A Thesis

Entitled

Retention of Brightness Discrimination

in Paramecia, *P. caudatum*

by

Catherine M. Mingee

Submitted to the Graduate Faculty as partial fulfillment of the
requirements for the Master of Arts Degree in Psychology

Adviser: Dr. Harvard L. Armus

Committee Member: Dr. Stephen Christman

Committee Member: Dr. Henry Heffner

Dr. Patricia Komuniecki, Dean
College of Graduate Studies

The University of Toledo

December 2009

An Abstract of
Retention of Brightness Discrimination
in Paramecia, *P. caudatum*

by

Catherine M. Mingee

As partial fulfillment of the requirements for the
Master of Arts Degree in Psychology

The University of Toledo
December 2009

Previous research into the possibility of learning in paramecia in this laboratory has shown that these organisms can learn to go to and remain in a specific location based on cathode shock reinforcement. The present experiment was designed to determine whether paramecia could retain (remember) the learned brightness discrimination task. The results indicate that the retention interval for this task in paramecia is shorter than six minutes. It is possible that paramecia can remember this task for longer than zero minutes but less than the interval of six minutes that was used during test. It is possible that remembering, for more than immediate testing, requires a central nervous system.

Acknowledgements

I would like to thank my committee members, Dr. Henry Heffner and Dr. Stephen Christman for their interest and suggestions on my work. I would also like to thank Dr. Gim Koay and Dr. Becky Gurney for their assistance with the computer program. A special thanks to my adviser, Dr. Harvard Armus, for his continued faith and support during the entire process.

Table of Contents

Abstract.....	iii
Acknowledgements.....	iv
Content.....	v
List of Tables.....	vi
List of Figures.....	vii
1 Introduction.....	1
2 Method.....	6
2.1 Subjects and apparatus.....	6
2.2 Procedure.....	7
3 Results.....	10
4 Discussion.....	15
References.....	17
Appendices	
A Time (in .5 sec) spent in correct side of trough during training.....	19
B Time (in .5 sec) spent in correct side of trough during testing.....	22

List of Tables

3.1 – Mean time (in .5 sec) spent in correct location during training trials.....	11
3.2 – Mean time (in .5 sec) spent in correct location during testing trials.....	12

List of Figures

3.1 – Percentage of time spent in correct location by zero retention interval subjects.....	12
3.2 – Percentage of time spent in correct location by 6 min retention interval subjects.....	13
3.3 – Percentage of time spent in correct location by 12 min retention interval subjects.....	14

Chapter 1

Introduction

Paramecium behavior has been previously studied for learning capabilities, with some support for the learning of tube-escape behavior as well as discrimination learning. Tube-escape behavior allows the subject to enter a pool of medium after swimming through (escaping from) a small tube. Day and Bentley (1911) used capillary tubing that restricted the movement of paramecia in order to study swimming behavior. They counted reversals of the paramecia's body position as well as time between reversals as a measure of behavior modification. An increase of reversals and decrease of time between reversals suggested the paramecia were altering their behavior to avoid the sides of the tubing, which were assumed to be aversive. These findings supported the idea of learned behavior modification in paramecia. However, "flexibility", or the ability to change direction in a small space, could be an alternate explanation for the paramecium's behavior, rather than the behavior modification through learning that was originally proposed as an explanation (Buytendijk, 1919 as cited in French, 1940). This is because the paramecia may have become better at the movements necessary to reverse their bodies in the tight space of the tube allowing them to move more quickly; that is the paramecia may not have learned anything, they may have simply become more effective

in escaping, possibly as a result of sensitization or some other mechanism. French (1940) used tubing that opened into a pool at the bottom to study escape behavior. He found a decrease in the time it took for paramecia to escape with successive trials, which he related to trial and error learning. Applewhite and Gardner (1973) suggested the liquid medium caused ions to collect in the tube interior, influencing the paramecia's ciliary action and decreasing escape time. When the capillary tubes were cleaned before each subject, Applewhite and Gardner were not able to reproduce French's findings.

Gelber completed a series of investigations into paramecium behavior (1952, 1956, 1957, 1958). Paramecia, which feed on bacteria, were shown to approach a bare but formerly bacteria-coated platinum wire after training with the bacteria-coated wire. Non-reinforced subjects were trained with a bare, rather than a bacteria-coated, wire while other control subjects did not have any prior wire exposure. Counts of paramecia on and around sterile test wires revealed significantly more paramecia in the reinforced group when compared to either control group. This research purported to show an association between the wire and food reinforcement, supporting learning in paramecia. However, Jensen (1957a, 1957b) suggested Gelber's results were not supportive of learning in paramecia because the introduction of bacteria to the culture during reinforcement training and a lack of mixing of the culture between counts might have caused paramecia to be present in the test area for reasons other than learning. To explore these possibilities Jensen studied the effect of Gelber's reinforcement procedure in distilled water and found that there was a transfer of bacteria to the culture pool from the wire. He then mixed the culture using Gelber's swirling motion and found it be inadequate for distributing the bacteria throughout the culture. Jensen suggested that

Gelber's results did not show learning in paramecia because the paramecia were simply accumulating in the bacteria-rich portions of the culture, rather than approaching the wire. In an effort to clarify the contradictions between Gelber and Jensen, a replication of Gelber's work was attempted by Katz and Deterline (1958). Although results were similar to those of Gelber, the authors concluded that Jensen's explanation of paramecia presence due to bacteria was more plausible due to the observed behavior of the subjects. Katz and Deterline used an additional group, which they exposed to food material at the end of the trials, and found the paramecia gathered there with no learning experience. They concluded that Gelber's explanation of paramecia learning to move towards the wire could not be sufficient because groups with no learning exposure displayed similar behavior when bacteria were present. Although multiple studies had been completed to investigate learning in paramecia, the capabilities of the organism were still unclear as of the 1960s.

More recently, Armus and Montgomery (2001) found that cathode shock is attractive to paramecia and can be utilized as reward when conditioning paramecia; they also found that anode shock is aversive. It has been suggested that the attractive quality of the cathode shock is biologically driven by changes in the membrane potential of the paramecium. The changes in the membrane potential cause the paramecia to reorient in space and 'swim' towards the cathode stemming from a change in the direction of ciliary movement (Ogawa, Oku, Hashimoto & Ishikawa, 2006). By utilizing cathode shock Armus, Montgomery and Jellison (2006) found that paramecia are capable of brightness discrimination learning. In this study, as in more recent work by Armus and associates, experimental subjects were exposed to short but continuous bursts of 6.5 volt DC cathode

shock when they were in the designated brightness level and location. Time spent in the designated location was recorded and compared to a control group as well as to a paired shock group that received shock at the same times as the experimental subjects, regardless of their behavior. It was found that paramecia exposed to cathode shock stayed in the designated location significantly longer than the control groups did during test trials, when no shock was experienced. A possible alternate explanation for their findings was based on the possibility that paramecia might have exuded an attractive substance while experiencing the cathode shock, which continued to be present during the testing phase. To test this explanation the illumination level was reversed (from right to left or left to right) for the testing trials. The experimental group, which previously received cathode shock, changed side preference to the new 'cathode' location although no shock was received; control subjects did not exhibit this change in behavior. These results eliminated the possible secretion explanation. Armus, Montgomery and Gurney (2006) utilized the aversive anode shock and found evidence of discrimination learning as well as extinction. In this experiment the experimental group, which experienced anode shock, avoided the anode half of the trough during training trials but increased the time spent in it during the extinction trials, in which no aversive shock was experienced. This effect was not found in the non-shock control group. These studies suggest that paramecia are capable of learning based on both reinforcement and punishment.

The present study was designed to further knowledge of learning in single celled organisms. Since it has been established that paramecia are capable of learning, it seemed logical to test their retention capabilities. Gelber (1958) looked at retention in paramecia using the previously discussed procedure and found retention in paramecia to

last 3 h. Because of the previously mentioned potential flaws in the experimental design the 'retention' may not have been the result of a learned behavior. Huber, Rucker and McDiarmid (1974) looked at tube escape behavior and found retention in escape speed after as much as 150 min. Although this study found retention in escape speed, the initial findings could be explained as a practice effect rather than true learning. As in the training trials it is possible that the 'retention' of escape speed was due to the paramecia's increased capability at swimming in the small space of the capillary tube rather than learning of the actions necessary for quick escape.

Chapter 2

Method

2.1 Subjects and Apparatus

Subjects were 306 paramecia (*P. caudatum*) from the laboratory-maintained colony, divided into six groups as described below. The colony was housed in a glass jar filled with Ward's cereal culture medium, as directed by the supplier, and kept loosely covered. The colony was given two to three organic wheat grains each week to supply bacteria on which paramecia feed. The room temperature varied from 70 - 74°F. The colony was originally obtained from Ward's Natural Science Establishment, Rochester, NY 14692.

The apparatus was constructed from square cross-section quartz tubing that had one side removed to create a trough. The tube ends were sealed with stainless steel blocks, and the entire trough was glued to a microscope slide using Goop brand adhesive, manufactured by Eclective Products, Inc. Electrical stimulation was provided by a Mallory recto power supply, model 12RS6D, set at 6.7 V DC at .74 mA with a duration of 60 ms and repeat frequency of 500 ms. Subjects were observed through a Leitz projection microscope, model 050222, under 22X magnification. The trough was divided into a light and dark side by a piece of gray transparent plastic filter that was placed under the appropriate side of the trough on the microscope stage. Twenty four paramecia

were run before the microscope light bulb exploded. It was replaced with an identical but unused bulb. However, it is possible that the illumination levels for these 24 subjects were lower than for the remaining 87 subjects. The illumination levels of the 250 w CSI lamp were 44132 lux without the filter and 12917 lux with the filter. These illumination levels provided contrast while still allowing the experimenter to see the paramecium in the dark side. Of the final 111 subjects used 24 were exposed to the old illumination levels; the other 87 subjects were exposed to the new light levels.

2.2 Procedure

An eyedropper was used to place a small drop of medium from the main colony onto a microscope slide that was on the stage of a microscope. The blunted needle of a syringe was inserted into the droplet and a single paramecium was ‘scooped’ up and transferred to the mid-point of the prepared trough, which had been filled with approximately 0.15 ml of distilled water and had been placed on the stage of the projection microscope. If a single paramecium was not caught the procedure was repeated until successful, however, after three unsuccessful attempts were made the trough was emptied, cleaned and refilled to prevent an excess of medium in the trough. Subjects were divided into one of three retention interval groups: zero min retention (immediate testing), 6 min retention interval or 12 min retention interval. For each of the retention intervals there was a control group as well as an experimental group. Individual paramecia were observed in the trough for ten 90 s training trials with no intertrial interval. During these trials experimental subjects received reinforcing cathode shock for staying in an “appropriate” location as described below; control subjects did not receive shock. Subjects were then exposed to the neutral stimulus for the duration of the

retention interval before retention was tested. The neutral stimulus was the illumination level the subject was not conditioned to; light for those subjects conditioned to dark and dark for those subjects conditioned to light. Subjects were left on the microscope stage with the filter in the appropriate neutral setting for the entire retention interval. Retention procedure was similar to the training procedure, but no shock was used. The correct side of the trough was the cathode side which consisted of a combination of location (left or right) as well as illumination level (dark or light). Thus, there were four possible combinations (dark/right, dark/left, light/right, and light/left). Both location and illumination were counterbalanced. A computer program recorded the total time of reinforcement each subject received during each trial; that is, the total time (in .5 sec) per trial that each subject remained in the reinforced half of the trough. Time was signaled to the computer by the researcher using a toggle switch to indicate the paramecium's location. Each paramecium was discarded at the completion of its session and the trough was thoroughly cleaned with distilled water.

Although 306 subjects were initially used, the following exclusion criteria were applied to limit the subject pool: each subject had to experience both brightness conditions within the ten training trials and each subject had to be visible at the end of the last testing trial. Applying these criteria reduced the subject pool to 146 paramecia. The subject pool was then further limited to include only the experimental subjects that had shown learning during the training trials. Subjects that spent more time in the reinforced location during the last two training trials, as determined by the mean, when compared to the mean of the first two training trials were said to have displayed learning. This was done because retention of the subjects was of interest and retention cannot logically be

looked at in subjects that did not learn the task in the first place. This resulted in 111 total subjects, 19 in each of the three control groups and 18 in each of the three experimental groups.

Chapter 3

Results

An independent samples t-test was completed to determine whether there was a preference for either the light or dark side of the trough. There was no significant difference in the times spent in either side for the experimental and control subjects combined or tested separately. The 111 control and experimental subjects that remained after application of the final exclusion criteria were compared using independent samples t-tests at two different times to determine if learning had taken place. The means of trials 1 and 2 combined were not significantly different between the control and experimental groups ($t_{109} = .22$, $p = .82$) while the means of trials 9 and 10 taken together were found to be significantly different ($t_{109} = 6.35$, $p < .05$). This pattern continued when the control and experimental subjects were broken down into the three retention intervals and retested. The means of training trials 1 and 2 taken together did not significantly differ from the control for any of the three retention intervals, 0, 6 and 12 minutes ($t_{35} = 1.00$, $p = .32$, $t_{35} = .43$, $p = .67$ and $t_{35} = .92$, $p = .37$), respectively. The means of training trials 9 and 10 taken together did significantly differ from the control for all three of the retention intervals (0: $t_{27} = 3.58$, $p < .05$, 6: $t_{28} = 3.68$, $p < .05$, 12: $t_{35} = 3.69$, $p < .05$). This suggests that learning did take place in all three groups of experimental subjects.

The means for these analyses can be seen in table 3.1.

Table 3.1: Mean Time (in .5 sec) Spent in Correct Location during Training Trials

	Mean of Trials 1 & 2	Mean of Trials 9 & 10
All Control subjects	87.3	85.9
All Experimental subjects	84.8	138.1
Zero retention Control	73.3	84.4
Zero retention Experimental	91.8	128.9
6 min retention Control	88.4	85.1
6 min retention Experimental	79.8	145.3
12 min retention Control	100.1	88.1
12 min retention Experimental	82.8	140.1

Retention was tested by looking at the time the subject spent in the correct location during the first testing trial. It was possible that extinction or forgetting would take place quickly; therefore, the first testing trial was of considerable interest as it would represent the paramecia's initial location choice. When all three levels of retention were grouped as either control or experimental subjects it was found that experimental subjects spent a significantly longer time in the correct location, $t_{93} = 2.26$, $p < .05$. The zero retention interval shows that the experimental subjects stayed in the correct location for more time than the control subjects, $t_{28} = 2.42$, $p < .05$. The control and experimental subjects in the 6 and 12 minute retention interval groups did not perform at significantly different levels from their controls, $t_{31} = 1.22$, $p = .23$ and $t_{30} = .29$, $p = .78$ respectively. Retention over all ten testing trials was also considered, however it was of less interest as extinction or forgetting was anticipated. An independent samples t-test was completed on the mean of the ten testing trials, grouping all control and experimental subjects, $t_{101} = 1.38$, $p = .17$. The mean of all ten testing trials was also looked at for the control and experimental subjects in their retention time groups. None of these comparisons was

found to be significant; zero min $t_{25} = 1.22$, $p = .24$, 6 min $t_{35} = 1.44$, $p = .16$, 12 min $t_{35} = .09$, $p = .93$. The means for these analyses can be seen in table 3.2.

Table 3.2: Mean Time (in .5 sec) Spent in Correct Location during Testing Trials

	Mean of Trial 1	Mean of Trials 1 - 10
All Control subjects	79.0	75.5
All Experimental subjects	100.5	84.3
Zero retention Control	73.3	74.3
Zero retention Experimental	111.8	87.4
6 min retention Control	74.8	70.7
6 min retention Experimental	95.9	85.3
12 min retention Control	88.9	81.3
12 min retention Experimental	93.7	80.2

The percentage of time spent in the correct location for each of the three retention levels can be seen in Figures 3.1 through 3.3.

Figure 3.1: Percentage of time spent in correct location by Control (n=19) and Experimental (n=18) subjects exposed to zero retention interval

Figure 3.2: Percentage of time spent in correct location by Control (n=19) and Experimental (n=18) subjects exposed to a six minute retention interval

Figure 3.3: Percentage of time spent in correct location by Control (n=19) and Experimental (n=18) subjects exposed to a twelve minute retention interval

Chapter 4

Discussion

Based on the previous research into paramecia's retention capabilities completed by Gelber (1958) and Huber et al. (1974) it was expected that paramecia would remember a learned task for a retention interval of several minutes. However, analysis of the data suggests that if a retention interval exists in paramecium it is less than 6 min long. Experimental subjects were able to learn the brightness discrimination task, however, only those that were immediately tested out-performed their control group counterparts in the first testing trial. A difference in time spent in the reinforced location by experimental and control subjects during the first testing trial of the 6 min retention groups can be seen, but the difference is not significant. At the 12 min retention level there is not a noticeable difference in performance during testing trial one. This suggests that extinction or forgetting had already taken place for subjects exposed to 12 min of a neutral stimulus.

Future work to investigate possible procedural parameters can be completed. For example, it is possible that the neutral stimulus, the non-reinforced brightness condition, was not actually neutral for all subjects. To test for this, a different neutral stimulus would need to be provided during the retention interval; however, it is not clear what an

acceptable alternate neutral stimulus could be. It is possible that paramecia are capable of remembering over shorter retention intervals. If so, work utilizing retention intervals ranging from a few seconds to multiple minutes should be looked at before a conclusion as to the retention capabilities of paramecia is drawn. It is also possible that retention, other than immediate retention, requires a central nervous system.

References

- Applewhite, P.B., & Gardner, E. T. (1973). Tube escape behavior of paramecia. *Behavioral Biology*, 9, 245-250.
- Armus, H.L., & Montgomery, A. (2001). Aversive and attractive properties of electrical stimulation for *Paramecium caudatum*. *Psychological Reports*, 89, 342-344.
- Armus, H.L., Montgomery, A., & Gurney, R. (2006). Discrimination learning and extinction in paramecia (*P. caudatum*). *Psychological Reports*, 98, 705-711.
- Armus, H.L., Montgomery, A., & Jellison, J. (2006). Discrimination learning in paramecia (*P. caudatum*). *The Psychological Record*, 56, 489-498.
- Day, L. & Bentley, M. (1911). A note on learning in paramecium. *Journal of Animal Behavior*, 1, 67-73.
- French, J. (1940). Trial and error learning in paramecium. *Journal of Experimental Psychology*, 26, 609-613.
- Gelber, B. (1952). Investigations of the behavior of *Paramecium aurelia*: I. Modification of behavior after training with reinforcement. *Journal of Comparative and Physiological Psychology*, 45, 58-65.
- Gelber, B. (1956). Investigation of the behavior of *Paramecium aurelia*: III. The effect of the presence and absence of light on the occurrence of a response. *The journal of Genetic Psychology*, 88, 31-36.
- Gelber, B. (1957). Food or training in paramecium? *Science*, 126, 1340-1341.

- Gelber, B. (1958). Retention in *Paramecium aurelia*. *Journal of Comparative and Physiological Psychology*, 51, 110-115.
- Huber, J.C., Rucker, W.B., & McDiarmid, C.G. (1974). Retention of escape training and activity changes in single paramecia. *Journal of Comparative and Physiological Psychology*, 86, 258-266.
- Jensen, D.D. (1957a). Experiments on “learning” in paramecia. *Science*, 125, 191-192.
- Jensen, D.D. (1957b). More on “learning” in paramecium. *Science*, 126, 1341-1342.
- Katz, M.S. & Deterline, W.A. (1958). Apparent learning in the paramecium. *Journal of Comparative and Physiological Psychology*, 51, 243-247.
- Ogawa, N., Oku, H., Hashimoto, K., & Ishikawa, M. (2006). A physical model for galvanotaxis of Paramecium cell. *Journal of Theoretical Biology*, 242, 314-328.

Appendix A

Time (in .5 sec) Spent in Correct Side of Trough during Training

Training Trials

Brightness	Side	Group	1	2	3	4	5	6	7	8	9	10
Dk	L	C	52	139	115	145	74	65	87	82	89	85
Dk	R	C	44	123	119	148	164	119	128	92	128	83
Lt	L	C	36	59	8	53	66	78	75	69	79	65
Lt	R	C	0	6	0	0	2	3	30	62	71	70
Dk	L	C	133	160	144	154	112	124	149	100	109	69
Dk	R	C	28	0	0	0	62	83	50	17	50	85
Lt	R	C	93	161	161	161	161	162	161	162	119	82
Dk	L	C	148	161	161	161	162	153	139	128	74	87
Dk	R	C	53	66	80	126	134	159	158	160	149	21
Lt	L	C	62	18	80	77	41	64	80	92	100	54
Lt	R	C	24	61	140	161	100	74	108	49	126	97
Dk	L	C	133	149	136	67	50	59	47	53	83	84
Dk	R	C	90	135	139	105	112	118	97	93	75	89
Lt	R	C	40	19	34	50	44	0	0	14	0	0
Dk	L	C	93	163	160	160	162	160	161	1257	135	119
Dk	R	C	103	88	85	118	84	86	96	91	85	75
Lt	L	C	0	12	27	91	94	130	98	86	83	109
Lt	R	C	45	45	24	0	50	57	66	59	73	76
Dk	R	C	42	0	14	93	73	0	0	40	67	162
Dk	L	C6	157	161	162	161	161	124	0	130	163	162
Dk	R	C6	4	0	4	5	19	12	38	48	41	47
Lt	L	C6	91	90	88	107	124	107	108	48	73	73
Lt	R	C6	0	6	0	0	9	37	5	0	0	0
Dk	L	C6	143	146	161	162	162	162	162	162	163	162
Lt	L	C6	155	163	162	162	163	162	162	163	161	161
Dk	R	C6	118	106	39	0	0	0	0	0	0	0
Lt	L	C6	80	114	112	78	162	163	162	160	155	162
Lt	R	C6	36	17	4	0	0	0	0	26	0	10
Dk	L	C6	159	133	153	130	0	72	144	162	162	128
Dk	R	C6	15	3	0	0	0	13	35	35	53	70

Lt	R	C6	7	0	6	68	134	54	121	0	0	0
Dk	L	C6	97	98	163	162	163	135	32	59	86	112
Lt	L	C6	113	113	52	89	164	114	113	78	110	94
Dk	L	C6	159	140	163	92	141	159	163	128	152	71
Lt	L	C6	133	162	162	162	162	162	163	147	163	163
Lt	R	C6	117	0	85	73	132	89	43	72	35	62
Dk	R	C6	0	5	9	0	8	0	0	0	40	9
Lt	L	C6	157	163	117	28	0	0	0	51	90	101
Dk	L	C12	108	95	91	66	73	86	78	67	76	70
Dk	R	C12	10	0	0	0	0	0	0	0	0	0
Lt	L	C12	96	104	128	94	58	35	92	69	82	100
Dk	R	C12	69	50	36	43	13	56	49	14	69	103
Lt	L	C12	47	20	0	0	0	0	0	0	0	16
Lt	R	C12	110	85	0	0	0	0	0	72	159	97
Dk	L	C12	110	70	76	153	141	106	106	103	93	71
Lt	R	C12	8	0	0	0	0	0	36	6	44	64
Dk	L	C12	161	163	164	163	161	161	161	162	161	163
Lt	L	C12	160	158	150	137	143	152	147	163	113	90
Lt	R	C12	0	0	0	43	62	31	70	110	90	60
Lt	L	C12	157	163	163	163	95	129	131	85	83	74
Lt	R	C12	114	100	89	56	58	69	69	104	96	81
Dk	L	C12	138	162	162	162	162	162	161	162	163	162
Lt	L	C12	160	163	164	164	164	163	162	161	160	160
Dk	L	C12	127	126	88	69	92	78	90	87	64	82
Lt	L	C12	140	141	82	122	162	161	154	162	107	123
Dk	L	C12	148	107	101	99	131	85	90	92	63	21
Lt	L	C12	106	126	163	163	95	118	76	110	92	95
Dk	L	IM	39	122	31	162	87	162	162	162	161	84
Dk	L	IM	0	10	161	50	140	158	42	0	64	160
Lt	R	IM	85	89	162	161	162	117	160	161	161	161
Lt	L	IM	118	162	162	161	79	162	161	162	160	151
Dk	R	IM	71	149	159	159	159	110	155	161	156	146
Dk	R	IM	145	161	161	159	160	161	161	162	161	160
Lt	L	IM	155	162	160	161	160	161	160	160	161	161
Dk	L	IM	34	161	134	161	136	162	162	161	162	162
Lt	L	IM	138	162	161	161	162	162	127	92	159	162
Dk	L	IM	144	147	105	83	100	125	25	93	162	160
Dk	R	IM	0	0	0	0	50	60	161	161	101	155
Dk	L	IM	125	152	158	153	163	152	148	161	155	155
Dk	R	IM	3	0	79	49	127	146	14	1	0	60
Lt	L	IM	70	39	0	0	0	0	0	0	0	123
Lt	R	IM	0	0	0	97	0	0	0	37	0	59
Lt	L	IM	52	0	0	0	0	0	0	0	14	162
Dk	L	IM	157	135	164	164	160	161	161	139	160	161
Dk	L	IM	156	163	164	164	145	154	142	162	161	161
Dk	R		6	0	39	116	162	142	160	161	159	162
Dk	R		6	101	149	164	126	162	160	157	60	123
Dk	R		6	126	72	141	88	161	162	162	148	161

Lt	R	6	21	53	100	49	34	84	160	114	160	161
Dk	R	6	0	6	64	160	56	0	0	56	22	72
Lt	L	6	86	161	72	17	16	126	49	119	161	162
Lt	R	6	0	8	4	129	161	161	162	161	161	162
Dk	L	6	104	121	139	161	161	161	161	160	161	161
Lt	L	6	58	67	101	162	74	121	139	56	110	61
Lt	L	6	157	160	160	161	162	161	160	161	160	160
Lt	L	6	123	8	131	154	129	162	162	161	161	162
Dk	R	6	0	0	23	129	116	58	0	114	34	148
Lt	R	6	0	48	2	68	161	163	162	162	162	160
Dk	L	6	126	136	129	163	162	163	163	88	150	162
Lt	R	6	151	164	164	154	0	0	0	137	162	160
Lt	L	6	159	157	152	147	164	163	163	162	161	161
Lt	L	6	132	130	141	163	44	4	127	112	155	164
Dk	R	6	25	24	163	162	163	163	164	163	163	163
Dk	R	12	151	161	161	161	161	161	161	163	161	162
Lt	R	12	86	162	84	161	162	160	160	161	162	161
Lt	R	12	61	160	90	95	162	162	162	162	162	162
Dk	R	12	116	162	161	161	162	160	160	160	161	162
Lt	R	12	62	77	0	0	0	54	143	26	126	28
Dk	L	12	107	155	161	161	161	161	161	162	162	163
Lt	L	12	60	138	119	162	162	161	162	162	162	162
Dk	R	12	32	35	0	26	35	0	25	114	50	162
Dk	R	12	1	0	0	0	0	0	57	9	60	0
Dk	R	12	0	0	57	0	0	0	71	161	162	163
Lt	L	12	162	139	130	145	152	162	162	161	161	162
Dk	R	12	158	162	150	161	161	163	163	162	162	162
Lt	L	12	79	146	76	111	107	119	97	163	163	74
Dk	R	12	13	120	0	158	89	162	163	163	70	96
Lt	L	12	50	0	54	140	56	119	163	162	163	163
Lt	R	12	0	0	0	0	44	0	22	60	162	163
Dk	R	12	0	0	59	10	120	151	163	162	162	163
Dk	L	12	79	147	31	0	82	163	163	163	163	163

Appendix B

Time (in .5 sec) Spent in Correct Side of Trough during Testing

Testing Trials

Brightness	Side	Group	1	2	3	4	5	6	7	8	9	10
Dk	L	C	100	87	87	101	84	78	86	99	72	97
Dk	R	C	102	92	113	92	49	51	66	90	79	80
Lt	L	C	93	79	81	52	77	70	61	88	43	68
Lt	R	C	55	81	52	69	57	57	58	73	58	76
Dk	L	C	69	111	86	74	65	81	100	84	74	83
Dk	R	C	62	62	69	77	88	80	80	75	54	75
Lt	R	C	41	70	72	76	66	74	87	65	60	72
Dk	L	C	73	105	84	88	78	60	73	91	67	117
Dk	R	C	44	153	155	125	119	147	78	93	80	92
Lt	L	C	85	74	90	106	52	89	90	85	54	42
Lt	R	C	59	63	54	61	69	51	67	63	60	73
Dk	L	C	55	109	78	79	78	109	80	101	72	80
Dk	R	C	75	30	62	82	57	58	63	74	80	28
Lt	R	C	0	29	0	85	43	21	0	60	0	0
Dk	L	C	130	116	117	114	95	73	97	135	128	82
Dk	R	C	66	119	104	41	0	106	80	124	116	86
Lt	L	C	82	101	99	69	90	96	75	107	77	86
Lt	R	C	41	74	72	86	68	91	55	49	54	96
Dk	R	C	161	129	0	0	0	0	0	0	0	54
Dk	L	C6	0	0	0	0	0	0	0	0	0	90
Dk	R	C6	71	93	86	87	105	98	85	102	100	88
Lt	L	C6	75	76	56	78	83	110	97	83	41	87
Lt	R	C6	0	0	0	0	0	0	0	40	50	0
Dk	L	C6	159	128	162	162	162	139	93	143	87	89
Lt	L	C6	80	67	114	74	91	73	95	98	96	82
Dk	R	C6	87	76	50	99	87	63	54	58	152	0
Lt	L	C6	153	77	0	1	87	0	76	93	161	161
Lt	R	C6	66	65	89	84	69	68	71	74	98	104
Dk	L	C6	77	75	80	83	69	56	57	58	90	52
Dk	R	C6	74	104	45	55	83	71	84	94	80	60

Lt	R	C6	0	0	0	0	0	0	0	0	83	153
Dk	L	C6	100	33	85	105	77	52	71	93	64	78
Lt	L	C6	50	72	71	87	69	71	61	81	102	86
Dk	L	C6	128	161	135	86	85	57	33	91	83	61
Lt	L	C6	57	71	69	42	112	61	71	65	101	86
Lt	R	C6	73	45	81	91	87	58	63	79	110	59
Dk	R	C6	81	76	97	82	53	75	66	87	80	54
Lt	L	C6	91	71	36	70	63	84	48	81	58	82
Dk	L	C12	112	75	76	60	64	71	79	75	33	66
Dk	R	C12	0	0	0	0	0	0	0	0	12	0
Lt	L	C12	56	102	47	73	72	81	68	56	72	71
Dk	R	C12	83	82	66	70	76	93	65	96	84	61
Lt	L	C12	81	72	64	90	82	64	83	71	69	68
Lt	R	C12	100	80	55	80	68	52	49	33	0	70
Dk	L	C12	100	56	52	123	79	68	103	65	69	106
Lt	R	C12	68	61	58	60	69	78	98	64	66	61
Dk	L	C12	107	163	103	130	113	100	86	73	76	65
Lt	L	C12	68	69	78	75	95	79	81	90	95	76
Lt	R	C12	72	71	93	78	92	92	94	3	78	51
Lt	L	C12	85	789	66	53	68	84	98	94	86	92
Lt	R	C12	108	128	80	111	106	111	88	136	81	61
Dk	L	C12	161	163	162	162	161	162	161	161	161	162
Lt	L	C12	161	161	162	162	135	46	139	85	56	88
Dk	L	C12	69	79	71	100	92	75	88	70	104	64
Lt	L	C12	60	82	69	68	69	84	92	78	101	82
Dk	L	C12	152	9	0	16	0	0	0	0	0	0
Lt	L	C12	46	78	100	72	87	79	100	59	60	95
Dk	L	IM	0	0	5	0	0	0	0	0	0	0
Dk	L	IM	9	0	0	0	0	71	87	65	28	51
Lt	R	IM	138	51	0	0	0	0	0	0	62	29
Lt	L	IM	161	12	161	161	159	159	161	161	161	32
Dk	R	IM	56	76	123	87	76	101	87	111	157	159
Dk	R	IM	161	163	162	162	162	62	162	83	162	128
Lt	L	IM	161	160	160	161	161	160	160	131	147	161
Dk	L	IM	161	126	102	133	36	42	47	105	46	77
Lt	L	IM	163	162	162	136	134	63	64	59	121	42
Dk	L	IM	87	99	94	71	0	6	69	121	105	128
Dk	R	IM	89	0	40	53	40	128	161	161	162	130
Dk	L	IM	149	112	40	112	120	51	70	77	67	73
Dk	R	IM	91	77	66	65	51	103	84	66	102	62
Lt	L	IM	162	162	162	162	160	81	127	61	75	66
Lt	R	IM	67	95	76	75	77	72	83	46	78	81
Lt	L	IM	161	130	62	85	74	84	78	85	38	76
Dk	L	IM	162	163	150	81	127	83	63	74	106	110
Dk	L	IM	35	28	33	122	49	60	24	0	5	133
Dk	R		6	159	161	148	157	158	160	152	7	108
Dk	R		6	158	37	3	0	10	98	49	112	73
Dk	R		6	130	92	102	70	100	65	48	81	73

Lt	R	6	55	78	92	67	79	73	71	75	78	42
Dk	R	6	64	45	64	59	49	52	66	70	73	49
Lt	L	6	116	160	162	162	162	162	139	145	162	133
Lt	R	6	0	0	0	0	54	75	66	71	162	161
Dk	L	6	160	161	160	139	154	103	91	160	78	65
Lt	L	6	45	47	60	103	63	6	66	70	115	60
Lt	L	6	158	159	160	161	161	161	159	151	151	161
Lt	L	6	57	96	106	35	91	63	69	114	86	123
Dk	R	6	51	36	47	45	70	51	73	70	68	77
Lt	R	6	15	48	14	24	40	97	113	59	91	34
Dk	L	6	161	162	145	141	53	49	72	43	92	75
Lt	R	6	8	58	86	32	65	100	94	52	89	42
Lt	L	6	145	147	5	2	98	112	137	81	57	84
Lt	L	6	84	88	88	99	66	63	64	48	77	66
Dk	R	6	161	145	4	33	24	53	52	60	38	43
Dk	R	12	155	119	79	102	83	103	82	103	90	67
Lt	R	12	139	162	161	161	161	161	161	162	161	161
Lt	R	12	152	0	71	63	68	46	92	55	63	23
Dk	R	12	52	72	54	74	42	67	62	0	18	0
Lt	R	12	19	65	44	42	81	47	20	16	43	37
Dk	L	12	159	161	136	102	128	73	127	55	11	103
Lt	L	12	160	161	160	123	66	34	4	37	33	44
Dk	R	12	28	80	86	51	53	83	87	73	83	87
Dk	R	12	94	73	67	93	92	97	54	41	102	75
Dk	R	12	82	0	33	51	58	118	40	70	79	82
Lt	L	12	60	85	69	43	80	79	70	80	102	113
Dk	R	12	160	162	162	162	162	161	163	162	163	85
Lt	L	12	0	65	35	0	0	44	0	0	0	0
Dk	R	12	161	20	30	62	34	31	127	54	66	64
Lt	L	12	89	86	78	75	71	76	82	96	35	87
Lt	R	12	0	0	0	0	0	0	0	72	51	70
Dk	R	12	85	94	155	110	163	163	163	162	162	163
Dk	L	12	91	89	86	48	59	18	103	89	135	163