

# THE GSR OF THE EARTHWORM AS A FUNCTION OF PHOTIC INTENSITY AND NUMBER OF TRIALS

Thesis for the Degree of M. A. MICHIGAN STATE UNIVERSITY Robert F. Morgan 1964 THESIS



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By

Robert F. Morgan

## AN ABSTRACT

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

MASTER OF ARTS

Department of Psychology

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Thirty-two earthworms were randomly assigned to one of four groups. Each group received four trials of the same photic intensity. Group intensities used were 480, 180, 80, and 0 foot candles. The subjects were sewn into sponges forty-eight hours before the test period to prevent escape from the electrodes during testing. Each worm was placed in an icebox for the entire test period, during which GSR readings were recorded every sixty seconds. After fifteen minutes in the icebox, four one-minute trials at an ITI of five minutes were given. Once all four groups had been run, additional worms were soaked in anaesthetic for twenty-four hours, and then run one at each photic intensity to control for the apparatus sensitivity. An attempt was made to restrict the daily running time and temperature range to keep the spontaneous activity level variation at a minimum.

The results showed a significant general adaptation over trials and a highly significant trialsXintensity interaction. Sensory or effector fatigue at the highest intensity level and central nervous habituation at a lower level were suggested as adaptation components since the middle intensity group showed no significant adaptation over trials. GSR amplitude was observed to increase significantly with increased photic intensity. The apparatus control unearthed a small artifactual error at the highest intensity level. This was corrected for in determining the above results.

The surgical and GSR technique used in this experiment appeared to offer an adequate approach to the exploration of the sensory capacities of <u>Lumbricus terrestris</u>.

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#### INTRODUCTION

Although experimental interest in the sensory capacities of the Annelid <u>Lumbricus terrestris</u> dates back to Darwin (1881), the total number of psychological studies is quite small. The total number of well controlled studies is even less. Hess (1924) observed earthworm approach and avoidance as a function of photic intensity, finding clear avoidance of 8600 ft. cd. and 3 ft. cd. sources. O. Mangold (1954) carefully investigated earthworm chemical thresholds and preferences by observing which chemically treated pine needles were pulled into the annelid burrows. His only consistent results were to quinine (avoidance) since there was much variability according to subject, number of presentations, and time of day.

Recently the earthworm photic response has been utilized in learning studies. Caldwell and Kailan (1955) found intense light to be a negative reward or "motivator" in a maze experiment. Stein (1962) studied the effects of spacing of training and ganglionic extirpation on annelid photic response to follow-up conditioning studies by Ratner and Miller (1959, 1962) in which a #2 photoflood was used as

an unconditional stimulus. Peeke, Herz, and Wyers (1963) used the Ratner and Miller conditioning technique to investigate the effects of partial reinforcement and number of trials.

In all the conditioning studies cited above, the portion of the earthworm photic response measured was an observed rearing and withdrawal of a fraction of an inch of anterior segments. While there was relatively high reliability in observing this response portion (Peeke <u>et al</u>. reports 97% agreement between 3 independent scorers), every observation was on an all-or-none basis. Individual response amplitude was not determined.

A better portion of the photic response to measure would be a more quantifiable one. One possibility is through the action of epidermal discharge effectors.

Millott (1943) remarked on the similarity of annelid visceral response to the autonomic system in vertebrates, emphasizing the high nervous control along the alimentary canal. In fact, according to the Australian Museum Magazine (1959), the Giant Australian Earthworms squirt jets of coelomic fluid from dorsal pores when disturbed. Vernon (1897) was the first to report metabolic discharge from the earthworm's epidermis to be under nervous control while Coonfield (1932) confirmingly found no discharge of mucous

or coelomic fluid in the absence of nerve cord. Coonfield never observed mucous emission to occur without a visible contraction of the body wall muscles. He concluded the mucous cell effectors were indirectly under nervous control through the contraction of the body wall muscles.

Since the previously measured response portion of rearing and withdrawal also depends on epidermal contraction, the discharge effectors should yield similar results. Furthermore, there is a more exact and quantifiable measurement technique for epidermal discharge effectors: that of the Galvanic Skin Response (GSR) apparatus.

The GSR technique dates back to 1888 and, over the last 40 years, has been studied in detail (Woodworth & Schlosberg, 1954). Davis (1930) discovered GSR amplitude, but not latency, to vary with stimulus intensity. Davis also demonstrated human GSR adaptation to a neon light flash at one minute intervals (mean resistance drop: 1st flash 1099 ohms, 2nd flash 268 ohms, 3rd flash 190 ohms). Human GSRs have been found to vary not only with stimulus intensity, number of presentations, mental set, size, type, and placement of electrodes, but also with rate of respiration and room temperature (Duffy & Lacey, 1946). Choice of appropriate GSR measurement units has long been a point of contention

between psychological camps. Schlosberg and Stanley (1952) put an end to much of the camp following by examining 24 GSR distributions and determining square root conductance (a reciprocal of resistance) to be the most desirable for statistical treatments based on normal distribution assumptions. The GSR has already been used as a psycho**physical** method. Hovland and Reiser (1940) measured GSR amplitude in response to varying auditory intensities, taking only 3 measurements at each intensity to avoid adaptation effects.

Although GSR techniques have not yet been applied to earthworm research, several electrical measurement techniques have been used on annelids over the years. Gray and Lissman (1938) took oscillographic measurements of the isolated nerve cord and observed the similarity of the electrical rhythm to the muscular locomotory rhythm. Galambos (1939) measured the electrical activity accompanying spontaneous contraction directly from the body wall. No more than 30 segments were measured at a time and the segments were clamped in a roughened paraffin trough. Kurtz and Schrank (1955) found the mean voltage between anterior and posterior ends of <u>Eisenia foetida</u> to be 14.0 mv. One problem confronted in the electrical measurement of earthworms was holding the subjects quiescent. In a normal

unrestrained state they will wriggle out from under electrodes, tape, and paste with appalling ease. Kurtz and Schrank had to anaesthetize their animals. Galambos used isolated segments and he had to clamp those in a paraffin trough. Gray and Lissman pinned the worm to cork and concentrated on the nerve cord. Coonfield (1932) wrote "A small glass rod was placed in the intestine of the worm to prevent its making disturbing movements during the experiments."

It would be more advisable, in the electrical measurement of un-anaesthetized annelids, to use a less damaging method of restraint.

Another important factor in any series of earthworm observations is the time of day. Baldwin (1917) measured definite daily activity cycles as did Ralph (1957), who also found a diurnal respiratory cycle (not highly correlated with activity). Activity is at a minimum from 5 A.M. to 1 P.M. Arbit (1957) found an earthworm group run through a T maze at night (8 to 12 P.M.) achieved criterion in significantly fewer trials than a day group (8 to 12 A.M.). Therefore, observations should not extend beyond a 6 or 8 hour daily range and should be entirely within either a high or low activity period. To reduce spontaneous activity, psychophysical studies might best be run in the low activity period

of morning and early afternoon.

If more than one stimulus presentation is to be used, the proper intertrial interval (ITI) becomes important. Botsford (1939) in investigating neuromuscular responses of the earthworm to a constant current stimulus found no electrical summation with an ITI of 5 minutes. Collier (1939) found peristalsis of segment preparations to cease within 3 minutes of the cessation of a strong tactile stimulus. Five minutes would appear to be a reasonable ITI for earthworm psychophysics.

A last research consideration is temperature. It has already been noted that human GSRs will vary with room temperature (Duffy & Lacey, 1946). Therefore, room temperature should also be kept within a restricted range. For earthworms the optimum preferred range is somewhat cooler than humans. In a recent study (author requested no citation) the photic withdrawal response of <u>Lumbricus terrestris</u> was found to occur 100% of the time only in the temperature range of 8° to 12° C. Above 20° C. not much responding was observed. Earthworm studies are apparently best prepared to combat the temperature problem in a cool, temperature-stable area such as an icebox.

Past studies have yielded the important GSR and earthworm considerations potentially involved in annelid psychophysics. An experiment consolidating them all to study the earthworm's photic response would introduce a new more precise approach into a growing comparative area.

## Problem

The present experiment was designed to study the effect of: (1) Photic Intensity, and (2) Number of Trials, on the GSR of the earthworm, <u>Lumbricus</u> <u>terrestris</u>.

#### METHOD

#### Subjects

Two hundred earthworms, <u>Lumbricus terrestris</u>, were obtained from the Homer Nelon Cutstone & Hardware Bait Supply in Lansing, Michigan. All worms were housed in a wooden colony box half filled with sphagnum moss, top soil, and wet rags. The colony box remained in an icebox at approximately  $6^{\circ}$  C. The sub-sample of 36 worms tested were apparently healthy specimens drawn haphazardly from the colony box.

#### Surgical Technique

Prior to surgery, worms were removed from the colony box with rinsed rubber gloves and placed in a small hold dish filled with wet sphagnum moss. The hold dish remained in a storage icebox as the worms, one at a time, went to surgery. Each animal in turn was placed in 2 ml. of .05 per cent solution of chlorotone until a tactile response could no longer be elicited. This took no longer than 15 minutes.

The worm was then laid parallel to and 0.5" from the long edge of a 4" by 6" foam rubber sponge. Using a curved needle and #50 black thread, the animal received the first

stitch through the epidermis just anterior to the clitellum. Stitches were continued every inch back to within a few segments of the anus. Throughout the operation, the inert animal was periodically wiped with a wet sponge to prevent drying. The entire operation including anaesthetic took nearly twenty minutes. By the end of the operation, subjects were usually beginning to stir.

After the operation, the worm sponge was replaced in the storage icebox, covered by a water saturated sponge and a wet rag and stocked with wet sphagnum moss deposited within reach of the prostomium. All subjects were allowed a 48 hour recovery period before testing.

The four earthworms slated for testing as apparatus controls were left in chlorotone solution for 24 hours before testing. This guaranteed quiescence if not mortality.

#### Apparatus and Testing Procedure

Each animal, having completed a 48 hour recovery period, was removed from the storage icebox, illuminated only by an 8 watt red bulb, and immediately placed in a cardboard holder. The finger electrodes were clamped on an inch apart, the anterior electrode in place just posterior to the clitellum. The worm in this apparatus was then placed on a

marked space at the bottom of the experimental icebox, directly below a water-filled glass bowl of 8" in diameter. The bowl acted as a heat filter for the higher intensity bulbs. The bulbs were screwed into a lamp socket 22" above the subject. Once the icebox door was shut, the room lights were turned on, a stop watch started, and GSR readings were made every 60 seconds for 34 minutes.

GSR readings, read to the nearest thousand ohms, were taken on the D.C. circuit of a Lafayette AC-DC GSR Amplifier Model 601A with finger electrodes. All timing was done with a Meylan stop watch. Data recording, dial reading, and the onset and cessation of the photic stimuli were all accomplished by the experimenter at a table three feet away from the icebox.

After fifteen minutes, the first photic stimulus was presented for 60 seconds. Three additional presentations of the same stimulus at the same duration were then presented at an ITI of five minutes. After the last presentation in the 34th minute, the animal was removed from the apparatus and discarded.

The following groups were run: Group 1 - #2 photoflood (480 f.c.) N=8 Group 2 - 300 watt bulb (180 f.c.) N=8

Group 3 - 150 watt bulb (80 f.c.) N=8 Group 4 - no bulb (0 f.c.) N=8 Anaesthetized animals: 480 f.c. N=1 180 f.c. N=1 80 f.c. N=1 0 f.c. N=1

Eight animals, assigned at random to one of the four groups, were run every day for four days. On the fifth day the four chlorotoned worms were run. All worms were tested in the six hour period from 10 A.M. to 4 P.M. According to thermometer readings taken before and after every subject's testing, the icebox temperature was kept within the range of  $4.5^{\circ}$  C. to  $11.7^{\circ}$  C. Figure 1.--Photograph of worm being sewn to sponge

Figure 2.--Photograph of sewn worm and surgical equipment

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Figure 3.--Photograph of sewn worm in electrodes and cardboard holder in front of the GSR unit

Figure 4.--Photograph of sewn worm in experimental icebox under heat filter and photic source

#### RESULTS

## Anaesthetized Animals

Only the chlorotoned earthworm exposed to four trials of the most intense bulb showed any observable GSR. This animal, showing a .01 GSR unit change, demonstrated that .01 GSR units as observed in the worms of group 1 may be artifactual. For this reason, tables and figures containing group 1 data (with the exception of Figures 7 and 8) have been corrected by subtracting .01 GSR units from every group 1 measurement.

All four chlorotoned animals showed the same GSR to their respective photic intensity across all four trials (see Table 2).

## GSR Adaptation Over Trials

An analysis of variance showed the number of trials to significantly affect GSR amplitude ( $p \langle .05$ ) although there was a highly significant intensityXtrials interaction ( $p \langle .0005$ ). Observation of Figure 5 bears this interaction out. Different intensity groups definitely appear to differ in quickness of adaptation. Statistical analysis employing t-test comparisons within groups show group 1 to have

significantly decreased its response amplitude by the fourth trial ( $p \langle .01 \rangle$ , group 3 to have significantly decreased its response amplitude by the third trial ( $p \langle .01 \rangle$ , and group 2 to have shown no significant decrease over four trials. In addition, group 1 appears to have dropped in amplitude quite suddenly and intensely on its last trial while group 3 shows a gradual drop from one trial to the next. At the end of four trials, only group 3, at the lowest photic intensity, has sunk to a GSR low enough to risk interpretation as random activity.

## GSR as a Function of Photic Intensity

An analysis of variance showed a highly significant difference between groups stimulated at different photic intensities (p < .0005). Figure 5 demonstrates this. Statistical analysis employing t-test comparisons between groups show groups to be significantly different from each other at all trials except groups 3 and 4 during the last two trials and group 1 and 2 during the last trial. These mergings are exclusively at points where significant adaptation has occurred. Figure 6 demonstrates the differential GSR curve over the four photic intensity levels observed.

#### Statistical Considerations

Bartlett's test for homogeneity of variance showed heteroscedasticity for the above data. Since Edwards (1960) points out that the F Test is in practice little influenced by heterogeneity of variance and since Schlosberg and Stanley (1952) consider the GSR data as it has already been transformed the most efficient for parametric analysis, the parametric analysis of variance has been maintained. However, the non-parametric Kruskal-Wallis One-Way Analysis of Variance was applied to the data as a check. Significance was confirmed for both the intensity variable (p < .001) and the trials variable (p < .02).

### GSR During Total Time in Apparatus

Figure 7 shows the mean curves for each of the four un-chlorotoned groups. Each curve follows a similar pattern, decreasing rapidly for fifteen minutes and then leveling off. The group 4 (0 intensity) readings, uninterrupted by photic stimulation, show a relatively completed leveling off at 22 minutes.

Figure 8 points out that much of the initial drop may be due to cooling of the animal from its short stay at room temperature. However, the chlorotoned worms do not show

as steep a drop as the normal animals, nor do they take more than 15 minutes to level off. This suggests that the 15 minute habituation period is needed both for the animal and the apparatus.











#### DISCUSSION

#### GSR Adaptation Over Trials

The results suggest that adaptation follows a very different course depending on the intensity of the photic stimulus used. The middle intensity group showed no significant adaptation while the higher intensity group showed a sudden significant response decrement on trial four and the lower intensity group showed a gradual response decline becoming significant at trial three. These data suggest a two factor interpretation of the adaptation differences. The early drop at low intensity may represent gradual central nervous habituation to the stimulus, while the sudden drop at high intensity may represent a sensory fatigue or, more likely, discharge effector fatigue.

Apparently two trials can be given at low, medium, and high intensity without danger of adaptation. This finding may apply directly to further GSR studies with earthworms.

### GSR as a Function of Photic Intensity

Hess (1924) found that photic intensities less than 3.0 foot candles may elicit earthworm approach, while sources

from 3.0 to 8600 foot candles elicited avoidance. Figure 6 specifies some more intensity points within this avoidance range while supplying a more quantifiable dimension of "avoidance." The curve suggests decreasing increments of response with increasing intensity. For this reason plus the fact that any photic source more intense than a #2 photoflood presents serious heat problems and is undoubtedly far brighter than anything the earthworm is likely to come in contact with in nature, it is probable further more detailed photic response mapping will be kept to the 0 to 500 foot candle range. The stability of the 180 foot candle group over trials indicates that this would be the best intensity for future conditioning and photic experiments on annelids.

## Anaesthetized Animals

Figure 8 shows the importance of an apparatus control in GSR research. The sensitivity of GSR apparatus is such that any strong photic or electric stimulus may cause a deflection over and above the subject's response. It is apparent from Figure 8 that group 1 was affected by the heat of the stimulus. In pilot work (1963) it was found that the #2 photoflood elicited a GSR that could be cut in half by placing the filled water bowl between subject and

source. This aqueous heat filter was retained and the source was moved 10" farther away from the subject. Despite these safeguards the apparatus control at this intensity showed an observable GSR. One explanation besides heat is the possibility of an electric reaction initiated in the moist epidermis by intense light. A dead animal control or its equivalent will give an indication of the direction and magnitude of this kind of error.

The chlorotoned worms also show the initial conductance drop to be due to more than annelid habituation. The most likely explanation is a cooling or temperature drop of the epidermis from room temperature to icebox temperature. This would follow from the study by Duffy and Lacey (1946) that stresses the importance of room temperature in GSR research. Luckily, as the 0 intensity group (Figure 7) shows, a relatively stable base line had been reached by the time the first photic stimulus was applied.

## Annelid Sewing Technique

Although it was a rare animal that had not broken at least one stitch by the time testing started, very few had to be discarded as seriously damaged or undone. Out of every ten worms sewn, eight could usually be run. The

stitches are more analogous to Pavlov's dog harness than Gulliver's Lilliputian straight jacket, since the fully stitched worm was capable of muscular contraction and expansion throughout all segments. In fact, the presence of an observable GSR proves this as Coonfield (1932) insists peristalsis is pre-requisite to epidermal discharge. The thick black thread served to keep the subject under the electrodes in the most humane method yet applied to the electrical measurement of annelids (not counting the subsequent discard procedure).

Note: The individual subject data (table 1) shows the sudden response drop of group 1 on trial 4 (figure 5) to occur for eight out of eight worms. In groups 2 and 3, five and six worms respectively, out of eight, showed a response decrement on trial 4 as compared to trial 1. Group 4, the unstimulated group, shows only two of the eight worms to have dropped in conductance on trial 4 as compared to trial 1. Thus the majority of the worms in each light stimulated group shows a response decrement by the fourth trial.

#### SUMMARY

The present experiment was designed to study the effect of photic intensity and number of trials on the GSR of the earthworm, Lumbricus terrestris. Thirty-two earthworms were randomly assigned to one of four groups. Each group received four trials at the same photic intensity. Group intensities used were 480, 180, 80, and 0 foot candles. The subjects were sewn through their epidermis into foam rubber sponges 48 hours before the test period. This prevented escape from the electrodes during testing. Each worm was placed in the icebox for the entire test period, during which GSR readings were recorded every sixty seconds. After fifteen minutes in the icebox, the worm received four oneminute trials at an ITI of five minutes. Once all four groups had been run, additional worms were soaked in anaesthetic for 24 hours, and then run one to each photic intensity in an attempt to control for the apparatus sensitivity. The daily running time and temperature range were also restricted to keep the spontaneous activity level variation at a minimum.

The results showed a significant general adaptation

over trials and a highly significant trialsXintensity interaction. Sensory or effector fatigue at the highest intensity level and central nervous habituation at a lower level were suggested as adaptation components since the middle intensity group showed no significant adaptation over trials. GSR amplitude increased significantly with increased photic intensity. The apparatus control unearthed a small artifactual error at the highest intensity level. This was corrected for in determining the above results.

The surgical and GSR technique used in this experiment appeared to offer an adequate approach to the exploration of the sensory capacities of <u>Lumbricus terrestris</u>.

1 - Effector fatigue in the sense that the immediate sup ly of muccous or coelcaic fluid may become exhausted or greatly diminished after continued high level emission. Four 60 second trials at the highest photic intensity then may well have seriously diminished the mucous supply neccessary for the full GSR.

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APPENDIX

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8     .10     .08     .10       2     180     1     .04     .03     .01       2     .01     .01     .01     .01	.02
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Table	1GSRs	of all	subjects	in	groups	1	to	4
	with	group	1 data con	rred	cted			

Worm	Photic Intensity	GSR incr	ease (sq Trial 2	rt. con	ductance)
	(1.0.)	<u></u> .		<u> </u>	<u> </u>
1	480	0.01	0.01	0.01	0.01
2	180	0.00	0.00	0.00	0.00
3	80	0.00	0.00	0.00	0.00
4	0	0.00	0.00	0.00	0.00

Table 2.--GSRs of the anaesthetized worms

Table 3.--Means and standard deviations for groups 1 to 4 with groupldata corrected

N	Group	Photic Inten (f.c.)	sity	GSR Inc Trial l	crease (s <u>Trial 2</u>	sq.rt.com Trial 3	nduct.) <u>Trial 4</u>
8	1	480	<b>X</b> :	.082	.079	.084	.032
			•	(.039)	(.041)	(.034)	(.023)
8	2	180	x.	· <b>.</b> 039	.039	.036	.034
			<b>q</b> :	(.026)	(.033)	(.031)	(.033)
8	3	80	x: •	.016 (.012)	.010 (.008)	.001 (.007)	005 (.016)
8	4	Ο	X: 67	018 (.016)	010 (.016)	.005 (.013)	010 (.015)

Source of Variation	d.f.	Mean Square	F
Photic Intensity	3	.03817	20.858##
Error term	28	.00183	
Trials	3	.00247	3.049#
Intensity X Trials	9	.01085	13.395##
Error term	84	.00081	

Table 4.--Analysis of variance of GSR data from groups 1 to 4 with groupldata corrected

## p (.0005 # p ( .05

Table 5.--Statistical analysis employing the Kruskal-Wallis One-Way Analysis of Variance to determine the

effect of heteroscedasticity on the significant results of parametric analysis

Factor	Groups	d.f.	Н	Pζ
Intensity	1,2,3,4	3	80.79	.001
Trials	1,2,3#	2	8.20	.02

# Since the question of interest here is possible adaptation to the photic stimulus over trials, only those groups actually exposed to a photic stimulus are relevant.

_						_
	Group-trial		Group-trial	<u>t</u>	P	
	1-1	vs.	1-2	0.20	N.S.#	
	1-1	vs.	1-3	0.06	N.S.	
	1-1	vs.	1-4	3.16	.01	
	2-1	vs.	2-2	0.00	N.S.	
	2-1	vs.	2-3	0.18	N.S.	
	2-1	vs.	2-4	0.34	N.S.	
	3-1	vs.	3-2	1.19	N.S.	
	3-1	vs.	3-3	3.00	.01	
	3-1	vs.	3-4	2.94	.02	

Table	6Statistical an	alysis	employi	ing t-te	est	comparisons
	to determine t	he effe	ects of	number	of	trials
	within groups					

# N.S. = p > .05

<b>G</b> roup-trial		Group-trial	<u>t</u>	p
1-1	vs.	2-1	2.66	.02
2-1	vs.	3-1	2.21	.05
3-1	vs.	4-1	4.69	.001
1-2	vs.	2-2	2.16	.05
2-2	vs.	3-2	2.42	.05*
3-2	vs.	4-2	3.23	.01
1-3	vs.	2-3	2.90	.02
2-3	vs.	3-3	3.16	.02*
3-3	vs.	4-3	0.73	N.S.#*
1-4	vs.	2-4	0.10	N.S.
2-4	vs.	3-4	2.99	.01
3-4	vs.	4-4	1.93	N.S.

Table 7Statistical analysis employing t-test comparisons
to determine the effects of photic intensity
between groups

# N.S. = p > .05
\* heterogeneous variances

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