Avoidance conditioning in *Limulus*¹

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Limulus has successfully been trained to respond to light using an avoidance-conditioning design. This success depends critically on an unusual harness employed in the experiment.

Makous (1969) recently reported in this journal a failure to train *Limulus* using a shuttle-box avoidance procedure with light as the conditioned stimulus. Makous also reported difficulties in establishing classical conditioning in one experiment although classical conditioning was established by Makous in a replication of Smith & Baker's (1960) experiment. We now present a preliminary report of a successful avoidance conditioning experiment. There is a peculiarity of the response emitted by *Limulus* which, if not accounted for, makes conditioning impossible to obtain.

METHOD

The animal was restrained in a holder which provided flowing sea water running at a depth of about 5 mm. The CS was a 150-W incandescent lamp placed about 25 cm from the animal. The animals might have been responding to the heat rather than the light. However, this does not seem to be the case as the well-practiced animals generalized to the turning on of the fluorescent room light at the end of the session. The US was a quasiconstant current of 50 mA ac. This is an extremely powerful shock; first-trial responses can be elicited with a current as low as 0.1 mA. The CS came on for 10 sec and the US for 1 sec which overlapped with the last second of the CS. The response was movement of the tail which was monitored by a string attached to a Mini-Torque Potentiometer (Giannini Controls No. 85153). Negligible force was required to turn the potentiometer and this is a crucial part of the apparatus. An ordinary sensitive potentiometer will not do. The wiper of the potentiometer was connected to a capacity-coupled shift trigger (Hunter No. 3205) which gave a relay closure whenever the tail movement exceeded a criterion magnitude

which depended on the speed of the response. Indefinitely slow responses required up to 5-mm movement while rapid responses of 1 mm would close the relay. If a criterion response was emitted, the animal either escaped or avoided the US, depending on the time of the response. A quasirandom intertrial interval was used; for some animals this was 50 to 100 sec, for others, 100 to 250 sec. The animals were in an otherwise dark, soundproofed room with a masking sound present. Animals were given 5 min to dark adapt and then 10 trials were run each day.

The essential element in achieving successful conditioning is the tail harness employed. The UR or escape reaction is invariably a rapid upward movement. In the early trials, some of the avoidance responses (AR) were also upward movements. We do not believe these to be true conditioned responses as the upwards movements drop out and in a well-practiced animal, the AR is almost always a downward movement of the tail. This response is not detectable without a proper harness, since if the animal's tail is already down, no further downward movement can occur. Therefore, weights were attached to the harness in such a way that there was zero net force acting on the tail when it was up as far as it could go, while there was 7-g force acting upward on the tail when it was all the way down. The force acting on the tail varied smoothly between these extremes. Thus, a downward AR is not simply a muscular relaxation as the animal had to work to move his tail down. The result was that the tail tended to float in between the extremes of position allowed and the shift-trigger detected movement in either direction. We also monitored tail movement on an oscilloscope and manually recorded the direction of movement. Response latency was measured with a Hunter Klockcounter.

RESULTS

We have successfully obtained avoidance conditioning in six animals to date. One additional animal failed to show any conditioned responses in 7 days of testing and was discarded. Four of the above

animals had been used in pilot work and were not naive when testing began under the present procedure. The other two animals required 80 trials to reach a criterion of nine consecutive avoidance responses. Response levels on the first 2 days averaged 35% and a typical learning curve describes the behavior. Response latencies were 3 to 4 sec with a decrease in variability as learning improved. There were occasional responses with latencies less than 1 sec. These were usually the result of spontaneous activity occurring before the CS went on and were not considered ARs. In a well-practiced animal, there is almost no spontaneous activity. Four animals have been maintained on an avoidance schedule after reaching criterion. Three of the four animals showed day-to-day variability, reaching an asymptotic performance near 80%. These animals would run at a high level (occasionally 100%) for a day or two, then drop off for a day, and then return to a high level. One animal of these four reached a very high asymptotic level. In 20 days of testing at 10 trials a day, he avoided successfully on 197 out of 200 trials. Only three reinforcements were necessary to maintain his behavior over 200 trials.

Two animals have been run on an extinction schedule after reaching criterion. At this time, the behavior of these animals does not appear different from those on the maintenance schedule, although we cannot now say whether or not extinction will finally occur.

In summary, avoidance conditioning can readily be established in *Limulus* with the appropriate technique. In animals with a stable level of asymptotic performance, psychophysical experiments might easily be done using a tracking procedure.

REFERENCES

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