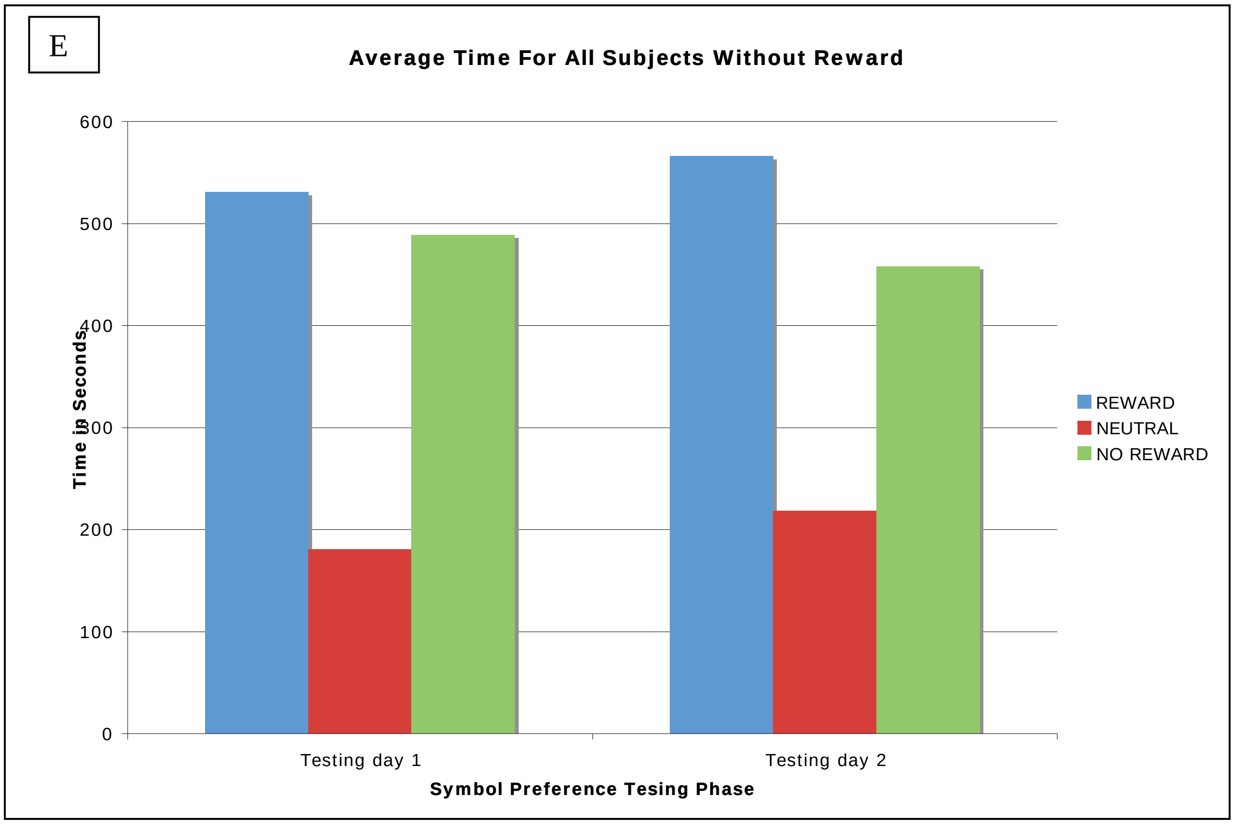
350
300
250
200
150
100
50
0
R 6-16-10
L 6-17-10
R 6-23-10
L 6-24-10
R 6-25-10
L 6-29-10
R 6-30-10
R 7-6-10 NO FOOD
L 7-7-10 NO FOOD
Date of Training with Reward Side Indicated
REWARD
NEUTRAL
NO REWARD

In figure E, shown is the average time spent in the “reward”, “neutral”, and “no reward” areas of the test tank for all test subjects. It can be observed that there is a trend indicating a time bias toward the reward area when food reward is absent (P>0.05).



CONCLUSION

Current results show no significant differences between time spent on the reward side of the testing environment and the non-reward side. There is however, a bias toward spending more time on the reward side (Fig. E). While not yet significant, this observation has lead to the belief that continued testing, and increased sample size while using the procedural alterations set forth in the methods section will net significant results.

Anecdotal evidence of crayfish behavior in the lab showed an aggressive response of crayfish in likely anticipation of being fed (personal observation). This observation lead to the design of an experiment that could test learned associations using visual symbols. Such work has been done in vertebrates such as the cod *Gadus morhua* L. Fish were trained to respond to a projected pattern of spots and the contrast of pattern and background then reduced until the minimum contrast required to elicit a response was established. (Anthony, 1981). However, since this kind of research for invertebrates is a novel idea there was no procedure set up to test such an association.

Alterations to the original experimental design and procedure consists of: 1) a larger experimental tank to allow for more accurate observations, 2) opaque walls to negate outside movement that might affect the behavior of the testing subject, surface disturbance dampeners to prevent a reaction from the testing subject to ripples on the surface of the water, 3) administration of food reward based where the subject must leave the reward area and then return in order to receive a reward, 4) and an acclimation period to allow the test subjects to become comfortable with the testing area and not react erratically to the transfer from isolation to the testing tank thereby skewing the results of the test. When the subjects were allowed to begin training without the acclimation period they tended to completely ignore the food rewards that were administered and so there could be no association of food with the reward symbol. Beyond that, if the subject became accustomed to being aggravated in that situation they could associate movement from one tank to another with that response and as a result the association that was being tested for would be neglected. Since the results of our study were dependent on time spent in the reward and non-reward zones, if the subject were to move erratically without any sense of purpose, any results that would have been obtained would not have been of any use since the association being tested for was not the one that was learned.

More tests are currently being run in the lab to increase the sample size. We believe that increasing the sample size will in fact result in significant and accurate findings (current $P=0.06$). Further testing of crayfish will consist of recognition of varying abstract shapes as well. We plan to test their ability to discriminate under more complex circumstances by giving alternative and conflicting shapes instead of a simple binomial choice (Fig. 6). There are also plans for conducting tests on females to see if there is a common theme to learn via operant conditioning, as well as plans to conduct tests with juveniles to see if their developing brains are capable of complex associations.

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