THE EFFECT OF SPACING OF TRAINING AND SURGICAL EXTIRPATION ON CONDITIONING IN EARTHWORMS

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THE EFFECT OF SPACING OF TRAINING AND SURGICAL EXTIRPATION ON CONDITIONING IN EARTHWORMS

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ABSTRACT

This study was designed to investigate in a factorial design, with earthworms, Lumbricus Terrestris, the effects of (a) removing the ganglia (anterior five segments removed) and (b) spacing of trials on (a) the conditioning of a withdrawal response and (b) reconditioning of this response after a rest period. The following experimental groups were used: (1) normal spaced, (2) normal massed, (3) operated massed, and (4) operated spaced.

Sixty earthworms were randomly assigned to groups: normal spaced, NS; normal massed, NM; operated spaced, OS; operated massed, OM; operated control spaced, OCS; and operated control massed, OCM. The forty operated Ss had their dorsal ganglia and anterior five segments removed while the ganglia of the remaining 20 Ss were left intact. Massed conditioning trials had a ten sec. inter-trial interval and spaced conditioning trials had a 90 sec. inter-trial interval.

The apparatus consisted of a clear, plastic tube into which the S was placed. The tube and a bell buzzer were mounted on a board with a photo-flood light centered above the tubes. Vibration of the bell buzzer provided the CS, and light from the photo-flood provided the US. For groups NS, NM, OS, and OM, the CS was presented for 6 sec., during the last 2 sec. of which the US was presented. The occurrence

and latency of a withdrawal response during the first 4 sec. of a vibration were recorded. Groups OCS and OCM received 6 sec. of vibration alone and the occurrence and latency of a withdrawal response during the first 4 sec. were recorded.

Groups NS, NM, OS, and OM received 80 conditioning trials followed by a 20 min. rest and ten post-rest conditioning trials. Groups OCS and OCM received 80 stimulus presentations followed by a 20 min. rest and ten post-rest presentations.

The results clearly showed conditioning in groups NS, NM, and OM on both measures of conditioning, while group OS did not show conditioning. The performance of control groups, OCS and OCM, clearly showed the operation did not account for the conditioning found in groups OS and OM. Group NS conditioned better than group NM on both measures of conditioning. Group OM clearly showed conditioning while group OS did not condition at all. Comparisons between the operated and normal groups showed that groups NM and OM do not differ significantly, while group OM conditioned and group OS did not condition at all. All groups showed a decrement in performance on the post-rest trials.

The results of this experiment showed that (a) spaced trials facilitated conditioning for normal Ss; (b) removing the "cerebral" ganglia did not affect conditioning with massed trials; (c) removing the "cerebral" ganglia prevented conditioning with spaced trials; (d) a rest period in the apparatus lead to a post-rest decrement for all groups.

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The writer is deeply indebted to Dr. Stanley C. Ratner for his advice in formulating this experimental problem and for his continued cooperation in all matters dealing with this thesis.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Until recently the investigation of classical conditioning in annelids has been largely neglected. The marine annelid Hydroides dianthus, received some attention from Yerkes (10), who conditioned two specimens of Hydroides dianthus to react negatively to photic radiation. In 1912, Yerkes (11) found that a manure worm, Allolobophora foetida, was capable of acquiring certain modes of reaction which involved a definite direction of movement and the association of two stimuli. Yerkes then removed the worm's anterior ganglia and after further experimentation with the animal concluded that performance of the worm in a T-maze was not dependent on the ganglia. Heck (8, pp. 213) trained a number of Eisenia foetida and Lumbricus terrestris and found that the removal of the ganglia did not prevent the learning of a sample turning response. However, at present no information is available concerning the effect of ganglia removal on annelid performance, using a classical conditioning paradigm.

In 1957, Ratner and Miller (4) clearly demonstrated classical conditioning of a withdrawal response in earthworms, Lumbricus terrestris, under massed training conditions using a vibratory stimulus as the CS and light as the US.

The fact that the mean per cent of CRs during the last 10 conditioning trials only reached 42.50 per cent may be due to the massing of the conditioning trials. However, past studies have not demonstrated a significant difference in performance of Ss under conditions of massed and spaced training with invertabrates.

Problem

The purpose of the present experiment is to investigate in a factorial design, with earthworms, Lumbricus terrestris, the effects of (a) removing the ganglia (anterior 5 segments removed) and (b) spacing of trials on (a) the conditioning of a withdrawal response and (b) reconditioning of this response after a rest period. For this purpose the following experimental groups were used: (1) normal spaced, (2) normal massed, (3) operated massed, and (4) operated spaced.

II. METHOD

Subjects

Sixty earthworms, Lumbricus terrestris, varying in size from 121 to 240 segments were studied. The worms were obtained from a plot of ground on the Michigan State University campus and were placed in the apparatus within one hour after removal from their natural habitat. During this transfer period Ss were kept in sphagnum moss.

Forty of the Ss had their pharyngeal ganglia (anterior 5 segments removed) removed while the remaining 20 Ss' ganglia were left intact.

Apparatus

Two photographs of the apparatus are shown in Fig. 1 and 2 respectively. Two identical units were constructed so that two Ss could be run at the same time. One S was run during the inter-trial interval of the other. The main part of the apparatus consisted of 2 clear, round, Koroseal tubes vented on the top and sides with 1 mm. holes at 3 cm. intervals. Each tube was 58 1/4 cm. long with an inside diameter of 8 mm. Each tube was mounted on a separate plywood base, with both ends fastened together by thin copper wire, thereby producing a circular runway for the animal. Each tube base was 24 cm. square with 6 1/2 cm. sides over

Figure 1. A photograph showing the apparatus used in this study.

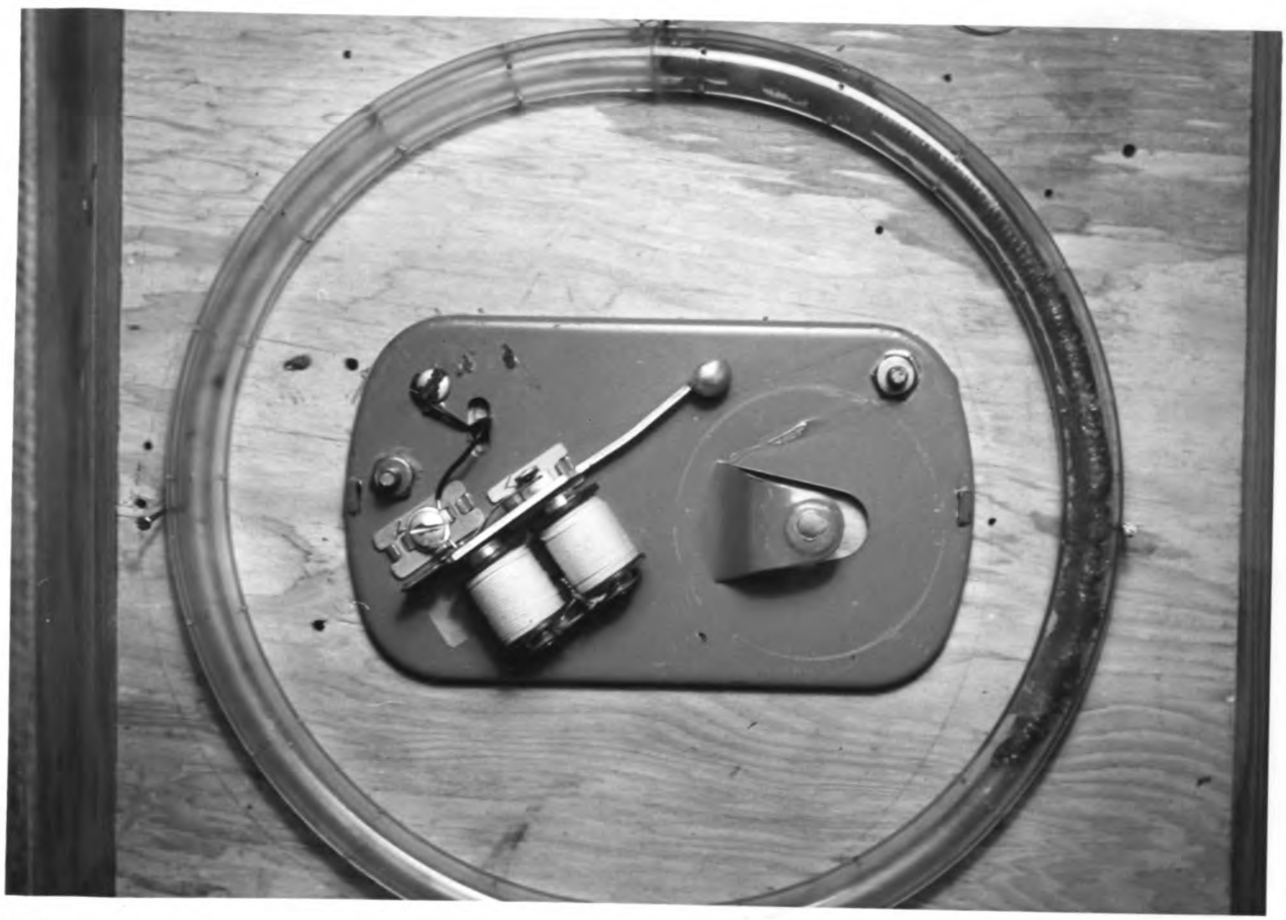

Figure 2. A close-up photograph showing an S in one of the two identical S housing tubes.

which a lid could be placed to shield one S from the unconditioned stimulus (US) while the other S was receiving a trial. A 6 volt d.c. door bell, with the bell removed, was attached securely on the base at the center of each subject tube and was used as the conditioned stimulus (CS). Each door bell was separately controlled by a toggle switch permitting only a single CS source to be operated by the timer at any one time. One #2 (G.E.) photo flood bulb in a 30.5 cm. aluminum reflector was mounted 43 cm. above and midway between both subject tubes and constituted the source of the US. An 8 candle power, dark red bulb positioned 1 cm. below and at the outer edge of the US reflector, provided general illumination.

The CS and US were timed and automatically presented by three interval timers. Inter-trial-intervals were timed with a stop watch. CR latent periods were measured by a timing clock which was activated by the CS timer and stopped by a micro switch operated by the experimenter.

All parts of the apparatus that could have produced noise or vibration were padded with foam rubber 1 cm. thick. This includes the stop watch holder, subject enclosure, lid and timing clock. To further control extraneous vibration, all the equipment was placed on a 73 x 71 x 1 cm. plywood base which was clamped securely to a fairly rigid table.

Procedure

Ss in the operated groups were placed on a disecting board and the first 5 segments were cut off with a sharp razor blade. Immediately following the operation Ss were again placed in sphagnum moss for a 10 min. interval before being placed in the apparatus. At this time the previously sectioned segments were examined to make certain the ganglia were present. If the ganglia was not present, both Ss were discarded and the above procedure repeated.

The tube which was to contain the S was moistened with .6 cc. of water immediately prior to S's entry. The S was transported from the sphagnum moss by hand the the S's anterior portion was placed in one end of the tube permitting the S to advance into the Koroseal tube. The ends of the tube were then fastened together with thin copper wire in such a way that the S had a complete circle to transverse. The above procedure was followed for each S in each tube.

An adaptation period which allowed the S to become adapted to the 8 candle power general illumination light started immediately after both Ss were in their respective tubes, and continued for 20 minutes preceding experimental study.

The Ss were randomly assigned to one of six groups consisting of 10 Ss each: The normal subject massed training group (NM), the normal subject spaced training group (NS), the operated subject massed training group (OM), the operated subject

spaced training group (OS), the operated subject spaced training control group (OCS), and the operated subject massed training control group (OCM).

Group NM was given a total of 90 conditioning trials with a 20 min. rest interval between trials 80 and 81. The inter-trial-interval for this group was 10 sec. for each trial. The vibratory stimulus (CS) was presented for 6 sec., after the first 4 sec. of vibration the light (US) was presented for 2 sec. Thus, the CS and US overlapped for 2 sec. and were terminated together. A withdrawal response occurring during the first 4 sec. of the CS, prior to the presentation of the US, was recorded as a conditioned withdrawal response.

The unconditioned response (UR) to light in normal Ss consisted of a rearing and withdrawal of the anterior segments of S's body. If Ss were moving at the time of stimulation the response consisted of an abrupt stop followed by the above response. In operated Ss the UR was either identical to the above or characterized by a forward movement of the anterior segments. Thus, a response of either type which occurred prior to the US was recorded as a conditioned response (CR). The latent period which intervened between the onset of the CS and the beginning of the CR was recorded. This measure was introduced because it is generally a more sensitive measure of response strength than frequency of CRs. (3, pp. 328)

For group NS, trials were administered in the same manner as for group NM, except that the inter-trial interval was 90 sec.

Groups OM and OS differ respectively from groups NM and NS in that Ss in groups OM and OS underwent the operation procedure previously described.

Groups OCS and OCM were employed to determine the number of responses which might occur as a result of the operational procedure sensitizing the animals to the vibratory stimulus. Ss in these groups were treated in the same manner as groups OM and OS except the US was not presented at any time. The 20 min. rest interval between trials 80 and 81 applied to all groups.

III. RESULTS

Conditioning

Two measures of conditioning were used for each group during the entire procedure, viz: percentage of CR's and response latency. Figure 3 presents the percentage of CR's during conditioning for the normal groups, groups NM and NS, for each block of ten trials and Fig. 4 shows this for the operated groups, groups DM and DS. To determine if conditioning occurred within each group binomial tests (7, pp. 36-42) were made for each group by comparing the percentage of CR's in block I (trials 1-10) with the percentage of CR's in block VIII (trials 71-80), for each S. The binomial tests yielded significant one-tailed probabilities for groups NM ($p = .001$), NS ($p = .01$), OM ($p = .05$), and OS ($p = .05$). That is, there was a significantly higher percentage of CR's on the last block of trials than on the first block for groups NS, NM, and OM. This was taken as evidence for conditioning. Group OS had a significantly higher percentage of CR's on the first block of trials than on the last block of trials. This indicated a lack of conditioning. Table 1 in the appendix presents details of performance for each group.

Figures 15 and 16 show the median latencies of CR's for the normal and operated groups for each block of ten trials.

Due to the non-homogeneity of the latency scores throughout this study, median latency scores were computed and used in all the statistical analyses. Figure 7 shows the learning curves for groups NS, NM, OS, and OM. The scores in Fig. 7 consist of total median latencies. That is, the median latency scores were computed using the total number of trials, therefore any block of 10 trials in which the total responses did equal 50 per cent were given a median latency score of 4.0 secs. Binomial tests were again used to determine if conditioning occurred for the four groups as evidenced by this latency measure. The median latency for the first block of trials (trials 1-10) was computed for each S and compared, as stated before, with the median latency of the eighth block of trials (trials 71-80). The binomial tests yielded significant one-tailed probabilities for groups NM ($p = .01$), NS ($p = .01$), OM ($p = .05$), and OS ($p = .01$). It can be seen in Figs. 5 and 6 that groups NM, NS, and OM showed decreases in latency indicating conditioning, whereas group OS showed an increase in latency of response, indicating the absence of conditioning. Table 1 in the appendix presents additional data for these groups.

In summary, the conditioning groups NS, NM, and OM clearly showed conditioning, on both measures of conditioning, while group OS did not show conditioning on both measures of conditioning.

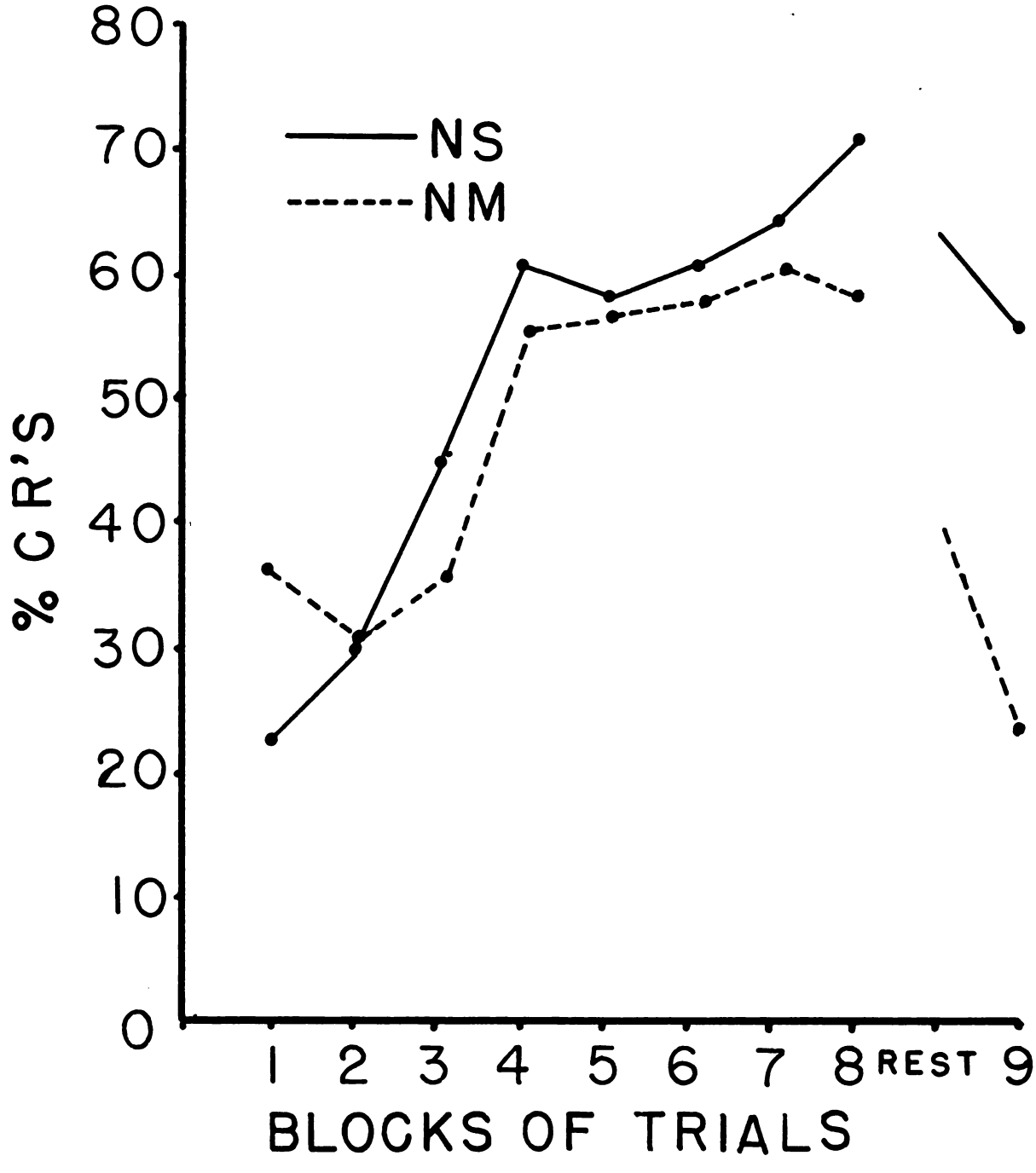

Figure 3. Percentage of CR's for groups NS and NM.

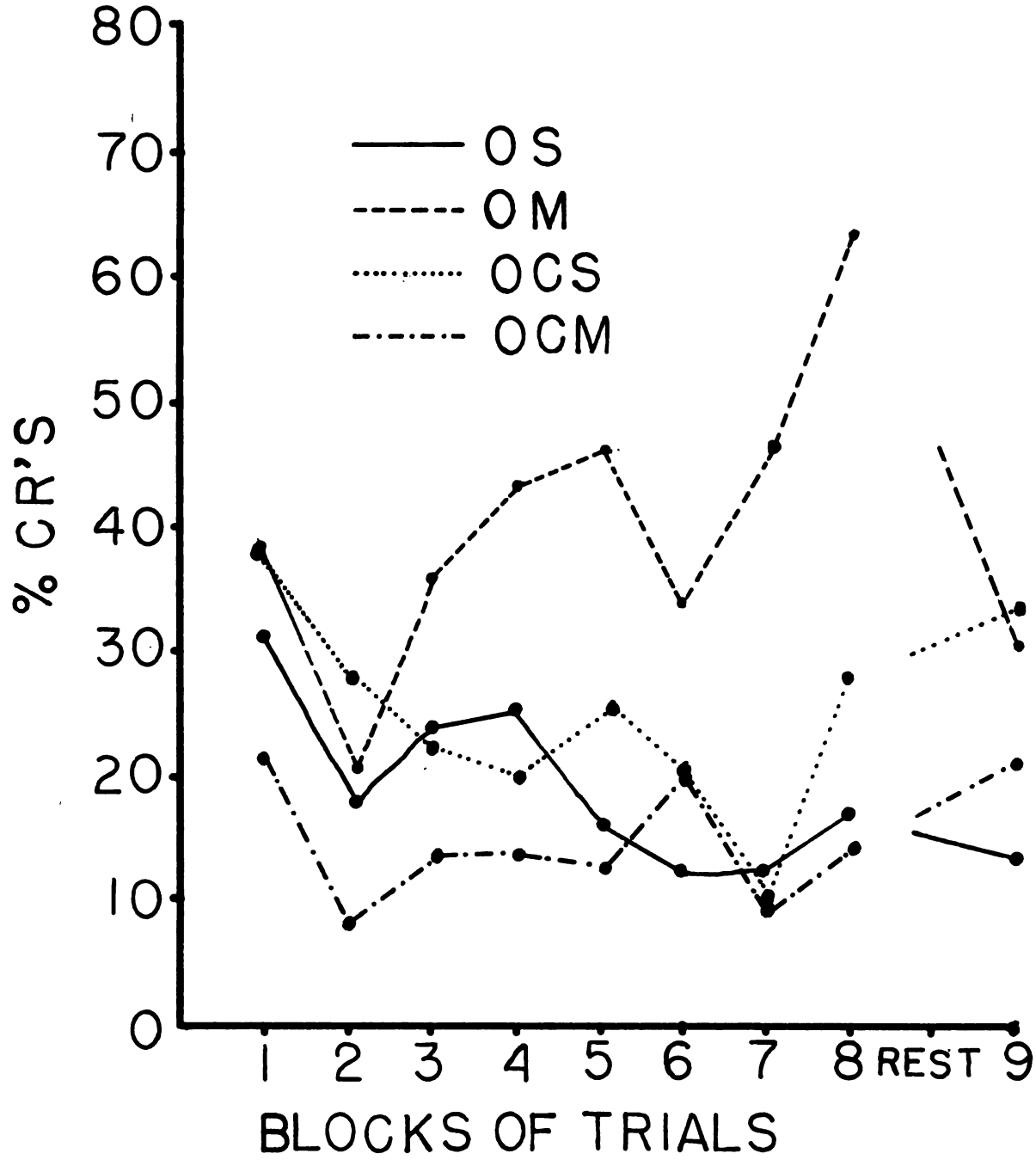

Figure 4. Percentage of CR's for groups OS, OM, OCS, and OCM.

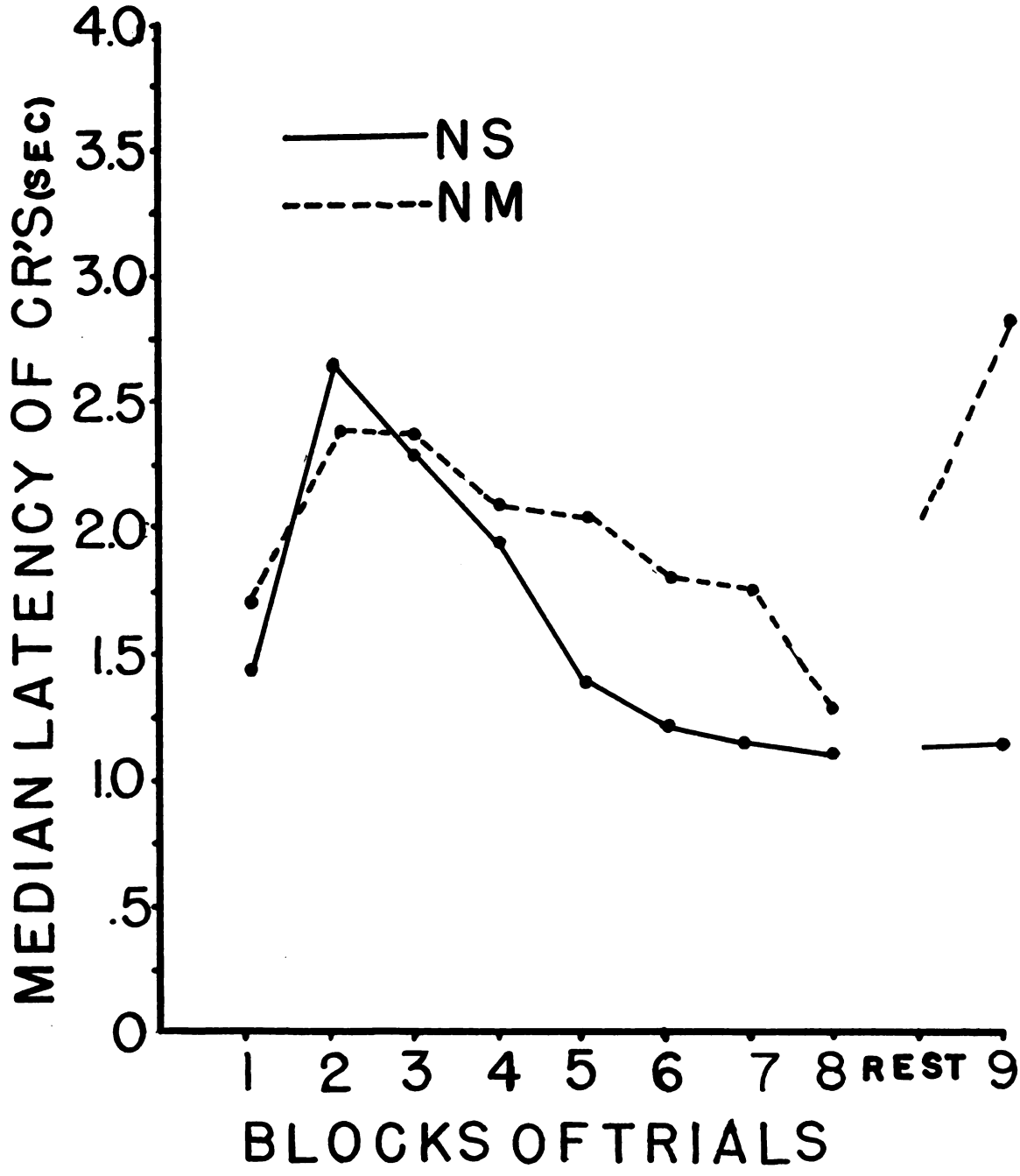

Figure 5. Median latency of CR's for groups NS and NM. Median latencies computed only from trials in which CR's occurred.

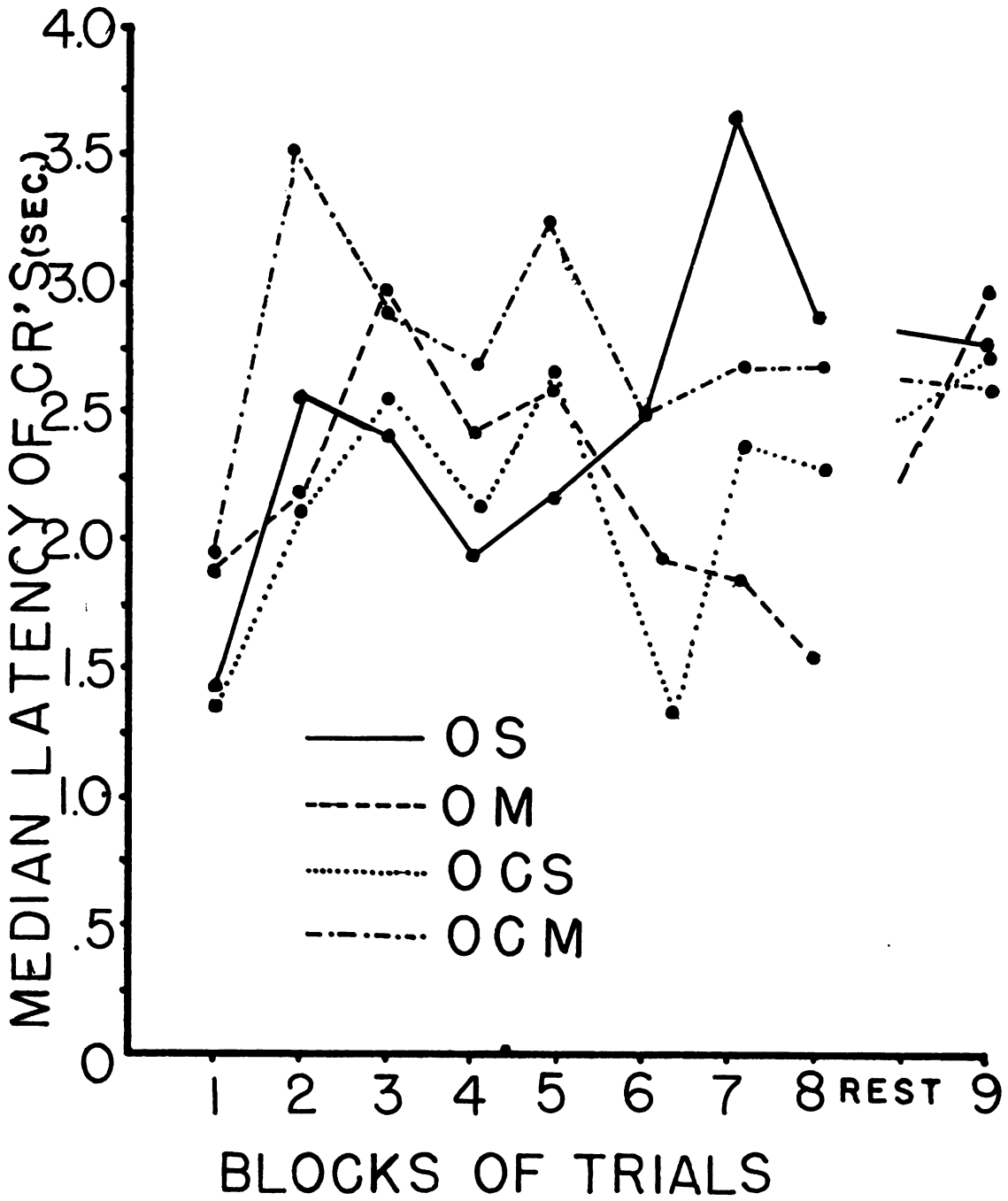

Figure 6. Median latency of CR's for group OS, OM, OCS, and OCM. Median latencies computed only from trials in which CR's occurred.

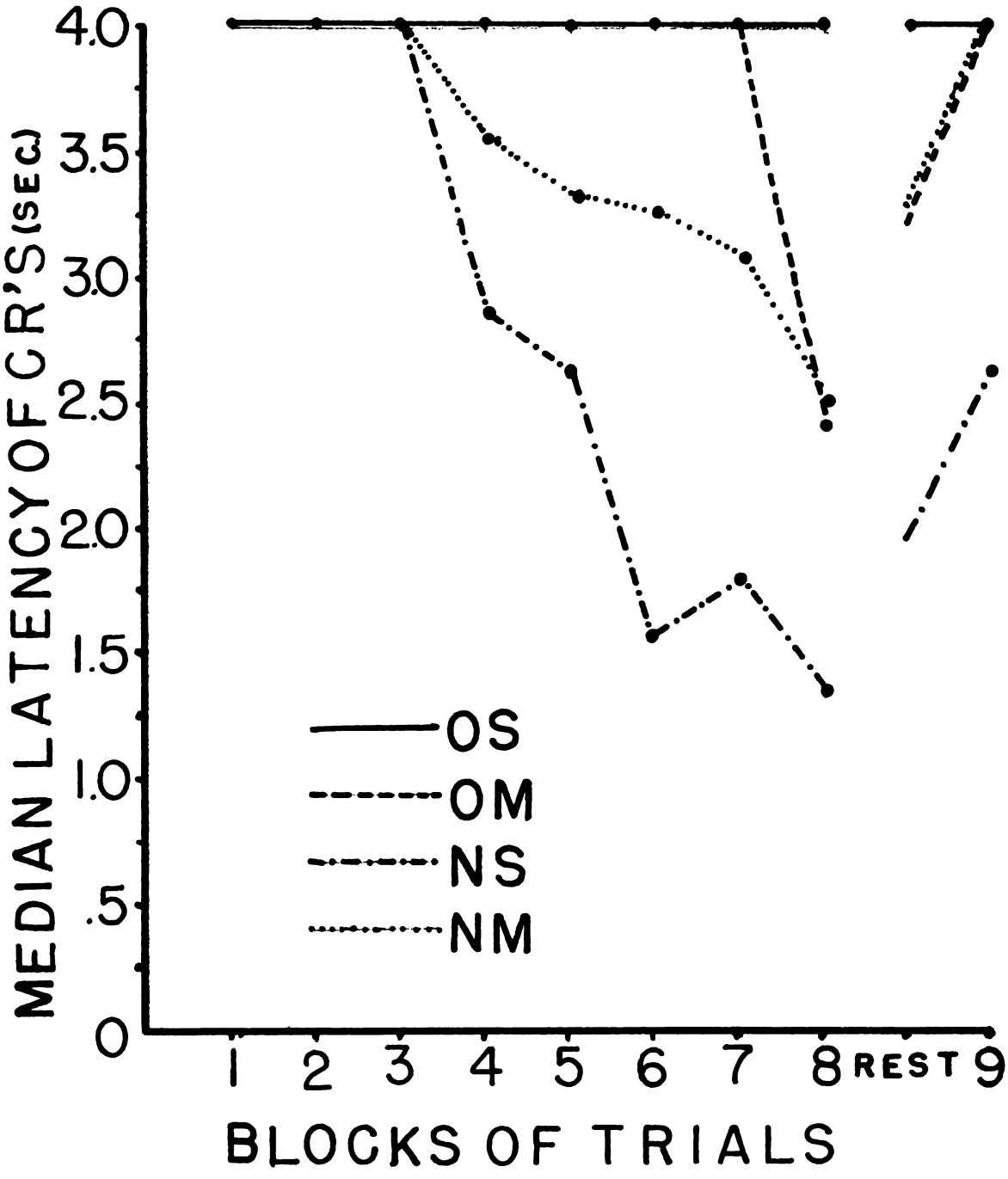

Figure 7. Median latency of CR's for groups NS, NM, OS, and OM. Median latencies computed from all trials for each block.

Sensitization Control for Conditioning

Since the operation per se might have accounted for any behavioral change shown by groups OS and OM during conditioning trials, for example by sensitizing them to vibration, two operated control groups were run with vibration alone. One group was run with massed presentations of vibration and the other with spaced presentations. The data for these groups, OCS, operated control spaced, and OCM, operated control massed, are shown in Figs. 4 and 6. It can be seen that these groups showed almost no overall increase in percentages of responses and little change in overall response latency after the first block of trials.

The Kruskal-Wallis one-way analysis of variance (7, pp. 184-193) was employed to test the over-all differences between groups, because of non-homogeneity of variance as tested by Bartlett's test (9, pp. 194-195). Group OS made a median of 15 CR's, Group OM a median of 31 CR's, Group OCS made a median of 17 CR's and Group OCM a median of 11.5 CR's for trials 1-80. That is the Ss in group OM made significantly more CR's than Ss in groups OS, OCS, and OCM, ($H = 16.16$ $p < .01$). See also Table 2 in the appendix.

In terms of median latencies of CR's made during trials 1-80, group OS made a median latency of 2.59 secs., group OM made a median latency of 2.0 secs., group OCS made a median latency of 2.07 secs., and group OCM made a median

latency of 2.66 secs. That is the Ss in groups OM made significantly shorter scores than Ss in groups OS, OCS, and OCM, ($H = 13.96$ $p < .01$). See also Table 2 in the appendix.

Comparing Massed Spaced Groups

In order to investigate the effects of the spacing of training on conditioning, the massed groups were compared with the spaced groups at the end of learning in that this was where the difference would be expected to occur. Mann-Whitney U tests (4, pp. 116-127) were applied to the total number of CR's made by the Ss in each group for trials 71-80. This test (henceforth referred to as U test) was employed because of non-homogeneity of variance as tested by Bartlett's test of homogeneity of variance (9, pp. 194-195). Group NS made a median of 8.85 CR's and group NM made a median of 6.70 CR's for trials 71-80. A U test of the difference between these groups yielded a significant value of U, ($U = 26$, $p < .05$). That is, the normal spaced group made reliably more CR's than the normal massed group.

Group OS made a median of 1.5 CR's and group OM made a median of 6.5 CR's for trials 71-80. A U test of the difference between these groups yielded a significant value of U ($U = 1.5$, $p < .001$), indicating the operated spaced group made significantly fewer CR's than the operated massed group.

In terms of latencies, group NS had a median latency of .84 secs. and group NM had a median latency of 1.33 secs. for

trials 71-80. A U test of the difference between these groups yielded a significant value of U (U = 20.5, $p < .05$). That is the normal spaced group made reliably shorter latencies than the normal massed group.

Group OS had a median latency of 3.12 secs. and groups OM had a median latency of 1.58 secs. for trials 71-80. A U test of the difference between these groups yielded a significant value of U, (U = 3, $p < .001$). That is, the operated spaced group made significantly longer latency scores than the operated massed group. Table 3 in the appendix presents additional information for the above groups.

In summary, the normal spaced group conditioned better than the normal massed group on both measures of conditioning. The operated massed group clearly showed conditioning while the operated spaced group did not condition at all.

Comparing Normal and Operated Groups

To evaluate the effects of the operation on conditioning each operated group was compared with its similarly trained normal group. Mann-Whitney U tests (7, pp. 116-127) were again used to evaluate the difference between the groups using the data from trials 21-80. The first two blocks of trials were omitted due to the previous finding (5) that the CS, vibration, elicited some avoidance responses early in conditioning and thus contaminated the data for the first two blocks of trials.

The median of the total number of CR's for trials 21-80 for group NS was 43.5 and the median of total number of CR's for trials 21-80 for group OS was 11. A U test of the difference between total values of CR's for groups NS and OS yielded a significant value of U (U = 11, $p < .001$). That is, group NS made reliably more CR's than group OS during conditioning.

The median of the total number of CR's for trials 21-80 for group NM was 33.5 and the median of the total value of CR's for group OM was 27 for trials 21-80. A U test of the difference between total values of CR's for groups NM and OM yielded a non-significant value of U (U = 49.5, $p > .05$).

The median of the latencies of CR's for trials 21-80 for group NS was 1.31 secs. and 2.48 secs. for group OS. A U test of the difference between total values of CR's for groups NS and OS yielded a significant value of U (U = 8, $p < .001$). That is, group NS made reliably shorter latencies than did group OS during conditioning.

The median of the latencies for trials 21-80 for group NM was 1.86 secs. and 2.02 secs. for group OM. A U test of the difference between these groups yielded a non-significant value of U (U = 52.5, $p > .05$).

In summary, comparisons between the operated and normal massed groups showed that the massed groups (NM and OM) did not differ significantly on either measure of conditioning.

However the difference was highly significant between the operated and normal spaced groups on both measures of conditioning. That is, the normal spaced group was the best and the operated spaced group showed no conditioning.

Post-rest Conditioning

Data from trials 81-90 in Figs. 3 and 4 show the percentage of CR's after rest for the normal and operated groups. In all groups the percentage of CR's dropped considerably after rest.

Statistical analysis of the post-rest conditioning data were made by binomial tests comparing the percentage of CR's in block VIII (trials 71-80) before rest with the percentage of CR's in block IX (trials 81-90) after rest. The binomial tests yielded significant one-tailed probabilities for groups NM ($p = .001$), NS ($p = .001$), OM ($p = .001$), while a non-significant probability was obtained for group OS ($p = .172$). That is, there was a significantly lower percentage of CR's on the ninth block of trials than on the eighth for groups NM, NS, and OM. This was taken as evidence for a lack of recovery after rest. Table 5 in the appendix presents additional information for these groups.

Data from trials 81-90 in Figs. 5 and 6 show the median latency of CR's after rest for the normal and operated groups. In all groups the median of latency scores increased considerably

after rest. Due to the non-homogeneity of the latency scores, median latency scores were computed and used in the statistical analyses. Binomial tests were used to determine if there were an effect of a recovery after rest. The median latency for the eighth block of trials (trials 71-80) was computed for each S and compared as stated before, with the median latency of the ninth block of trials (trials 81-90). The binomial yielded significant one-tailed probabilities for groups NM ($p = .001$), NS ($p = .001$), OM ($p = .01$), while a non-significant probability was obtained for group OS ($p = .377$). That is, there was a significantly higher latency on the ninth block of trials than on the eighth for groups NM, NS and OM. This was taken as evidence for a lack of recovery after rest effect. Table 5 in the appendix presents additional data for all groups.

In order to determine whether the spaced and massed training groups differed in the amount of change after rest, U tests were applied to the total number of CR's made by Ss in each group for the ninth block of trials (trials 81-90). Group NS made a median of 6.5 CR's and group NM made a median of 2.75 CR's for trials 81-90. A U test of the difference between these groups yielded a significant value of U, ($U = 15.5$, $p < .01$). That is, the normal spaced group made reliably more CR's than the normal massed group during the recovery trials.

Group OS made a median of .5 CR's and OM made a median of 2.83 CR's for trials 81-90. A U test of the difference between these groups yielded a significant value of U ($U = 22$, $p = .025$).

In terms of latencies, group NS had median latency of 1.14 secs. and group NM had a median latency of 2.94 secs. for trials 81-90. A U test of the differences between these groups yielded a significant value of U ($U = 19.5$, $p < .025$). That is, the normal spaced group made reliably shorter latencies than the normal massed group.

Group OS had a median latency of 1.5 secs. and group OM had a median latency of 3.11 secs. for trials 81-90. A U test of the difference between these groups yielded a non-significant value of U ($U = 32$, $p > .05$). That is, the operated spaced group made shorter latency scores than the operated massed group, but the value of the difference was not reliably different.

In summary, all the conditioning groups showed a decrement in performance on the post-rest trials. This decrement in Ss performance was significant in groups NS, NM, and OM, while the Ss in group OS did not show a significant decrement in post-rest trials. Comparisons between the normal massed and spaced groups showed the Ss in group NS performed significantly higher than Ss in group NM on both measures of recovery. The comparisons between operated massed and spaced

groups showed Ss in group OM performed significantly higher than Ss in group OS on the total number of CR's measure. However, the difference between group OS and OM was not significant on the median latency of response measure of recovery.

Relationship Between S's Length and Values of UR's

In order to investigate the relationship between the S's length as measured by the number of S's segments and total number of UR's, rank order correlation coefficients (7, pp. 202-213) were computed. The data for the massed and spaced groups were combined.

Group NM made a median of 70.5 UR's and Ss in this group had a median length of 170 segments. Group NS made a median of 64.5 UR's and Ss in this group had a median length of 163.5. The correlation between lengths and number of UR's was significant ($r_s = .53$, $p = .01$). Also see Table 7 in the appendix.

Group OM made a median of 45.5 UR's and Ss in this group had a median of 167 segments. Group OS made a median of 31.5 UR's and Ss in this group had a median of 180.5 segments. The correlation between lengths and number of UR's was significant ($r_s = .38$, $p = .05$). See also Table 7 in the appendix.

IV. DISCUSSION

Conditioning

Figures 3, 4, 5, 6, and 7 clearly indicate the presence of conditioning in groups NM, NS, and OM, and an absence of conditioning in groups OS. The statistical tests yielded significant differences in Ss' performance from the first block to the last block of conditioning trials using both total CR's and median latency scores.

The data from the operated control groups, OCS and OCM, support the interpretation that the operation *per se* could not have accounted for the increased avoidance responses in group OM. Groups OCS and OCM, tested only with the vibratory stimulus showed lower performance levels than group OM, and did not differ from group OS, which did not condition.

The large number of responses and the short latency of responses shown by all groups during the first and/or second blocks of trials is interpreted as a reaction to the vibration (CS). Data from a previous study by Ratner and Miller (3), using a proper control group indicated that the vibratory stimulus elicited UR's during the early part of experimental trials. That is, neutral stimuli may produce responses but not for long because of sensory adaptation (3, pp. 71-82). Sensory adaptation, sometimes referred to as stimulus satiation,

refers to the fact that with continued or repeated presentation of a stimulus, the stimulus loses its eliciting or directing power.

Of particular interest was the lack of conditioning in group OS. A recent classroom study (5) as discussed below has essentially verified this finding of no conditioning if operated Ss are trained under spaced conditions. Three tentative hypotheses may be advanced to account for the absence of conditioning in group OS. First, the finding may be due to a sampling error. The verification of the data found in group OS by the classroom study mentioned above did not support the sampling error hypothesis. The second hypothesis is that time in the apparatus may have led to a biological deterioration of the operated spaced group. That is, they were in the S tube for approximately four hours while the operated massed Ss were in the apparatus for approximately one hour. The operation combined with the time in that apparatus may have had deleterious effects. This hypothesis was not supported by data obtained from the classroom study above (5) in which an operated group received massed training after a two-hour rest period in the apparatus. This OM group conditioned readily.

The third hypothesis which may explain the lack of conditioning in group OS would impute a "remembering or memory"

function to the pharyngeal ganglia. This hypothesis seems relevant since the operated massed group did show conditioning, which shows the operated Ss are capable of learning conditioned responses. That the highest level of conditioning was present in group NS also seems to uphold the interpretation that the pharyngeal ganglia is necessary for spaced conditioning. That is, we would expect group OS to condition to a higher level than group OM, since group NS conditioned to a higher level than group NM.

An experimental design which might throw light on the lack of conditioning in group OS would involve a long conditioning procedure in which blocks of massed trials are interspersed with blocks of spaced trials. According to the third hypothesis above we would expect the massed group to condition to a higher level than the spaced group.

Comparing Massed and Spaced Groups

Figures 3, 4, 5, and 6 clearly show a difference in performance between the massed and spaced training groups for both measures of conditioning. That is, the spaced group, NS, conditioned significantly better than the massed group, NM. Group OM conditioned significantly better than group OS, which did not condition at all. Until the present study the massed-spaced training effect had not been clearly demonstrated among invertabrates. Yerkes (10) for example, found spacing did not affect performance, using an instrumental paradigm.

However, the finding that group NS conditioned to a higher degree than group NM is consistent with other findings on the effects of massing vs. spacing in a conditioning situation (3, pp. 336-350).

Comparing Normal and Operated Groups

The lack of a significant difference between groups NM and OM is consistent with findings by Yerkes (11), who trained one worm Eisenia foetida in a T maze, and Heck (8, pp. 213), who trained a number of Eisenia foetida and Lumbricus terrestris. These workers found that removal of the ganglia did not prevent the learning of a simple turning response. There are no data in the literature which bear on the behavior of the OS group, which showed no conditioning at all. The hypotheses relating to the failure of the operated-spaced group to condition were presented earlier.

Post-rest Conditioning

Figures 3, 4, 5, 6, and 7 clearly indicate the absence of any recovery after rest in groups NM, NS and OM. That is, as opposed to recovery there was a significant decrement in Ss' performances after rest. The statistical analyses shows this decrement was greater than could be expected by chance alone. Group OS did not show a significant increment or decrement in performance since they did not condition.

The significant decrement found on post-rest trials in groups NM, NS, and OM was not what one would expect. That is, typically massed practice followed by a rest leads to improvement in performance and spaced practice followed by a rest leads to little or no change in performance (3, pp. 336-350).

The interpretation of the post-rest decrement is that the rest period served as an extinction session. Since the conditioning trials were presented at regular intervals for all groups and since the rest took place in the conditioning apparatus for all groups, it was possible that some temporal conditioning took place which led to the occurrence of CR's during the rest period in the absence of the US. Thus extinction was present.

This hypothesis may be tested by conditioning a group of Ss and observing during a rest period the number of responses occurring during that interval of time in which the CS previously occurred.

The graphs and statistical analyses of post-rest performance lend some support to this hypothesis. The Ss in group NS performed significantly better than Ss in group NM on both measures of post-rest conditioning.

Group NS gave a significantly higher post-rest performance than group NM and this finding is consistant with the previous hypothesis accounting for the lack of a recovery after rest. That is, the massed group with an inter-trial

interval of 10 seconds experienced a greater number of extinction trials in the 20 minute rest-interval than the spaced group. However, the difference between the groups may simply be a function of the greater degree of conditioning which took place for group NS.

The comparisons between group OS and OM show Ss in group OM performed significantly better than Ss in group OS on the total number of CR's measure. However, the difference between OS and OM was not significant on the median latency of CR's measure of recovery. This finding was consistent with the lack of conditioning in group OS. That is, operated Ss are not capable of conditioning under conditions of spaced training.

Relationship Between S's Length and Total Number of UR's

The significant positive correlations found between S's length (expressed in number of segments) and total responses to the US is of interest in view of a previous finding by Ratner and Miller (4) in which the only significant correlation between S's length and total number of responses was found in a control group which received the US alone. This correlation coefficient was negative. In the above study, length was measured in metric units. Probably the most logical reason for the discrepency between the study mentioned above and the present study was due to the more reliable measure of length used in the present study, namely counting the S's segments.

V. SUMMARY

This study was designed to investigate in a factorial design, with earthworms, Lumbricus Terrestris, the effects of (a) removing the ganglia (anterior five segments removed) and (b) spacing of trials on (a) the conditioning of a withdrawal response and (b) reconditioning of this response after a rest period. The following experimental groups were used: (1) normal spaced, (2) normal massed, (3) operated massed, and (4) operated spaced.

Sixty earthworms were randomly assigned to groups: normal spaced, NS; normal massed, NM; operated spaced, OS; operated massed, OM; operated control spaced, OCS; and operated control massed, OCM. The forty operated Ss had their dorsal ganglia and anterior five segments removed while the ganglia of the remaining 20 Ss were left intact. Massed conditioning trials had a ten sec. inter-trial interval and spaced conditioning trials had a 90 sec. inter-trial interval.

The apparatus consisted of a clear, plastic tube into which the S was placed. The tube and a bell buzzer were mounted on a board with a photo-flood light centered above the tubes. Vibration of the bell buzzer provided the CS, and light from the photo-flood provided the US. For groups NS, NM, OS, and OM, the CS was presented for 6 sec., during the last 2 sec. of which the US was presented. The occurrence

and latency of a withdrawal response during the first 4 sec. of a vibration were recorded. Groups OCS and OCM received 6 sec. of vibration alone and the occurrence and latency of a withdrawal response during the first 4 sec. were recorded.

Groups NS, NM, OS, and OM received 80 conditioning trials followed by a 20 min. rest and ten post-rest conditioning trials. Groups OCS and OCM received 80 stimulus presentations followed by a 20 min. rest and ten post-rest presentations.

The results clearly showed conditioning in groups NS, NM, and OM on both measures of conditioning, while group OS did not show conditioning. The performance of control groups, OCS and OCM, clearly showed the operation did not account for the conditioning found in groups OS and OM. Group NS conditioned better than group NM on both measures of conditioning. Group OM clearly showed conditioning while group OS did not condition at all. Comparisons between the operated and normal groups showed that groups NM and OM do not differ significantly, while group OM conditioned and group OS did not condition at all. All groups showed a decrement in performance on the post-rest trials.

The results of this experiment showed that (a) spaced trials facilitated conditioning for normal Ss; (b) removing the "cerebral" ganglia did not affect conditioning with massed trials; (c) removing the "cerebral" ganglia prevented

conditioning with spaced trials; (d) a rest period in the apparatus lead to a post-rest decrement for all groups. A positive correlation was found between Ss' length and values of UR's.

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APPENDIX A

TABLE 1

A statistical analysis employing the Binomial Test to determine if the increase or decrease in S's performance during conditioning trials is due to chance alone.

Group	Scores	Trials	No. of S's showing poorer performance on trials 81-90 than on 1-10	No. of S's showing better performance on trials 81-90 than on 1-10	Confidence level
NS	CR's	1-10 71-80	1	9	p = .01
NS	Latency	1-10 71-80	1	9	p = .01
NM	CR's	1-10 71-80	0	10	p = .001
NM	Latency	1-10 71-80	2	8	p = .05
OS	CR's	1-10 71-80	8	2	p = .05
OS	Latency	1-10 71-80	9	1	p = .05
OM	CR's	1-10 71-80	2	8	p = .05
OM	Latency	1-10 71-80	2	8	p = .05

TABLE 2

A statistical analysis of the differences between the operated conditioning groups and the operated control groups, in order to determine whether the operation may have accounted for conditioning. The Kruskal-Wallis one-way analysis of variance test was employed.

Group	Scores	Trials	Rank		Test	Confidence Interval
OM			R_1	340.5		
OCS			R_2	189.5		
OS			R_3	175.5		
OCM	CR's	1-80	R_4	89.5	H = 16.16	$p < .01$
OM			R_1	253.0		
OCS			R_2	244.0		
OS			R_3	191.0		
OCM	Latency	1-80	R_4	132.0	H = 13.96	$p < .01$

TABLE 3

A statistical analysis of thedifferences in conditioning between massed and spaced groups employing the Mann-Whitney U Test.

Group	Scores	Trials	Sum of the ranks	Test	Confidence level
NS NM	CR's	71-80	R_1 129.0 R_2 81.0	U = 26.0	p < .05
NS NM	Latency	71-80	R_1 134.5 R_2 75.5	U = 20.5	p < .05
OS OM	CR's	71-80	R_1 56.5 R_2 153.5	U = 1.5	p < .001
OS OM	Latency	71-80	R_1 58.0 R_2 152.0	U = 3	p < .001

TABLE 4

A statistical analysis of the differences in conditioning between normal and operated groups employing the Mann-Whitney U Test.

Group	Scores	Trials	Sum of the ranks	Test	Confidence level
NS OS	CR's	21-80	R_1 144.0 R_2 66.0	U = 11.0	p < .001
NS OS	Latency	21-80	R_1 147.0 R_2 63.0	U = 8.0	p < .001
NM OM	CR's	21-80	R_1 105.5 R_2 104.5	U = 49.5	p > .05
NM OM	Latency	21-80	R_1 102.5 R_2 86.5	U = 52.5	p > .05

TABLE 5

A statistical analysis employing the Binomial Test to determine if the increase or decrease in S's performances after rest is due to chance alone.

Group	Scores	Trials	No. of S's showing poorer performance on Trials 81-90 than on 71-80	No. of S's showing better performance on Trials 81-90 than on 71-80	Confidence level
NS	CR's	71-80 71-90	10	0	p = .001
NS	Latency	71-80 71-90	10	0	p = .001
NM	CR's	71-80 71-90	10	0	p = .001
NM	Latency	71-80 71-90	10	0	p = .001
OS	CR's	71-80 71-90	7	3	p = .172
OS	Latency	71-80 71-90	6	4	p = .377
OM	CR's	71-80 71-90	10	0	p = .001
OM	Latency	71-80 71-90	9	1	p = .01

TABLE 6

A statistical analysis of the differences in recovery after rest between massed and spaced training groups employing the Mann-Whitney U Test.

Group	Scores	Trials	Sum of ranks		Test	Confidence level
NS NM	CR's	81-90	R_1 R_2	139.5 70.5	$U = 15.5$	$p < .01$
NS NM	Latency	81-90	R_1 R_2	135.5 74.5	$U = 19.5$	$p < .025$
OS OM	CR's	81-90	R_1 R_2	77.0 133.0	$U = 22$	$p < .025$
OS OM	Latency	81-90	R_1 R_2	87.0 123.0	$U = 32$	$p > .05$

TABLE 7

A statistical analysis of the relationship between S's length and values of UR's, employing a rank order correlation coefficient.

Group	Scores	Trials	D^2	Test	Confidence level
NM x NS	CR's	1-80	631.00	$r_s = .53$	$p = .01$
OM x OS	CR's	1-80	822.00	$r_s = .38$	$p = .05$

APPENDIX B

"Raw data in folder."

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6 Suppls.