THE EFFECTS OF SPACING OF TRAINING AND EXTIRPATION OF CEREBRAL GANGLIA ON THE UNCONDITIONED RESPONSES OF EARTHWORMS

Thesis for the Degree of M. A.

MICHIGAN STATE UNIVERSITY

Donald Gerald Stein

1962

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By

Donald Gerald Stein

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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THE EFFECTS OF SPACING OF TRAINING AND EXTIRPATION OF CEREBRAL GANGLIA ON THE UNCONDITIONED RESPONSES OF EARTHWORMS

by Donald Gerald Stein

Ninety-six earthworms were divided into 6 surgical treatment groups. Half of the animals in each group received massed presentation of a strong photic stimulus while the other half received spaced presentation of the US. Frequency, direction, and latency of URs were recorded for all groups. Results showed that normal worms made fewer URs under massed trial conditions than under spaced trials although the direction of URs did not change. Duration of ITI did not appear to affect the degree of adaptation of the UR to light for the normal, intact worm.

No differences in latency, frequency or direction were obtained for _Ss_ with either sub- or suprapharyngeal ganglia removed for comparisons made within the same ITI. These _Ss_ were consistently positive to the US as compared to the negative URs of the intact animal. No evidence of specificity of function of the cerebral ganglia was obtained regarding the worm's UR to light.

Operated worms did not differ from normal _Ss_ under massed trial conditions. However, extirpation of neural

tissue and long ITI were found to decrease frequency and increase latency of URs for the operated groups. Direction of the URs was not affected by spacing of training. Adaptation of the UR occurred under both massed and spaced trials but appeared more pronounced under spaced trials.

Results cannot be interpreted in terms of existing statements of neural function of invertebrates. At the descriptive level, results suggest that operated worms become response-bound while normal worms remain stimulus-bound with regard to reactions to light.

Stanley C. Ratner
Associate Professor
Sept. 17, 1962

TO DAREL

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ACKNOWLEDGMENTS

The author would like to express his gratitude to Dr. Stanley C. Ratner for his guidance and help which was freely given whenever needed, and to Dr. Donald Montgomery of the Physics Department whose loan of valuable equipment made this research possible. The author would also like to thank Drs. C. Hanley and A. Barch for their careful review and criticism of the experimental design.

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INTRODUCTION

Use of the Annelid, _Lumbricus terrestris_, as an experimental subject is not a recent phenomenon. Darwin (1881), attempted some research into the "habits and intelligence" of the earthworm, but his studies were rather crude and poorly controlled and not much data could be gleaned from them. Yerkes (1912), using one subject, demonstrated that an earthworm can learn to make a discriminatory response in a simple T-maze when differential reinforcement to each side is given. For the most part, early research on annelid behavior dealt with the attempt to modify rather than specify the worm's responses to its environment. In addition, little emphasis was placed on the study of the neural mechanisms involved in the organism's behavioral responses.

The physiology and morphology of _L. terrestris_ are well understood due to the efforts of the comparative physiologists and zoologists (Hess, Prosser, Stevenson, _et al_.). Researchers in these areas provided much information as to the classes of stimuli to which the organism is sensitive. However, these workers were not generally interested in the peculiar characteristics of the earthworm's behavior and the conditions under which it could be made to vary.

One of the early attempts to specify the function of the "cerebral ganglia" in earthworms was made by Heck (as reported in Prosser, 1950). Following Yerkes (1912), he found that earthworms are capable of learning a simple T-maze, and that removal of the "cephalic ganglia" does not result in the loss of an "acquired habit" until the new segments have been regenerated. Hess (1924) studied the reactions of Lumbricus terrestris to light of varying intensity under several surgical conditions. He found that removal of the cerebral ganglia caused earthworms to become "definitely positive in their reactions to lights of moderate intensity and that this behavior also occurs when the suprapharyngeal lobes are separated, although to a lesser degree" (p. 541). He further determined that the cerebral ganglion "is the controlling factor in negative reactions to strong and moderate stimuli." Prosser (1950) in a review of his own research states that the cerebral ganglia of the earthworm control the animal's behavior and motor reactions to various types of interoceptive and exteroceptive stimulation.

Much of the research dealing with the functions of the ganglia has been contradictory. For example, some workers reported that extirpation of the cerebral ganglia caused no differences in behavior (Buddenbrock, Friedlander, Steen-Maxwell), while others have reported that removal of the sub- and suprapharyngeal ganglia has modified behavior in several ways (Wayner and Zellner, Ratner and Miller, Ratner). In many of these studies, however, sample size was very small, the responses being observed were not clearly specified, and, in several cases, the procedures were difficult to understand. It is possible that these factors would explain some of the discrepant findings reported by these workers.

In more recent research, Wayner and Zellner (1958) demonstrated that removal of the suprapharyngeal ganglia is related to a decrease in the number of negative responses at a choice point in the simple T-maze. They theorized that such removal increases reactive inhibition, therefore decreasing the number of alternations at the choice point. Swartz (1929), using a T-maze with electric shock in one arm of the maze, found that worms formed an "association" with the correct (shockless) direction of turning. The "habit" was not lost after removal of the suprapharyngeal ganglia, and worms from which the anterior segments had been removed learned the habit (p. 33). Bharucha-Reid (1961) attempted to demonstrate that directional behavior can be influenced by differentially removing either the right or left lobe of the suprapharyngeal ganglia. She reported that when the worms were placed in a choice situation, they would turn to the side opposite that from which the lobe had been removed. However, study of her procedures suggested that the ganglia of her subjects had regenerated so her conclusions are not firm.

Until 1959, all of the research dealing with learning in the earthworm and the functions of the ganglia in such learning, had utilized the instrumental learning situation;

for example, the T-maze. Ratner and Miller (1959a, b) utilized classical conditioning procedures for the study of annelid behavior. In the first study (1959a), it was demonstrated that a withdrawal response to strong photic stimulation could be conditioned to occur to a weak vibratory stimulus. It was also found that the duration of the intertrial interval influenced the percentage of conditioned responses. That is, animals trained under spaced trials showed a higher percentage of CRs than animals trained under massed trial conditions. The more recent studies (1959b, 1962) were designed to investigate the effect of both removal of "cephalic ganglia" and spacing of trials on conditioning of a withdrawal response. It was found that the non-operated (normal) spaced trials group had the highest percentage of CRs while the operated spaced trial group did not condition at all. The operated massed trials group had about 60 per cent CRs which was significantly different from the operated control groups and the operated spaced trials groups whose responses were almost negligible.

Ratner (1962) showed that decerebrate worms condition when tested under massed trials even when given a long delay in the apparatus. When conditions were reversed from massed to spaced trials, the percentage of CRs decreased for the operated worms. Ratner concluded that duration of intertrial interval and not confinement in the apparatus was responsible for the decrease in the percentage of CRs for decerebrate worms. Thus, "failure in conditioning of decerebrate worms trained with spaced trials. . .is based on some particular relationship between decerebration and spacing of training." It was further determined that removal of the anterior five segments of the worm inhibited not only the CRs but suppressed the URs to the photic stimulus as well. However, no specification of the role of the sub- and suprapharyngeal ganglia in the unconditioned responses of the earthworm has been undertaken. Defining the function of each of the neural centers in the responses of *Lumbricus terrestris* would be a contribution to the neurophysiology of learning and the mechanisms involved in it.

Problem

The present experiment was designed to study the effect of: (1) differential extirpation of the suprapharyngeal and subpharyngeal ganglia, and (2) spacing of trials on the unconditioned response to strong photic stimulation of the earthworm, *Lumbricus terrestris*.

METHOD

Apparatus

The part of the apparatus which held the worm during the experiment consisted of clear, round, non-toxic "Tigon" plastic tubing with a 3/8-inch internal diameter. The tubing was ventilated by small holes located at 1/2-inch intervals on both the sides and the top of the tube. Both ends of the tube were fastened together with a piece of clear plastic of a larger internal diameter to form a complete circle. The tube was then placed in a square plywood box 10 inches on each side and 2.5 inches deep. To increase reflectance from the photic source and insure equal stimulation on all sides of the tube, the floor and sides of the box were covered with translucent plastic sheeting.

A standard #2 photoflood lamp in an aluminum reflector was mounted 39.5 inches above the table top, the bulb itself being 24.5 inches directly above the tube. This source provided the photic stimulation used as the unconditioned stimulus (US). At all times during the experiment, an 8 watt dark red bulb was used to provide a low level of illumination for the experimenter. Previous research (Hess, 1929) showed that earthworms of this species are insensitive to the red end of the spectrum.

Presentation of the US was automatically timed by Meylan interval timers. One timer presented the US every 6 seconds and the other every 88 seconds. In both cases presentation consisted of two second durations. Latency of the unconditioned response (UR) was timed by a Lafayette timer activated by a microswitch operated by the experimenter when the onset of the UR was observed to occur.

In order to prevent any extraneous vibration from influencing S's responses, all parts of the apparatus that could have produced vibration or were sensitive to it were padded with pieces of foam rubber. This included the base on which the tube rested, the experimenter's writing surface, and the Lafayette timer.

To reduce any increase in temperature produced by the photic source, the floor of the box on which the tube rested was sprayed with cool water before and between trials, especially for the 88 second ITI groups which remained in the tube and apparatus for one hour and forty minutes. An attempt was made to keep room temperature below 65° F. during the time the experiment was in progress.

Subjects

Ninety-six earthworms, Lumbricus terrestris, with an average weight of 3.8 grams, were studied. The annelids were obtained from the Tanner Bait Supply Company in New York State. Upon receipt, the worms were taken from their shipping cartons and placed in a large plastic container filled with fresh sphagnum moss and humus which was kept in a refrigerator at a temperature of 40° F., ±3°. Approximately 250 worms were kept in this container from which the sub-sample studied was drawn.

Surgical procedure

Immediately prior to the surgical treatment, a number of worms were haphazardly selected from the large container using a forceps to grasp them. Each animal was then placed in a small non-toxic plastic freezer carton with a bed of moist sphagnum moss and a ventilated lid. The animals chosen were then weighed and randomly assigned to one of the following surgical treatment groups:

a) normal, non-operated (Normal)
b) sham-operated dorsal side (SD)
c) sham-operated ventral side (SV)
d) anterior five segments removed (5-seg-R)
e) suprapharyngeal ganglia removed (Sup-R)
f) subpharyngeal ganglia removed (Sub-R)

Each of the surgical treatment groups contained 16 worms and each of these groups was subdivided after surgery into a group which received massed trials and a group which received spaced trials. This variable will be discussed below. Excepting the 5-seg-R group, Ss were placed on a piece of non-toxic plastic sheeting and were anesthetized with 2 ml. of .008 per cent solution of chlorotone (6.25 gms. chlorobutynol crystals per 125 cc. distilled water). Anesthetized animals were allowed to remain in the solution approximately 10 minutes, or until tactual stimulation with a glass probe produced no response of contraction or movement. When no response could be elicited, S was removed from the anesthetic with a forceps and placed on a styrofoam dissecting board covered with a clean and thoroughly moistened cloth. The anterior portion of the animal was held in place by a strip of white paper placed over S and pinned on either side to the board. The board with S on it was then placed on the stage of a binocular dissecting microscope. This allowed E to observe the ganglia more clearly and facilitated extirpation of the neural tissue.

In the SD and Sup-R groups, an incision was made on the dorsal side of the animal from the first to the fifth or sixth anterior segment. If S were in the sham dorsal group (SD), the incision was made and S was checked for bleeding and damage to the suprapharyngeal ganglia. If bleeding occurred or if the ganglia appeared to be disturbed in any way, the animal was rejected and another chosen. After the "operation" the worm was returned to its individual container and placed in the refrigerator for 48 hours. Animals whose ganglia were removed were incised in the same manner except that small hemostats were attached to the musculature on either side of the incision to hold it open and expose the ganglia. The circumpharyngeal connectives were severed, the sub- or suprapharyngeal ganglia removed, the hemostats removed, and the animal returned to its container and placed in the refrigerator for the 48-hour recovery period.

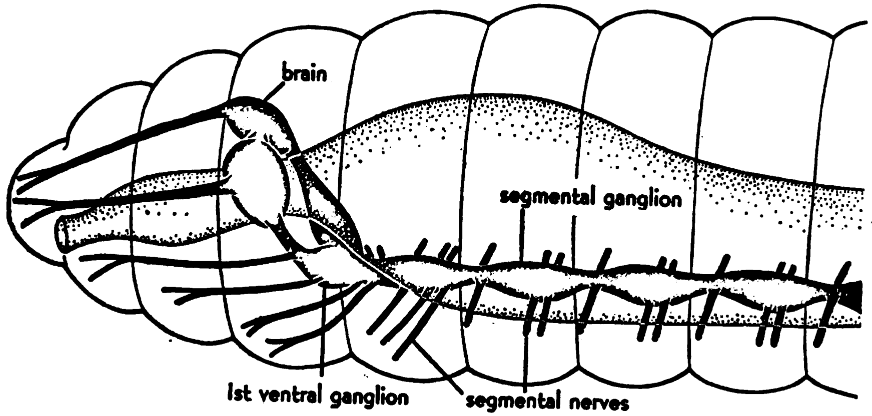


Figure A.--Diagram of cerebral ganglia in the earthworm, *Lumbricus terrestris*.

A similar procedure was carried out for the sham ventral (SV) and Sub-R groups except that the incision was made on the ventral surface from the second to the seventh or eighth segments.

In the 5-seg-R group, no chlorotone was administered. The animals were placed on a plastic sheet moistened with tap water and the anterior five segments were removed with a very sharp razor blade. The sectioned segments were then examined to make certain that the ganglia had been completely extirpated. The S was then returned to its individual container, placed in the refrigerator and given the 48-hour recovery period as were all other Ss used in the experiment.

The non-operated normal animals (Normal) were simply weighed, placed in their containers and returned to the refrigerator for the same amount of time allowed for the recovery period of the operated groups.

Testing procedure

After the 48-hour recovery period, S was removed from its container and placed in the Tigon plastic tube which had been moistened with .6 cc. of tap water immediately prior to S's entry. To help eliminate contamination from salts and other substances on the E's hands, all Ss were handled with rubber gloves while being placed in the tube. The worm's anterior portion was placed in the tube while gently stimulating the posterior portion of the animal. When S was completely in the tube, the ends were fastened together so as to form a complete circle for S to transverse. The tube with S inside was then placed on its base and a 10-minute "dark adaptation period" was allowed for each animal prior to the onset of the trials.

The eight Ss assigned to the massed trial condition from each surgical group were given a total of 60 trials with an intertrial interval of 6 seconds. The US was presented on each trial. Thus, for Ss in these groups the US was presented for two seconds followed by the 6 second ITI period. Responses to the US were observed during the 6 second period, after which another US presentation occurred. The entire procedure including the habituation to the tube and darkness required 18 minutes for each animal. The Ss assigned to the spaced trial condition (88 second ITI) were also given a total of 60 trials in which the US was presented on every trial. Thus, for Ss in these groups the US was presented for 2 seconds followed by the 88 second ITI period. Responses to the US were observed during the first 6 seconds of the 88 second period, after which another US presentation occurred. The entire procedure for this group, including the habituation period, took one hour and 38 minutes for each animal. In both conditions, the US was presented for a total of 2 seconds on each trial. No subjects were given both massed and spaced trials.

Measurement

The UR can be described as follows: (1) a withdrawal

and/or contraction extending approximately for 1/4 to 1 inch in length of the anterior segments of S's body.
(2) A rapid forward movement of approximately the same length of the anterior portion of S's body. If the worm happened to be moving at the time the US was presented the response consisted of an abrupt stop followed by either of the responses described above. A response occurring before 6 seconds had lapsed from the onset of the US was recorded as an unconditioned response. Prostomial movement was not considered to be a UR. In operated subjects a forward movement was observed to occur more often than a withdrawal response. Therefore, the direction of S's response was recorded as either a forward (F) or withdrawal (W) reaction. The time lapsed between the onset of the US and the beginning of the animal's response was recorded for all groups and has been designated as latency of response. Records were also taken of the number of times a worm doubled back or turned around while in the tube. During the 6 second ITI, an assistant recorded latency, frequency and direction of URs, thus allowing E to observe the worm for the entire 6 second ITI period.

In order to reduce judgmental bias, the identity of the group to which S belonged was not known by E (except 5-seg-R) until after the last trial had been completed. Animals studied on any given day were chosen by the date in which they were placed in their individual containers.

RESULTS

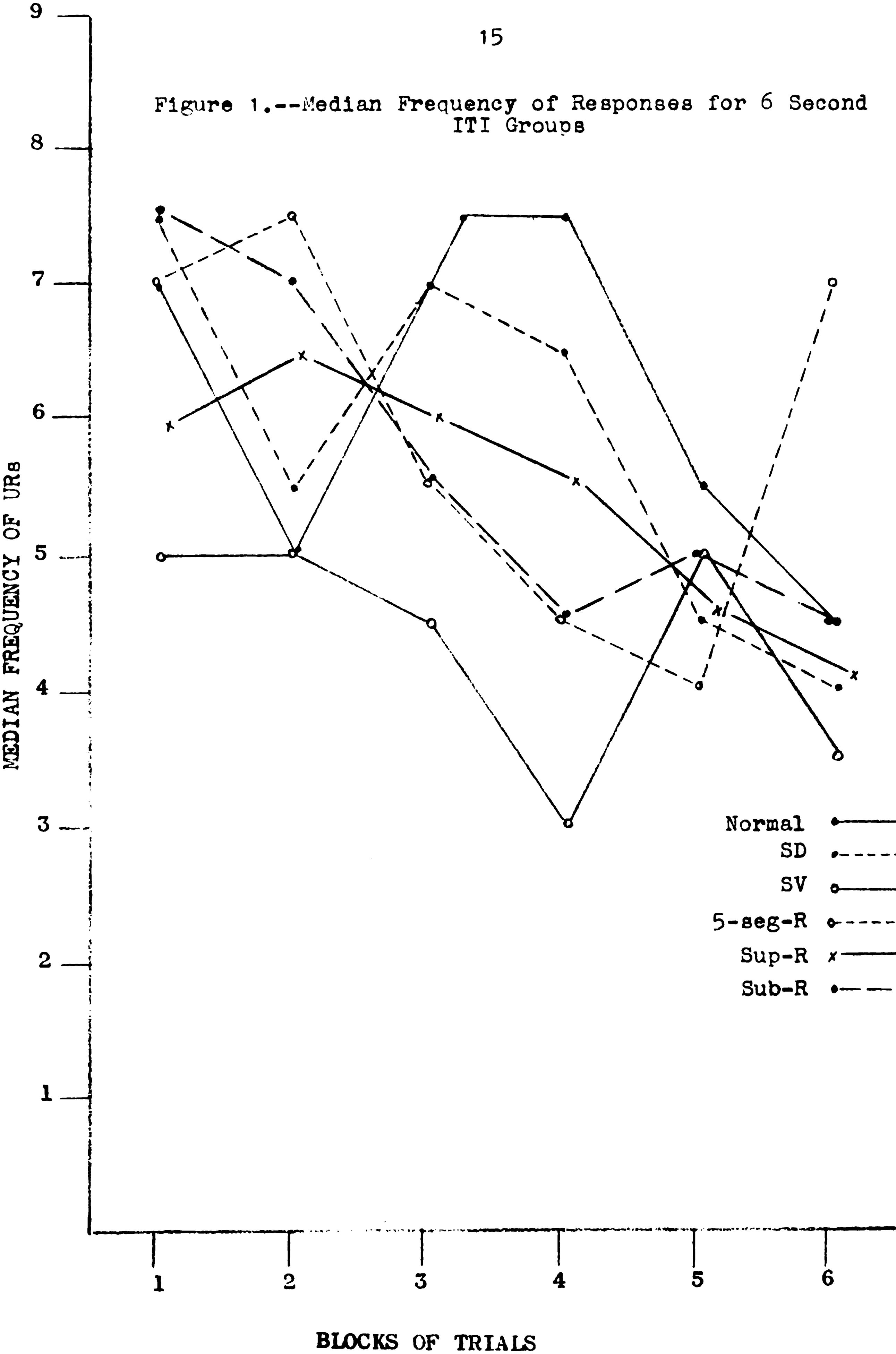
Frequency of unconditioned responses

Three measures of S's URs were taken for all groups: frequency of response, direction of response, and latency of response. Figures 1 and 2 present the median frequency of URs for the Normal, SD, SV, 5-seg-R, Sup-R, and Sub-R groups subjected to the 6 second and 88 second ITI.

To determine if spacing of trials affected the total number of URs to light, the Wilcoxin test (Wilcoxin, 1949) for groups of replicates was used. The median frequency of URs was 33 URs for the massed trial groups and 24 URs for the spaced trial groups. The sum of the ranks of frequency of URs was found to be 369.0 for the massed trial group and 261.0 for the spaced trial group. The probability of obtaining a rank total of 369 as compared to a rank total of 261 is less than 0.01. Thus, it was concluded that the groups given massed trials had a significantly greater total number of URs to light than did Ss given spaced trials.

Observation of Figures 1 and 2 suggests that an interaction exists between surgical treatment and intertrial interval. That is, it appears that the effects of surgical treatment on total frequency of URs vary according to the temporal conditions under which Ss are tested. This observation was tested by using a non-parametric test for interaction as described in Wilcoxin (1949). A highly significant

Figure 1.--Median Frequency of Responses for 6 Second ITI Groups



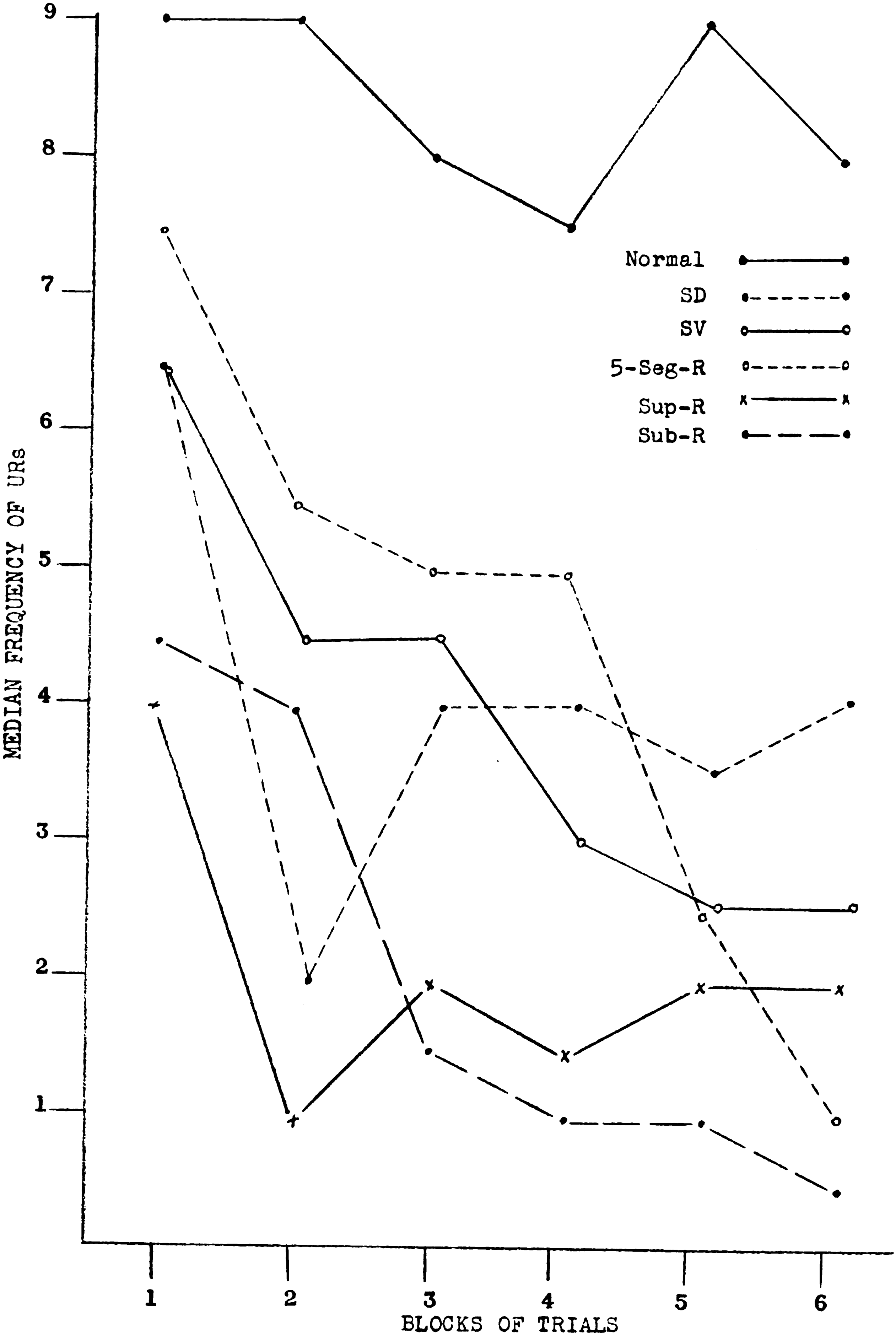


Figure 2.--Median Frequency of Responses for 88 Second ITI

interaction ($X^2_r = 21.12$, p .001) was obtained.

To determine if spacing of trials affected total frequency of URs between groups which received the same surgical treatment, Mann-Whitney U tests (hereafter referred to as U tests) were made by comparing the total frequency of URs in each group given the 6 second ITI with its equivalent group given the 88 second ITI. Table 1 presents the median total frequencies, U values, and the probabilities associated with them for each comparison. It can be seen that the Normal massed trial group differed significantly from the Normal spaced trial group in the total number of URs to light (U = 8.5, p .007). Thus, for the Normal animals, frequency of URs was greater under spaced trials than under massed trials. Both the Sup-R and Sub-R massed trials groups differed significantly from their spaced trials counterparts (U = 5.5, p .002; U = 7.0, p .003 respectively). In both cases the frequencies of URs was significantly higher for Ss tested with massed trials than for Ss tested with spaced trials. No significant differences were obtained for the SD, SV, or 5-seg-R comparisons.

To determine if "surgery" had an effect on the total frequency of URs, U tests were performed comparing some massed trials groups with other massed trials groups and some spaced trials groups with other spaced trials groups. Table 2 in the Appendix shows the comparisons that were carried out between groups tested with massed trials. Not all comparisons were made because some groups obviously did not differ. Thirteen _U_ tests were performed and no significant differences were obtained for the massed trial groups. The same statistical procedure was carried out for those _Ss_ tested with spaced trials. All possible comparisons were made and Table 3 in the Appendix presents the median total frequencies, the _U_ values, and associated _p_ values. Comparisons between the Normal group and each of the other spaced trials groups, including groups SD, SV, 5-seg-R, Sup-R, and Sub-R, shows that in all cases the _U_ values were highly significant. Total frequency of URs in the Normal group were significantly greater than the total frequency of URs for each of the surgically treated groups. In addition, significant _U_ values were obtained when the sham-operated _Ss_ were compared with their ganglion extirpated counterparts (e.g. SD--Sup-R, SV--Sub-R). In both cases the total frequency of URs was greater for the sham-operated animals. A significant difference in total frequency of URs was found comparing the Sub-R and 5-seg-R groups. No significant differences were found between sham-operated _Ss_ nor were there any significant differences between sham-operated animals and those with their anterior five segments removed. No significant differences were found between Sup-R and Sub-R _Ss_ although it appeared as if there were a "trend" towards greater frequency of responding in the Sup-R group. When 5-seg-R _Ss_ were compared with Sup-R and Sub-R respectively, it was found that no differences in total frequency of URs existed between the 5-seg-R and Sup-R animals.

In summary, frequency of URs was greater in the surgically treated Ss given massed trials than for the equivalent surgical groups given spaced trials. This relationship, however, did not hold for the Normal Ss whose total frequency of URs was depressed by massed trials. See Figures 1 and 2.

Direction of response

To assess whether ITI affected the direction of S's response, the Fisher exact probability test was used (Siegel, 1956). An S was assigned to category F (forward) if greater than 50 per cent of its total URs were forward. If greater than 50 per cent of its URs were withdrawals, S was assigned to category W (withdrawal). Table 4 presents the comparisons made and their significance levels. In comparing Ss receiving massed trials with their spaced trials counterparts, no significant differences were obtained. It can be concluded that in this sample spacing of trials (ITI) did not influence the direction of URs.

To determine the effects of surgical treatment on the direction of the response, the Fisher exact probability test was again employed. Now, instead of comparing groups tested under different ITIs, groups tested under the same ITI were individually compared. The same criterion described above was used to assign Ss to either the F (forward) or W (withdrawal) category. Within the 6 second ITI significant differences in total frequency of forward response were obtained between the Normal and ganglionic tissue removed groups, i.e. Sub-R, Sup-R, and 5-seg-R animals. Although a highly significant difference was obtained between the SD and Sup-R groups (p .01), no significant difference was obtained between the SV and Sub-R groups. No significant differences were found between the Normal and sham-operated groups (SD, SV). Similarly, comparisons between groups in which the ganglia had been removed (5-seg-R, Sub-R, Sup-R) yielded no significant differences. An interesting finding was that the 5-seg-R group (with both sub- and suprapharyngeal ganglia removed) did not differ significantly from either the Sub-R or Sup-R groups.

When the same comparisons were made between groups given the spaced trials, the <u>identical</u> results were obtained. Table 5 in the Appendix presents the comparisons that were made and the probabilities of obtaining the observed differences. The fact that the results were identical for both the massed trials and spaced trials groups is taken as evidence that surgical removal of the "cerebral ganglia" and not spacing of trials (ITI) affects the direction of <u>S's</u> URs to light.

<u>Latency of response</u>

In order to determine if spacing of trials had an effect on latency of URs, the Wilcoxin test for groups of replicates was used (1949). The median latency of URs was 3.31 seconds for the massed trial groups and 6.00 seconds for the spaced trial groups. The sum of ranks of median latency of URs was found to be 241.5 for the massed trial groups and 388.5 for the spaced trial groups. The probability of obtaining a rank total of 388.5 as compared to a rank total of 241.5 is less than 0.01. Thus, the overall latencies of the URs were significantly shorter for the massed trial _Ss_ than for _Ss_ tested under spaced trials (see Figures 3 and 4). This is consistent with the finding that _Ss_ tested under massed trials have a significantly greater total frequency of URs than _Ss_ given spaced trials.

_U_ tests were used to compare latencies of URs between groups given massed trials or spaced trials. Both the Sup-R and Sub-R massed trial groups were found to have significantly shorter latencies than their spaced trial counterparts. Median latencies of the Normal spaced trial group and Normal massed trial group were _least_ different of all the groups compared (median latency spaced = 2.31, median latency massed, 2.32). A finding consistent with the frequency data was that no significant differences were obtained when 5-seg-R massed _Ss_ were compared with their spaced trial counterparts. In all the other comparisons no significant differences were obtained. The "trend," however, is towards shorter overall latencies for operated _Ss_ tested under massed trials. Although spacing of trials affects the latency of URs in the Sup-R and Sub-R _Ss_, it does not seem to affect the overall latency of URs for _Ss_ with both supra- and subpharyngeal ganglia removed (5-seg-R group). Table 6 in the Appendix presents the _U_ values and other relevant information.

Figure 3.--Median Latency of Responses for 6 Second ITI Groups

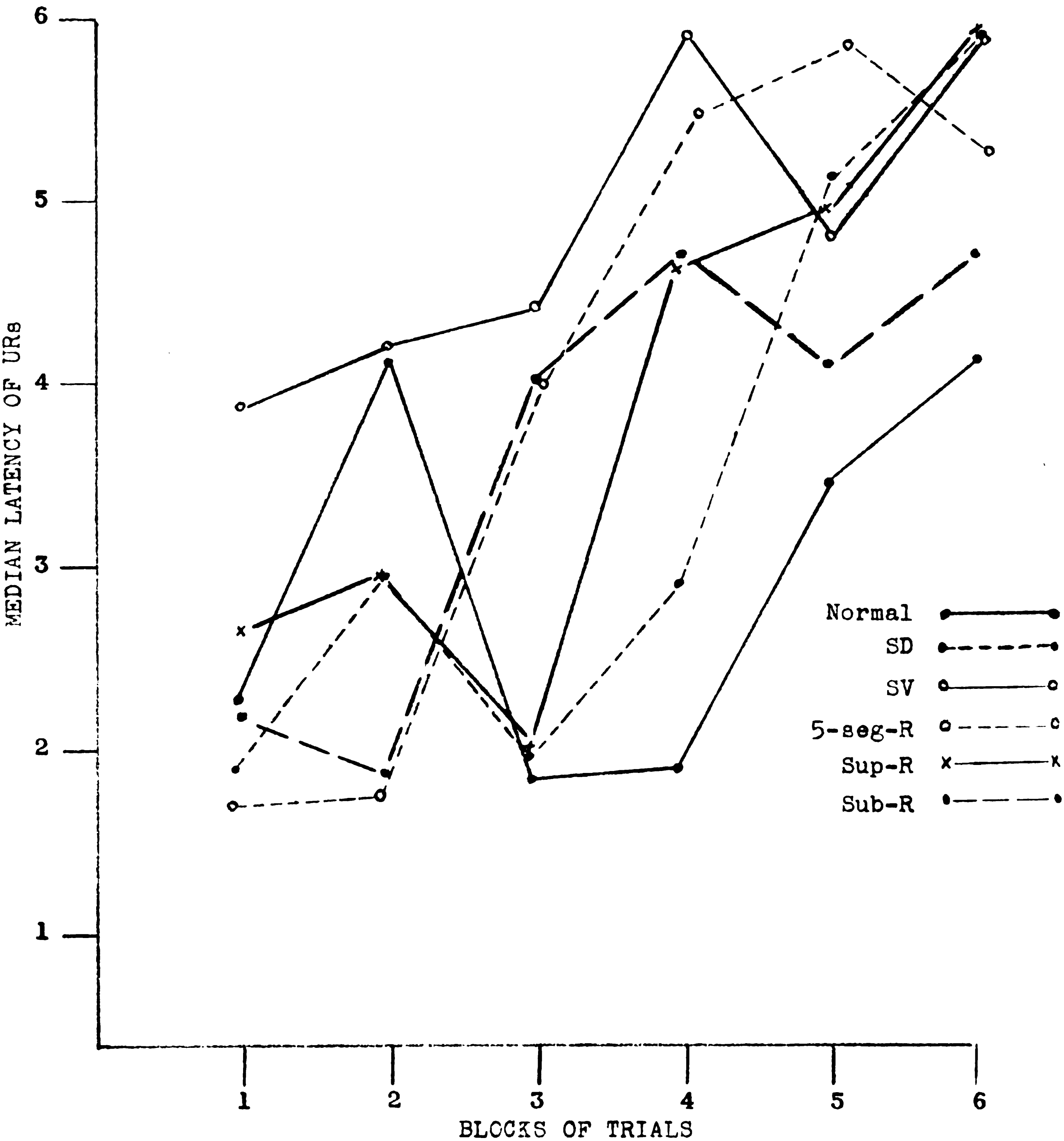
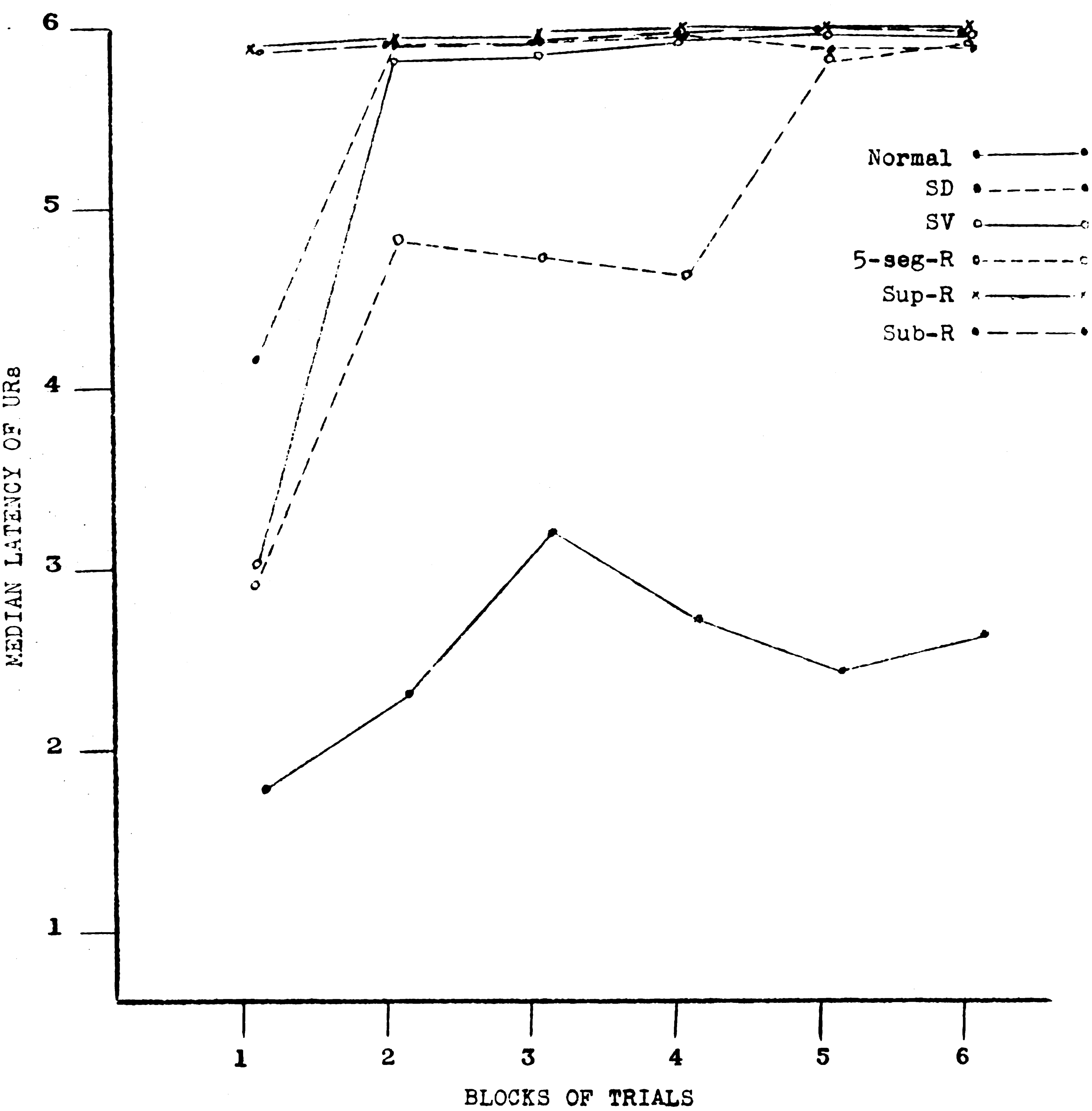


Figure 4.--Median Latency of Responses for 88 Second ITI Group



To determine the effects of "surgery" on overall latency of URs *U* tests were made between the groups *within* each ITI. Under spaced conditions, highly significant differences were obtained between the Normal and all the other remaining groups. Normal animals had significantly shorter latencies than did the sham-operated (SD, SV) and ganglionic extirpation groups (Sup-R, Sub-R, 5-seg-R). The *Ss* most similar to the Normal group were in the 5-seg-R group. Other comparisons between sham-operated and ganglia removed groups yielded non-significant findings. Table 7 presents the comparisons, and the probabilities associated with them.

Latencies of response were similarly tested for those *Ss* given massed trials. Within the 6 second ITI no significant differences in overall latency were obtained between any of the groups. However, observation of the data suggests that there is a "trend" towards longer latencies in the groups that were subjected to anesthesia and surgical treatment. Table 8 shows the comparisons and other relevant information.

Adaptation

It can be seen in Figures 1 and 2 that median frequency of URs diminishes over blocks of trials. A test was made to determine whether there were significant differences in frequency of URs and in latency of URs between the first block of trials (1 - 10) and the last (51 - 60). In the massed trials condition the number of *Ss* making an equal number more URs on trials 1 - 10 were compared with the number of *Ss* in the *same* group making more URs on trials 51 - 60. The binomial test as described in Siegel (1956) was used to analyze the data. On the basis of the results, it can be concluded that adaptation to the UCS occurs in both massed and spaced trial conditions. Further, it appears that adaptation, measured in terms of decrement of URs over trials, is more pronounced under spaced than under massed trials. Table 9 in the Appendix presents the *p*-values obtained.

As several previous studies have been carried out using "decerebrate" worms (Ratner and Miller, Ratner) *Ss* in the 5-seg-R groups were compared to see if ITI affected frequency of responding over trials. The frequency of URs over trials 1 - 10 for the 88 second ITI group was compared with the frequency of URs of *Ss* tested under the 6 second ITI. No significant differences were found between massed and spaced *Ss* with the anterior five segments removed on trials 1 - 10 ($U = 30.5$, $p = .439$). The same subjects were then compared on the last block of trials (51 - 60) and a highly significant difference in frequency of URs was obtained ($U = 7.5$, p $.005$). 5-seg-R *Ss* given massed trials made significantly *more* URs during the last block of trials than did the equivalent spaced trial animals. This was taken as evidence that adaptation occurs at a greater rate under spaced trial conditions than under massed for "decerebrate" worms (average frequency of URs massed = 5.87, average frequency spaced =

1.25; sum of ranks massed = 92.5, sum of ranks spaced = 48.5).

Since the latency measure was not clearly defined and was not independent of the frequency measure on any given trial, it is felt that the latency measure could not provide adequate information as to the effects of spacing of trials on adaptation of the UR to light. Therefore, no analysis comparing latency of URs at onset of the experiment with latency of URs on the last block of trials will be made at this time.

DISCUSSION

Behavior of the normal animal

The variable of spacing of trials was found to have a strong effect on the unconditioned responses of the normal earthworm, Lumbricus terrestris. Worms tested for 60 trials with a 6 second intertrial interval made less URs than those animals tested with an 88 second ITI. However, the median latencies of the URs of these groups did not differ. It is felt that the latency measure was less meaningful than the frequency measure because the latency measure is less well defined in terms of the time of onset of the UR and includes reaction time of the experimenter. Thus, it is concluded that the normal groups did differ in URs as a function of the ITI. This finding is consistent with a report by Clark (1960), on the unconditioned responses of marine worms. He reports that these animals showed a greater rate of adaptation under massed trials than under spaced trials. That is, the total frequency of URs to light was less for animals tested under massed trials than for animals tested under spaced trial conditions.

The finding regarding the effect of spacing of trials on URs is of interest because it can be used to explain the effects of spacing of training on conditioning. That is, spaced conditioning trials have been found to lead to better conditioning than massed trials. Ratner and Miller (1960) reported that normal, intact worms trained under spaced trials showed a higher percentage of CRs than animals trained under massed trial conditions. Calvin (1939) reported that for the acquisition of a CR in rats, 3 trials per minute were superior to 9 or 18 trials per minute. The present results suggest that higher percentages of CRs obtained under spaced trial conditions are due to the fact that organisms make more URs under spaced trials than under massed trials and therefore make more conditioned responses. The frequencies of URs cannot be evaluated for the typical conditioning study because they are usually not reported.

The normal worms were found to make almost all negative responses to photic stimulation. That is, a rapid withdrawal response occurred at the onset of the light (US). Hess (1924), Nomura (1926, as reported in Warden, et al., 1940), and Prosser (1950) report similar findings that normal, intact worms show a negative phototaxis to moderate and strong photic stimulation.

Over the 60 test trials, neither group of normal worms showed adaptation of the withdrawal response to the photic stimulation. That is, frequency of URs did not change comparing the first block of trials with the last. Clark (1960) reports marked adaptation to photic stimulation within 60 trials for normal marine worms under both massed and spaced trial conditions. The difference between the present study and Clark's may be due to differences between them in test conditions, media in which the US was presented, and/or species of annelid studied.

<u>The differential functions of the supra- and subpharyngeal ganglia</u>

The unconditioned responses (URs) of the group with the suprapharyngeal ganglia removed did not differ from the URs of the group with the subpharyngeal ganglia removed. Specifically, the Sup-R groups did not differ from the Sub-R groups in frequency, latency, direction, or rate of adaptation comparing groups within the same ITI. Kovaleva (1961) in a recent paper reports that worms with the suprapharyngeal ganglia removed show an increase in activity and oxygen consumption while those with the subpharyngeal ganglia removed consume less oxygen and show a decrease in activity as a result of loss of muscle tension in the body wall. His findings regarding activity would suggest that the Sup-R and Sub-R groups in the present study should differ. Such differences were not found and the reasons are not clear.

The present results regarding no differences in response to light by Sup-R and Sub-R groups within the same ITI are in disagreement with the theories of Hess (1924), Kovaleva (1961), and Nomura (1926). These workers hold that the sub- and suprapharyngeal ganglia serve differential functions. Specifically, they hypothesize that the suprapharyngeal ganglia serve an inhibitory function and the subpharyngeal ganglia an excitatory function. However, the results of the present study show no evidence for specificity of function of the cerebral ganglia with regard to the earthworm's URs to light.

The general functions of the cerebral ganglia

The results of the present study clearly indicate that removal of any neural tissue changes direction of the URs to light regardless of ITI. This finding is supported by the work of Hess (1924) and Nomura (1926) who also reported that removal of the cerebral ganglia (specifically the supra-pharyngeal ganglia) changes the direction of the UR from negative to positive under moderate or intense photic stimulation. Ratner and Miller (1959) and Ratner (1962) also report similar findings for "decerebrate" worms (5-seg-R group). Nomura's theory (as reported in Warden, *et al.*) that positive phototaxis is increased as a result of removing the suprapharyngeal ganglia is not supported by the findings of the present study since non-significant differences regarding direction of response were obtained regarding direction of response for comparisons between Sup-R, Sub-R, and 5-seg-R groups. These findings, however, cannot be attributed to surgical shock, handling, or anesthesia since the sham-operated groups behaved like normals with regard to direction of response.

The effect of removal of any neural tissue on URs has been found to depend on the ITI under which the earthworm is tested. Operated worms tested with massed trials (ITI = 6 seconds) do not differ in frequency or in latency of URs as compared with normal and sham-operated animals. This finding is consistent with the work of Ratner and Miller (1959), who reported that operated animals tested under massed trials did not differ significantly in percentages of URs and CRs from normal, intact worms given massed trials. The sham-operated groups (SV and SD) also did not differ from the normal and operated massed trial groups on frequency or latency of URs. This suggests that the findings of no differences among the experimental groups is reliable regarding these measures. The sham dorsal and Sub-R groups were found to show significant adaptation although no other massed trials groups showed adaptation of the UR. These results cannot be interpreted at the present time.

Extirpation of neural tissue and long ITI (88 seconds) were found to significantly affect the URs of the earthworm. That is, under spaced trial conditions total frequency of URs decreased and overall latencies increased for animals with the cerebral ganglia removed as compared with normal, intact worms given spaced trials. In addition, sham-operated worms had higher latencies, lower frequencies, and showed more adaptation than normal animals. Although the sham-operated worms differed from the normal animals, they were still significantly more responsive than those worms with either the sub-or suprapharyngeal ganglia removed. However, the hypothesis that the anesthetic might have accounted for the differences between normal and sham-operated groups does not appear to be tenable due to the fact that under massed trial conditions, non-significant differences were obtained on comparisons among all of the massed trial groups.

A finding which is difficult to interpret in the light of present theory of neural function is that worms with both sets of ganglia removed did not differ in total frequency or latency of URs from the sham -operated animals given spaced trials. The 5-seg-R animals also differed in total frequency of URs from the Sub-R group but not the Sup-R group. Kovaleva's report (1961) that worms with the suprapharyngeal ganglia removed are more responsive while those with the subpharyngeal ganglia removed are less responsive is not consistent with the findings of the present study of non-significant differences between groups tested under massed trials. Thus, the reasons for differences between 5-seg-R and Sub-R animals given spaced trials are not clear.

A recent study by Ratner (1962) showed that changes in the URs of worms given spaced training could not be due to length of time the animals remained in the apparatus. He was able to demonstrate that conditioning of decerebrate worms could occur even after a long delay in the apparatus. Subjects in Ratner's study were given either massed or spaced trials after a long delay in the tube (40 minutes) with spacing conditions being reversed after 60 trials. Decrease in frequency of URs was shown to be the result of long ITI rather than amount of time spent in the tube. Thus, it would appear that in the present study, lack of responding over trials in the ganglia removed groups given spaced training could not be attributable to length of time the animal remained in the apparatus.

The findings of the present study again demonstrate that the impaired worm under spaced trials is less responsive than either the normal worm under spaced trials or the impaired worm under massed trials. Ratner (1962) reported similar findings using 5-seg-R worms. The interpretation of the interaction between surgery and ITI on URs to light does not follow from existing statements about neural function in invertebrates. At the descriptive level, it appears that <u>the impaired organism becomes MORE response-bound</u> as compared to the normal, intact organism which is more stimulus-bound. That is, the impaired worm tends to continue what it had been previously doing prior to the onset of the following US. Thus, under massed trial conditions the operated animal which responds to the US continues to do so as a result of the rapid, repeated presentation of the US which occurs every 6 seconds. Under spaced trial conditions, the US is presented less frequently (every 88 seconds) and therefore the worm is inactive for a greater amount of time than it is active. Under these conditions the operated animal will continue to remain inactive when the photic stimulus is presented.

Adaptation to the US

Analysis of the results indicates that adaptation of the UR to photic stimulation occurs for surgically treated animals in both massed and spaced trial conditions. Specifically, some of the groups showed a decrease in frequency of URs comparing the first block of trials with the last. However, this relationship was not consistent for all the groups tested. Normal, intact worms showed no decrease in frequency or increase in latency under either massed or spaced trial conditions. This finding is not consistent with the work of Clark (1960) who reported that adaptation of a UR to light occurs to a greater extent under massed trial conditions. In general, operated animals showed less adaptation under massed trials than under spaced trial conditions. However, some of the specific findings are not clear. That is, under massed trial conditions the Sub-R group and SD group showed adaptation while the Sup-R group did not. However, under spaced trial conditions the Sup-R group did not show evidence of adaptation of the UR to light. It would be expected that adaptation to the photic source would occur more rapidly in the massed trial groups since these animals were subjected to more rapid intense stimulation (every 6 seconds) over a shorter period of time (8 minutes) than their spaced trials counterparts (90 minutes). The results regarding adaptation cannot be interpreted at this time.

Since Ratner (1962) used decerebrate worms in an earlier study, the 5-seg-R massed trial and 5-seg-R spaced trial animals were compared in order to determine if adaptation occurred to a greater extent under massed or spaced training for decerebrate worms. Frequency of URs for the first and last block of trials were compared for these two groups. No significant differences were obtained on trials 1 - 10 comparing massed and spaced decerebrate worms, while highly significant differences were obtained comparing the two groups on the last block of trials. These results are consistent with those of Ratner who reported that percentage of URs decreases over trials especially for those _Ss_ given spaced training. As reported earlier, these results cannot be attributed to the length of time the animal remained in the apparatus.

SUMMARY

The present experiment was designed to study the effects of spacing of trials and differential extirpation of the cerebral ganglia on the unconditioned responses of the earthworm, Lumbricus terrestris. Ninety-six worms were randomly divided into 6 surgical treatment groups. Each of the surgical treatment groups contained 16 worms, and each of these groups was subdivided after surgery into a group which received massed trials and a group which received spaced trials. Each animal received a total of 60 trials in which the US was presented either every 6 seconds or every 88 seconds. The frequency, direction, and latency of the UR were recorded for each S.

The results showed that normal worms make fewer URs under massed trials than under spaced trials. The UR to strong photic stimulation in the normal worms was consistently negative. These animals also showed no adaptation of the UR to light under either massed trial or spaced trial conditions.

Worms with either the sub- or suprapharyngeal ganglia removed showed no differences in frequency, latency, direction, or adaptation of the UR when comparisons were made within the same ITI. The results of the present study offer no evidence for specificity of function of the cerebral ganglia with regard to the worm's URs to photic stimulation.

Results indicate that removal of any neural tissue changes the direction of the UR regardless of the ITI. The effect of removal of neural tissue on frequency of URs was found to be dependent on the intertrial interval under which the animals were tested. Operated worms given massed training did not differ from normals and sham-operated worms in frequency or latency of URs. Extirpation of neural tissues and long ITI were found to significantly affect URs. Latency is increased, total frequency of URs decreased, and adaptation of the UR to light is more frequent among groups and is more pronounced. Operated animals, including the sham-operated groups, were much less responsive under spaced training than under massed training.

Adaptation to the photic stimulus for operated Ss occurred under both massed and spaced trials but appeared to be greater under spaced trial conditions.

The results cannot be interpreted in terms of the existing statements about neural function of invertebrates. At the descriptive level, the results suggest that operated worms become response bound as compared to the normal, intact organism which remains stimulus bound with regard to its reactions to photic stimulation.

APPENDIX

Table 1.--Statistical analysis employing the Mann-Whitney U test to determine if spacing of trials (ITI) affects the *frequency* of URs to light.

Groups	Median of Total Frequency	U Value*	Level of Significance
Normal (6 sec) Normal (88 sec)	35.0 49.5	8.5	p .007
SD (6 sec) SD (88 sec)	37.5 26.5	18.5	p .097
SV (6 sec) SV (88 sec)	25.5 25.0	25.0	p .253
5-seg-R (6 sec) 5-seg-R (88 sec)	31.0 23.5	19.5	p .117
Sup-R (6 sec) Sup-R (88 sec)	28.5 15.5	5.5	p .002
Sub-R (6 sec) Sub-R (88 sec)	35.5 13.5	7.0	p .003

*The n's used to evaluate the U values for each comparison were 8 and 8.

Table 2.--Statistical analysis employing the Mann-Whitney U test to determine if "surgery" affects frequency of URs to light under massed trial conditions.

Groups	Median of Total Frequency	U Value*	Level of Significance
Normal SD	35.0 37.5	31.0	p .480
Normal SV	35.0 25.5	21.5	p .164
Normal 5-seg-R	35.0 31.0	30.5	p .480
Normal Sup-R	35.0 28.5	29.0	p .399
Normal Sub-R	35.0 35.5		
SD SV	37.5 25.5	15.5	p .052
SD Sup-R	37.5 28.5	28.0	p .360
SV Sub-R	25.5 35.5	19.0	p .097
5-seg-R Sup-R	31.0 28.5	31.5	p .520
5-seg-R Sub-R	31.0 35.5	29.0	p .399
Sup-R Sub-R	28.5 35.5	27.0	p .323

*The n's used to evaluate the U values for each comparison were 8 and 8.

Table 3.--Statistical analysis employing the Mann-Whitney U test to determine if "surgery" affects frequency of URs to light under spaced trial conditions.

Groups	Median of Total Frequency	U Value*	Level of Significance
Normal SD	49.5 26.5	6.5	p .003
Normal SV	49.5 25.0	0.0	p .0001
Normal 5-seg-R	49.5 23.5	4.0	p .001
Normal Sup-R	49.5 15.5	0.0	p .0001
Normal Sub-R	49.5 13.5	0.0	p .0001
SD SV	26.5 25.0	31.5	p .520
SD 5-seg-R	26.5 23.5	31.0	p .480
SD Sup-R	26.5 15.5	14.0	p .032
SD Sub-R	26.5 13.5	7.5	p .005
SV 5-seg-R	25.0 23.5	32.0	p .520
SV Sup-R	25.0 15.5	13.5	p .032
SV Sub-R	25.0 13.5	4.0	p .001
5-seg-R Sub-R	23.5 13.5	13.0	p .025
5-seg-R Sup-R	23.5 15.5	17.5	p .080
Sup-R Sub-R	15.5 13.5	26.0	p .287

*The n's used to evaluate the U values for each comparison were 8 and 8.

Table 4.--Statistical analysis employing the Fisher Exact Probability Test to determine if duration of ITI affects the <u>direction</u> of URs to light.

Group	Freq. of <u>Ss</u> making 50% Withdrawal Rs	Significance Level
Normal (6 sec)	8	NS
Normal (88 sec)	8	
SD (6 sec)	7	NS
SD (88 sec)	8	
SV (6 sec)	6	NS
SV (88 sec)	6	
5-seg-R (6 sec)	0	NS
5-seg-R (88 sec)	0	
Sup-R (6 sec)	1	NS
Sup-R (88 sec)	3	
Sub-R (6 sec)	3	NS
Sub-R (88 sec)	3	

Table 5.--Statistical analysis employing the Fisher Exact Probability Test to determine if surgical treatment affects the <u>direction</u> of URs to light.

	6 Second ITI		88 Second ITI	
Groups	Freq. of Ss Making 50% Withdrawal Rs	Level of Significance	Freq. of Ss Making 50% Withdrawal Rs	Level of Significance
SD SV	7 6	NS	8 6	NS
SD Sup-R	7 1	p .01	8 3	p .025
SV Sub-R	6 3	NS	3 2	NS
Sup-R Sub-R	1 3	NS	3 2	NS
5-seg-R Sup-R	0 3	NS	0 2	NS
5-seg-R Sub-R	0 3	NS	0 2	NS
Normal SD	8 7	NS	8 8	NS
Normal SV	8 6	NS	8 6	NS
Normal 5-seg-R	8 0	p .005	8 0	p .005
Normal Sup-R	8 1	p .005	8 3	p .025
Normal Sub-R	8 3	p .025	8 2	p .01

Table 6.--Statistical analysis employing the Mann-Whitney U test to determine if duration of ITI affects the *latency* of URs.

Groups	Grand median of Overall Latency	U Value*	Level of Significance
Normal (6 sec) Normal (88 sec)	2.32 2.31	31.0	p .480
SD (6 sec) SD (88 sec)	3.39 6.00	14.0	p .032
SV (6 sec) SV (88 sec)	6.00 6.00	26.0	p .287
5-seg-R (6 sec) 5-seg-R (88 sec)	3.05 6.00	22.0	p .164
Sup-R (6 sec) Sup-R (88 sec)	4.07 6.00	6.5	p .003
Sub-R (6 sec) Sub-R (88 sec)	3.58 6.00	9.0	p .007

*The n's used to evaluate the U values for each comparison were 8 and 8.

Table 7.--Statistical analysis employing the Mann-Whitney *U* test to determine if surgery affects the *latency* of URs under *spaced trial* conditions.

Groups	Grand Median of Overall Latency	*U* Value*	Level of Significance
Normal SD	2.31 6.00	8.5	p .007
Normal SV	2.31 6.00	4.0	p .001
Normal 5-seg-R	2.31 6.00	14.5	.041 p .052
Normal Sup-R	2.31 6.00	0.0	p .00001
Normal Sub-R	2.31 6.00	0.0	p .00001
SD SV	6.00 6.00	27.5	p .323
SD Sup-R	6.00 6.00	20.0	p .117
SV Sub-R	6.00 6.00	20.0	p .117
5-seg-R Sup-R	6.00 6.00	19.0	p .097
5-seg-R Sub-R	6.00 6.00	18.0	p .080
Sup-R Sub-R	6.00 6.00	31.5	p .520

*The n's used to evaluate the *U* values for each comparison were 8 and 8.

Table 8.--Statistical analysis employing the Mann-Whitney U test to determine if surgery affects the latency of URs under massed trial conditions.

Groups	Grand Median of Overall Latency	U Value*	Level of Significance
Normal SD	2.32 3.39	29.0	p .399
Normal SV	2.32 6.00	19.0	p .097
Normal 5-seg-R	2.32 3.05	26.0	p .287
Normal Sup-R	2.32 4.07	25.0	p .253
Normal Sub-R	2.32 3.58	27.0	p .323
SD SV	3.39 6.00	20.0	p .117
SD Sup-R	3.39 4.07	34.5	p .520
SV Sub-R	6.00 3.58	23.0	p .323
5-seg-R Sup-R	3.05 4.07	31.5	p .520
5-seg-R Sub-R	3.05 3.58	31.0	p .480
Sup-R Sub-R	4.07 3.58	27.0	p .323

*The n's used to evaluate the U value for each comparison were 8 and 8.

Table 9.--Statistical analysis employing the Binomial Test to determine if there is a difference in frequency of URs between the first block of trials (1-10) and the last (51-60).

Groups	A*	B**	p	N
		6 Second ITI		
Normal	5	3	.145	8
SD	6	2	.045	8
SV	4	4	.363	8
5-seg-R	4	4	.363	8
Sup-R	5	3	.145	8
Sub-R	6	2	.045	8
		88 Second ITI		
Normal	5	3	.145	8
SD	6	2	.045	8
SV	6	2	.045	8
5-seg-R	8	0	.002	8
Sup-R	5	3	.145	8
Sub-R	8	0	.002	8

*Number of Ss making more URs on trials 1-10 than on trials 51 - 60.

**Number of Ss making the same number or more URs on trials 51-60 than on trials 1-10.

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