A television technique for quantifying conditioned and unconditioned responses of planarians

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A technique for quantifying responses of the planarian, using television recording, is described. The planarian swims in a watch crystal filled with spring water, with a TV camera directly under the crystal aimed upward. Shock is administered via four electrodes placed 90 deg apart around the perimeter of the crystal. Magnitude of response to shock is expressed in terms of percent reduction in body length. Latency of response onset is also available by counting synchronizing pulses equalling 1/60 sec. Data from two worms which received 20 shocks/day for 5 days indicated that the magnitude and latency measures were similar for the two animals and were reasonably reliable. The average latency of the UR to shock was about 100 msec, similar to that of the human eyeblink. Extension of the technique for studying CRs was also described, indicating that greater sophistication in quantifying URs and CRs in planarians is now available.

Behavioral studies of learned and unlearned responses of planarians to visual, vibratory, and electrical stimuli have traditionally used a dichotomous response measure, response present or response absent. Tests of hypotheses and statements of conclusions, consequently, have been based upon percentages of trials on which the response occurred (e.g., Kimmel & Garrigan, 1973; Thompson & McConnell, 1955). While there is little doubt that this measurement strategy has yielded important findings which have influenced current physiological theories of classical conditioning (e.g., Eisenstein, 1967), the unavailability of a continuous quantitative response measure has hindered efforts to relate conditioning phenomena observed in traditional Pavlovian preparations to those seen in the planarian (Kimmel & Garrigan, 1973).

This paper describes a measurement technique utilizing video tape recording of the planarian's behavior in a restricted swimming space. Two continuously quantitative measures of response strength on each CS or US trial are described: (1) relative amount of reduction of body length and (2) latency of response onset. A television camera, monitor, and video tape recorder are the only major items of equipment required.

SWIMMING SPACE AND SHOCK CIRCUIT

A circular wristwatch crystal, 2 cm in diam, is glued into a hole cut in a 10 cm x 7 cm x 4 mm piece of translucent white Plexiglas. The Plexiglas is clamp mounted to a test stand 20 cm under a second piece of 4-mm Plexiglas, 15 cm², which is mounted to the stand 4 cm below a 75-W flood lamp. The translucent Plexiglas between the lamp and the watch crystal provides a diffuse light background for television

monitoring and recording. The planarian swims freely in spring water which fills the watch crystal.

Four stainless steel electrodes, .3 mm in diam, are located 90 deg apart around the perimeter of the watch crystal, with their tips in the water. The shock circuit, shown schematically in Figure 1, delivers the 200-Hz output of an audio generator (Hewlett-Packard Model 205 AG), via an R-C network² and standard switching-timing apparatus, to the four electrodes. Adjacent electrodes are opposite in polarity, but because of the time constant (θ) of the R-C network, coupled with the 200-Hz output of the audio generator, the phase of the sine wave shifts 90 deg at a rate of 200 times/sec. This generates a more homogenous electrical field throughout the water in the watch crystal than would be obtained without the R-C network.

Figure 1. Shock circuit and R-C network for administration of ac shock via four electrodes in swimming space. The upper portion of the figure contains a block diagram of the arrangements, and the lower portion shows a schematic of the main components of the R-C network. T_1 is the output transformer of the audio oscillator; CT is the center tap of this output; T_2 is a 1:10 transformer permitting description of the output of the R-C network.

Figure 2. 35-mm photograph of television monitor showing swimming space with shock electrodes separated by 90 deg and worm swimming with a heading of about 60 deg azimuth. The diagonal lighter segment in the photograph results from the helical scan of the Ampex recorder. This photograph of the television monitor shows a planarian at onset of electric shock (see text for explanation).

Figure 3. Photograph of television monitor showing planarian at point of maximum contraction following shock (see text for explanation).

TELEVISION RECORDING

A Sony AVC 3200 Vidicon television camera is mounted directly under the watch crystal, shooting upward. To obtain clear focus and fill the television monitor screen with the picture of watch crystal, the lens of the camera is located approximately 5 cm below the crystal. Figure 2 is a photograph of the screen of the television monitor (Panasonic Model AV-2200S), showing the circular swimming space, the four shock electrodes, and a freely swimming worm. This photograph (and that in Figure 3) were taken with a 35-mm camera at 1/15 sec and f/5.6, with the video tape held in stop action between two synchronizing pulses. A number of details of the planarian's anatomy are shown, including the head and auricles.

An Ampex VR-5100 video tape deck is used to record the video signal from the television camera. Administration of shock is recorded on the audio channel of the video tape. In addition to permitting continuous recording of the planarian's swimming behavior and reactions to stimuli, the video tape recorder may be stopped and moved forward and back manually, in order to obtain measurements of body length on single frames. Since the video signal is recorded at a rate of 60 frames/sec, latency of response onset is also measurable in units of 1/60 sec.

RELATIVE REDUCTION IN BODY LENGTH

To illustrate the manner in which measurements of relative amount of reduction in body length are obtained, Figures 2 and 3 present photographs of single

video frames at the onset of unsignaled shock (Figure 2) and at the point of maximum contraction immediately following (Figure 3). The shock used to elicit the response illustrated in Figures 2 and 3 had a duration of .2 sec and an intensity of 6 V ac, measured across the electrodes. Length measurements *made on the monitor screen*³ indicated that the planarian's body length reduced on the screen from 5.6 cm at shock onset to 2.5 cm at the point of maximum contraction, a reduction of 3.1 cm. Using the formula:

$$100 \times \left[\frac{\text{length at stimulus onset} - \text{length at maximum contraction}}{\text{length at stimulus onset}} \right],$$

we obtain a value of 55% for this response.

Parallax is not a significant problem since the depth of the water in the watch crystal is too small, relative to total focal distance, to permit the planarian to alter its distance from the television camera lens. Indeed, only minimal variations in focus are seen as the worm moves about the water. However, because the planarian's body is often curved longitudinally either at the moment of stimulus onset or at the point of maximum contraction, a flexible plastic ruler was used to obtain body length measurements. The ruler was curved manually to follow the main longitudinal axis of the worm's body to obtain measurements on such occasions. Occasionally, curvature out of the plane perpendicular to the orientation of the television was noticeable but unmeasurable. We made the working assumption that these occasions simply add some error unsystematically to the measures, although systematic study of the effect of known parameters of unconditioned and conditioned responding on responses of this type remains to be accomplished.

The first four columns of Table 1 summarize the basic descriptive statistics of the relative reduction measures for two different worms, each of which received an unsystematically ordered sequence of 20 shocks/day for 5 days, with intensities ranging from 3.5 to 6.5 V ac. As is shown in Table 1, the two worms did not differ in either mean relative reduction or in the variability of relative reduction. An intraclass correlation index of reliability, computed separately for each planarian, indicates that a substantial proportion of the variability in the relative reduction measures was associated with systematic rather than random effects. Table 1 also indicates that the reliability estimate was statistically significant in one planarian ($F = 1.67$, $p < .05$) but not in the other ($F = 1.35$, $.10 > p > .05$). Both worms showed symmetrical frequency distributions for the body length measures.

LATENCY OF RESPONSE ONSET

As noted, the video signal is recorded at a rate of 60 frames/sec, which permits a measurement to be made in units of 1/60 sec of the time between administration of the eliciting stimulus and onset of the response. Manual forward movement of the tape reels permits the number of synchronizing pulses to be counted as they pass across the monitor screen (one pulse per frame). Since the shock stimuli are recorded on the audio channel of the tape, it is relatively easy to locate the frame on which the audio representation of a shock becomes audible (similar to what is done in editing audio tape), and then to count one frame at a time until the first observable reduction in body length occurs. Latency of response onset is then the number of synchronizing pulses counted times 1/60 sec. These values are converted to milliseconds for presentation of the descriptive statistics in the last four columns of Table 1.

As is shown in Table 1, the two planarians differed only slightly in average latency of response onset, but both had average latencies close to 100 msec, i.e., not unlike the average latencies of unconditioned eyelid responses to air puffs in humans. As in the case of the measure of relative reduction of body length, the latency measures showed substantial reliability, although neither worm's separate reliability estimate was significant. Frequency distributions of the latency measures were slightly positively skewed.

CONDITIONED RESPONSES

Although the principal purpose of the present paper is to describe our basic television recording and scoring procedures for obtaining quantitative measures of relative reduction of body length and latency of response onset, using electric shock as the eliciting US, we decided to administer paired trials with a visual CS and an electric shock US in one animal, to extend the

Table 1
Basic Descriptive Statistics for Two Quantitative Measures

Subject	Mean	SD	Range	R*
Relative Reduction in Length				
1	33.92	13.10	59.80	.80**
2	33.82	13.14	65.80	.77
Latency of Response Onset				
1	90.52	28.84	133.33	.71
2	114.37	36.51	183.33	.79

$$*R = \sqrt{1 - \frac{SS_{Int.} \times Trials \times Days}{SS_{Total}}}$$

** $p < .05$

television methodology to the conditioning preparation. For this purpose, a 6-W dc bulb was mounted 2 cm above and 2 cm to the side of the watch crystal. The visual stimulus had a duration of 2 sec, terminating on paired trials with the onset of the .2-sec shock. The conditioning trials were administered approximately 3 weeks after the responses to shock described above were studied. Three initial presentations of the light alone were administered, followed by 28 paired light-shock trials, and then three extinction trials were run on light alone. The intertrial interval averaged about 2 min. No measurable responses to the light occurred during the first three light-only trials or on any of the first eight paired trials. On the ninth paired trial, a definite contraction response to the light occurred prior to the onset of shock (onset latency of this response was 27/60 sec). The added illumination resulting from the administration of the visual stimulus overloads the Vidicon television camera and produces a picture on the monitor resembling a negative photograph. This change in the appearance of the monitor picture has no influence on our ability to measure the amount of relative reduction of body length, which was 20% for the response on the ninth conditioning trial.

The animal made a total of seven responses to the light during the last 19 paired light-shock trials, as well as a response on the first extinction trial. Presentation of the light 3 days later resulted in yet another response on the first trial.

CONCLUDING COMMENT

Future research on classical conditioning of the planarian, using the television recording technique described above, may be expected to be conducted at a higher level of quantitative sophistication than has previously been possible. The two quantitative measures of response to the CS and US we have described may be utilized to examine the effects of the traditional parameters of classical conditioning on response strength, e.g., CS-US interval, CS intensity, etc. In particular, the trial-by-trial pattern of change in amplitude of the CR and UR may now be studied, as well as dynamic phenomena of more recent interest,

such as CR and UR diminution. To the extent that such research provides a definitive answer to the question of whether classical conditioning in the planarian is similar in detail to classical conditioning in more complex organisms, the television recording preparation will have more than repaid for its modest cost.

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NOTES

1. The subjects used in the work described were *Dugesia dorotocephala*, but the technique is applicable to other members of the genus.
2. Professor Sam Garrett, College of Engineering, University of South Florida, suggested the use of this type of circuit by personal communication.
3. Since these measurements are made on the TV monitor screen, they are much longer than actual size.

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