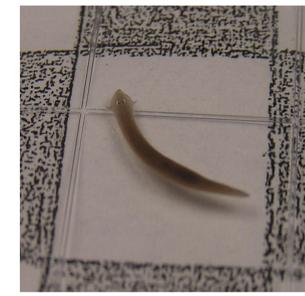


Dugesia tigrina (square length 1.3 cm)

Tests for Learning in the Flatworm *Dugesia tigrina*

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Dugesia tigrina (square length 1.3 cm)

Planarians (a family of flatworms) have a primitive nervous system that includes chemoreceptors located anteriorly on structures called auricles, and photosensitive eyespots (Asano *et al.* 1998; Ferrero and Bedini, 1989). Previous studies have investigated the flatworm *Dugesia tigrina*'s ability to associate an aversive stimulus with an unconditioned stimulus (Halas, 1962). We investigated the learning capabilities of *D. tigrina* through a series of choice tests following the simultaneous presentation of food with a variety of stimuli. Our experiments were similar to association tests performed on the nematode *Caenorhabditis elegans* (Torayama *et al.* 2007). The types of stimuli we addressed in our trials were texture, light, and novel chemical cues. We found evidence that *D. tigrina* are able to learn to associate light with the presence of food, but are unable to make such an association via texture differences or novel chemical stimuli. In addition, we also investigated *D. tigrina*'s response to operant conditioning. We utilized previous studies (Reynierse, 1967) and our own pilot studies that show *D. tigrina*'s negative phototaxis, and employed darkness as a reinforcer to attempt to increase the behavior of seeking out a stimulus (rough texture). Finally, we also examined the influence of several method variations on *D. tigrina*'s response to association and operant conditioning, such as training dish size and maintenance conditions. Our results suggest that *D. tigrina* are capable of limited learning, but that their behavior is largely intrinsically motivated.

Associative Learning

- Association trials consisted of a thirty minute group training period and a five minute individual trial period. (Our pilot studies indicated that *D. tigrina* would usually have fed, and still be feeding, thirty minutes after the introduction of food into the dish.)
- The five minute trials were conducted in a Petri dish that was laid on a piece of paper with a line through the middle of the dish, defining each side as "a" and "b" (Figure 1). Several of our experiments included further modifications to these Petri dishes. The time the flatworms spent in the experimental half of the Petri dish was recorded during the five minute trial.

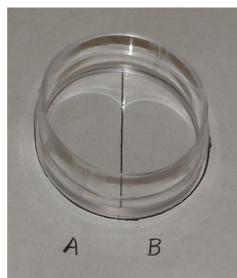


Figure 1: association trial environment

Association to Apple: No Association

- Our first experiment tested *D. tigrina*'s ability to associate a novel chemical and environmental cue with a favored food.
- The experimental group fed for half an hour on 0.75 ± 0.05 grams beef liver minced with 2.25 ± 0.5 grams apple; the control group fed for half an hour on 0.75 ± 0.05 grams beef liver.
- The trials consisted of five minutes in a divided Petri dish, in which half "a" contained 4.5 ± 0.5 grams of apple.
- The experimental group spent significantly less time in the side with apple, indicating that the flatworms could sense the apple.
- Results (Figure 2) indicated no significant difference between the experimental and control groups.

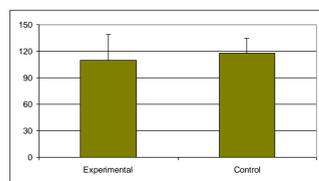


Figure 2: Seconds spent in the half of the dish containing apple for control and experimental subjects

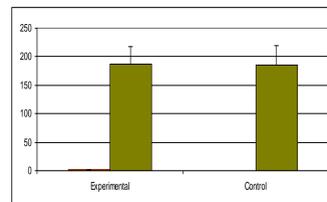


Figure 3: Seconds spent in the rough half of the dish for control and experimental subjects

Association to Texture: No Association

- We next did a similar experiment testing *D. tigrina*'s ability to associate a favored food with a textural cue.
- The experimental group fed for half an hour on a sanded half of a Petri dish; the control group fed for half an hour on a normal Petri dish.
- The trials consisted of five minutes in a divided Petri dish, in which half "a" was sanded.
- The experiments were repeated in smaller Petri dishes, 3.8 cm in diameter. The results were similar.
- Results (Figure 3) indicated no significant difference between the experimental and control groups. In addition, no group spent significantly more time on either side, leaving the possibility open that *D. tigrina* cannot sense a texture difference.

Association to Light: Positive Association

- Our next experiments were aimed at testing *D. tigrina*'s ability to associate light with a favored food.
- D. tigrina* are negatively phototactic; they tend to move away from light.
- All light association trials were performed in small Petri dishes that had half of the dish painted with a black coating (Figure 4); the light side was considered side "a."



Figure 4: light association trial environment

Part One: Habituation to Light

- We first tested to see if habituation to light affected *D. tigrina*'s performance in an association test.
- The control group was maintained under normal lab lighting conditions (14 hr dark: 10 hr light) and the experimental group was maintained for two weeks in the dark.
- Both groups fed for thirty minutes in a "choice" dish with the food located on the dark side of the dish. Trials were conducted in choice light/dark dishes.
- Results (Figure 5) indicated that habituation to light significantly increased the amount of time that specimens spent in the light (side "a").

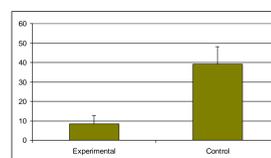


Figure 5: Seconds spent in the light for experimental (not habituated) and control (habituated to light) subjects

Part Two: Association to Light

- Next, we tested *D. tigrina*'s ability to associate light with food.
- Flatworms were maintained under normal lab conditions (they were not kept in the dark).
- The control group fed for half an hour in a normal, unaltered small Petri dish; the experimental group fed for half an hour in a choice dish where the food was located on the light side.
- Results (Figure 6) indicated that the association of the light side with food significantly increased the amount of time that specimens spent in the light (side "a").
- Habituation can be discarded as an explanation for these results; if habituation were the cause of the difference, then the experimental group would have spent less time in the light than the control group.

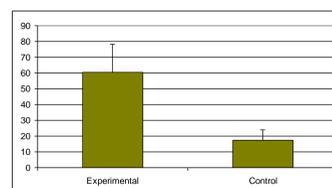


Figure 6: Seconds spent in the light for experimental and control subjects

Operant Conditioning: No Conditioning

- Our last experiments tested *D. tigrina*'s ability to learn, by operant conditioning, to prefer rough texture. From our knowledge that *D. tigrina* is negatively phototactic, we used the presence of bright light as positive punishment and the absence of bright light as negative reinforcement.
- Our experimental methods consisted of square Petri dishes with a grid which had been sanded in a checkerboard pattern, so that every other square was rough and every other square was smooth. The dishes were placed on a piece of paper with a checkerboard pattern, to make the rough squares easier to distinguish (Figure 7).
- We used paired trials, in which the experimental subject was in the "checkerboard" dish and the control subject was in an unaltered, but otherwise identical, Petri dish for the training time.
- The training time lasted for fifteen minutes, during which we turned a bright light off when the experimental subject was on a rough square and on when the experimental subject was on a smooth square.
- Extinction trials were performed immediately after training and consisted of placing both subjects in "checkerboard textured" Petri dishes with constant bright light for ten minutes.
- Results (figure 8) indicated no significant difference between the experimental and control subjects. Both groups showed no behavioral change during the training period, but did show an extinction for both groups. Further research is necessary to understand these results.



Figure 7: Operant conditioning trial environment

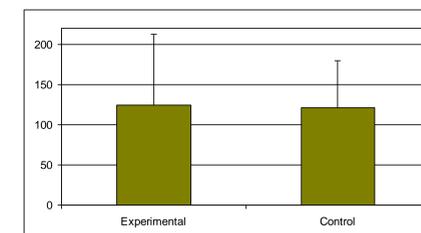


Figure 8: Seconds spent in rough squares during extinction by experimental and control subjects

Conclusions

- Our experiments found that *D. tigrina* can learn to associate a favored food with light, but that they are unable to associate a favored food with texture or a novel chemical cue.
- We were also unable to teach *D. tigrina* to prefer rough texture through operant conditioning.
- Our results indicate that *D. tigrina* can learn by association with a reinforcer – not just an aversive. However, the stimuli that they associate with a reinforcer is limited, and they seem most likely to learn when dealing with the stimulus that they find most important (light). While *D. tigrina* can learn, the great majority of its responses are instinctual behaviors that have not been significantly modified from their initial state.

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