CLASSICAL CONDITIONING OF THE HINDLIMB FLEXION REFLEX IN THE PARTIALLY DECEREBRATE ACUTE SPINAL CAT

by

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INTRODUCTION

The classical conditioning procedure appears to be very simple. Two stimuli, designated as the conditioned stimulus (CS) and the unconditioned stimulus (UCS), are presented in a temporally fixed sequence, the CS preceding and, usually, overlapping the occurrence of the UCS. Both stimuli may terminate simultaneously. After several paired presentations of these stimuli a response similar to the response to the UCS occurs after the CS. This response to the CS is termed the conditioned response (CR) and the response to the UCS is termed the unconditioned response (UCR).

The simplicity of the procedure is limited to the experimental operations. The neural or physiological mechanisms underlying conditioning have not yet been identified. A model system against which conditioning theories can be tested would be useful to elucidate these mechanisms. Major interest has centered around the search for a relatively simple neuronal system in which confounding influences, such as movement, light, and age are minimized. Such a system, whether an isolated section of a more complex system (ganglion, nerve, spinal cord) or the intact system of a relatively primitive organism, would be of value to conditioning theorists. This accounts for the vast amount of work expended in attempts to condition planaria and spinal animals.

The work by Thompson and McConnell (1955) on classical conditioning in planaria aroused a great deal of interest, but later investigators (Cummings and Moreland, 1959; Halas, James and Stone, 1961; Van Deventer and Ratner, 1964) have pointed out significant weaknesses in

the planaria conditioning experiments. Some specific variables, which significantly influence responsiveness of planaria to light (the CS used by Thompson and McConnell, 1955), have been shown by VanDeventer and Ratner (1964) to include temperature, dark adaptation and size of the animal. These factors were uncontrolled in the Thompson and McConnell study. In a review of studies on learning in paramecia and planaria, Jensen (1964) concludes that the results of these studies do not support the hypothesis that learning can occur in these animals. The possibility of a spinal conditioning model thereby assumes more importance.

The ability to develop a conditioned reflex without involvement of cortical structures was first reported by Lebedinskia and Rosenthal (1935). Their subject, a dog, had both cerebral hemispheres removed. The response conditioned was salivation to a metronome, the first CR appearing after 250 trials.

Five years later Shurrager and Culler (1940) reported evidence indicating the presence of conditioning of a spinal preparation. This report generated much controversy. Because portions of the technique subsequently have been incorporated into other experiments, the general method that Shurrager and Culler used is of interest.

The spinal cord of dogs was transected, the level of transection varying from second cervical to third lumbar levels. Both semitendinosus muscles of the hind limbs were dissected out with nerve and blood supply intact. Fine threads were attached to the fascia over a pit-like depression in the muscle and these threads and tendon were then attached to a kymograph lever.

In the conditioning procedure the CS was either a moderate electric shock or a stiff-bristle brush tap applied to the tail or to the fore-paw opposite the hind leg receiving the UCS. The UCS was an electrical shock of sufficient intensity to elicit full contraction of the semitendinosus muscle when applied to the homolateral hind paw. At the beginning of the conditioning series, thirty to sixty presentations of three CSs at 1 second intervals were administered as a control for the possibility of increased responsiveness of the semitendinosus muscle to a repeated CS. Following this series of the CS alone, another series of stimuli was presented in which the third CS in the above pattern was paired with the UCS. These paired presentations occurred every 15, 30, or 60 seconds.

The CR was a muscle twitch which was recorded on the kymograph.

After the establishment of the CR no stimuli were given for at least
10 minutes. The extinction procedure followed this rest period and
was identical to the conditioning procedure except the UCS was omitted.

The authors set three commonly accepted criteria as their criteria for conditioning. First the CR had to be distinguishable from the original response to the CS alone. Second, the occurrence of the CR had to be dependent upon paired presentations of the CS and UCS. Third, the CR had to diminish in frequency during the extinction procedure (repeated presentation of the CS alone).

One control procedure consisted of approximately 200 presentations of the CS alone at various time intervals. There was no evidence of a CR to the CS before pairing with the UCS. To meet the second criterion, Shurrager and Culler alternated a series of three UCS presentations with

three CS presentations or presented the UCS followed by two CS presentations. Neither of these combinations produced a CR. Regular extinction procedures reduced the CR to zero values. Extinction was effective whether initiated immediately or after a delay of several hours.

Other controls were included by Shurrager and Culler to establish that the results were not the result of recovery of spinal reflexes from anesthetic nor an artifact produced by direct electrical stimulation.

Shurrager and Culler plotted their data in a vincentized form, percent conditioned responses versus percent conditioning or extinction (i.e., successive tenths of the whole number of trials required for complete conditioning or extinction) trials. The extinction curve, when rotated 180 degrees, could almost be superimposed on the conditioning curve. Repeated series led to fewer trials to criterion (10 CRs in 10 successive trials) for both conditioning and extinction. These two observations led the authors to conclude they had demonstrated conditioning in a spinal preparation and they suggested that conditioning and extinction were based on similar mechanisms.

Shurrager and Culler (1940) employed several control procedures to eliminate nonconditioning phenomena, such as recovery from anesthesia, and these controls appear to have been adequate. Little criticism has been given to this aspect of the investigation by other workers. Many other aspects of the method and/or interpretation of their results, however, have been criticized. The usual starting point is that, of the 196 dogs that received the conditioning procedure, only 98 gave positive evidence of spinal conditioning. The remaining 98, or 50%,

failed to show any evidence of conditioning. The failure to demonstrate conditioning in 50 percent of the animals does raise serious questions. Shurrager (1947) has apparently made only one statement in rebuttal to this criticism: "It is not uncommon to find a normal animal which fails to learn a simple differential flexion response in hundreds of conditioning trials." Shurrager and Shurrager (1946) reported positive evidence of spinal conditioning in dogs but the total number of dogs used was not reported.

A second point of the Shurrager and Culler (1940) study that has been criticized has to do with the character of the CR. Although initially the CR was a very small localized muscle twitch, on later trials it became more diffuse and included more muscle fibers. This change in the nature of the response has been questioned because the usual progression seen in classical conditioning in the intact animal is one in which a diffuse general response gradually becomes more and more localized and restricted (Kimble, 1961; Hilgard and Marquis, 1940).

A third criticism leveled at the Shurrager and Culler experiment was that an increase in CS intensity failed to produce an increase in the magnitude of the CR (Pinto and Bromiley, 1950). This point has not been settled conclusively.

Since the initial investigation by Shurrager and Culler (1940) a variety of studies have been reported in which no evidence of spinal conditioning was found (Kellogg, 1947; Pinto and Bromiley, 1950; Forbes and Mahan, 1963). One of the most well known studies, by Kellogg, Deese, Pronko and Feinberg (1947), used an experimental preparation so different from that of Shurrager and Culler that it is difficult to make a meaningful comparison between the two studies. Kellogg

et al. used a chronic preparation similar to one which Shurrager and Culler had tried unsuccessfully (Shurrager, 1947). The CS consisted of two brief shocks 1 second apart delivered to one of the hind limbs. The UCS, a single brief shock delivered to the other hind limb, occurred 1 second after the termination of the CS. A major problem with this technique of presenting the CS and UCS is that it can elicit two antagonistic and mutually exclusive reflexes in the leg receiving the UCS. Thus a CS of moderate intensity would be expected to produce flexion of the leg stimulated by the CS and extension of the leg that was to receive the UCS. In the Kellogg et al. study the CS was of sufficient intensity to change the response pattern to one of flexion in both hind legs. During the course of the experiment, however, the flexion response to the CS in the leg stimulated by the UCS alternated in dominance with the crossed-extension reflex. The authors concluded that their data gave no evidence in support of spinal conditioning since, after approximately 600 trials the frequency of the flexion reflex in the UCS leg was close to zero.

Shurrager (1947) also had found a bilateral hindlimb flexion reflex in response to a strong CS. The magnitude of this response increased with further increases in CS intensity. It is difficult to understand why Kellogg et al. used these responses or what the implications of their results are for understanding spinal conditioning.

Kellogg (1947) stated that usually about 100 to 400 conditioning trials were required for a normal intact animal to reach 100 percent conditioning under the stimulus conditions employed by Shurrager and Culler (1940). The problem then is to explain the 100 percent conditioning which Shurrager and Culler reported was obtained in 30 to 40

conditioning trials. A search of the literature reveals no studies of the number of trials required to reach a conditioning criterion under different experimental conditions, especially the conditions employed in spinal conditioning. Since neither the Shurrager group nor the Kellogg group could get spinal conditioning in chronic animals, Kellogg suggested that the results of Shurrager and his associates was an artifact due to some post-operational factor. It would appear to be equally possible for the physiological deterioration in the chronic animal to affect important neural units involved in spinal conditioning and thus prevent the changes involved in conditioning.

Deese and Kellogg (1949) studied flexion response of the whole leg in chronic spinal dogs. Two brief shocks to the tail (CS) were followed by a shock to the right hind foot (UCS). The authors reported both the CS and UCS produced a twitch in both legs, and concluded that the response to the CS was not a conditioned response since it occurred to the CS alone without having been reinforced by the UCS. This was presented as evidence against spinal conditioning. It must be remembered that Shurrager and Culler (1940) used a response of much smaller magnitude than that in the Deese and Kellogg (1949) study and this response did not occur to the CS alone.

Forbes and Mahan (1963) conditioned intact cats to escape tail shock by hind limb flexion. There was no evidence of this response after cord transection or during etherization. The results, from only two animals, each of which was subjected to a different experimental procedure, were interpreted as evidence that the learning which occurred prior to cord transection was cortical and that spinal conditioning, if possible, is very difficult to achieve.

One is forced to conclude, after consideration of all the above reports, that they have provided no conclusive evidence in support of, or contradictory to, the establishment of spinal conditioning. The following are some of the weak points in this group of studies.

First, several studies used an unusual pattern of multiple stimuli as the CS. In addition, 1 sec. intervals were used between presentation of CS and UCS. The optimum interstimulus interval for some classically conditioned responses, as indicated by results from several studies (cf. Kimble, 1961, Table 13), appears to be close to 0.5 sec. Thus, 1 sec. can be considered a long interstimulus interval in these studies.

Second, most experiments have allowed the animal to recover from general anesthesia following cord transection. The foreparts of subjects have not been immobilized and Pinto and Bromiley (1950) reported that movements of the foreparts markedly affected the excitability of the isolated cord of a spinal animal.

Contrary to general opinion, Shurrager and Culler (1940) did run all controls necessary to show conditioning. Those studies which have failed to verify their results also failed to replicate the essential aspects of their experimental procedure, thereby limiting the generality of the negative results. Some results in the Shurrager and Culler work have been singled out because they do not match results from intact animals. One ordinarily would not presume to generalize results from one preparation to a vastly different preparation without experimental work, and therefore it seems rather illogical to reject results derived from a spinal animal, i.e., a preparation very different from the intact animal.

The results of Shurrager and Culler (1940) were supported in a pilot study by Fitzgerald and Thompson (1967). Using partially decerebrate spinal cats, the latter investigators presented a shock (CS) to the hip followed by shock (UCS) to the footpad. During acquisition training the amplitude of the flexion response in the conditioning group was significantly greater than in a control group given an identical number of stimuli in an unpaired pattern. A major portion of the response increase appeared in the first 20 trials, and an interstimulus interval of 0.5 sec. appeared to give better conditioning than any of a number of other interstimulus intervals used in this study although this could not be determined statistically.

The results of that pilot study led to the following investigation. The general procedure follows the early work of Shurrager and Culler, with specific modifications introduced to eliminate certain possible sources of error, such as large movements. The most drastic change introduced was surgically to render the subject comatose instead of allowing it to recover from anesthesia. As a result of this procedure there was very little skeletal muscle activity in the foreparts of the subject other than respiratory movements. Another change was to equate the tension of the individual muscles involved by using each muscle's response pattern to the UCS as a reference.

The purpose of this study was to test for spinal conditioning, using a method based on the original work by Shurrager and Culler (1940) with modifications introduced to meet most of the methodological objections from other investigators.

APPARATUS:

The Ss were anesthetized with ether and then immobilized by an RF lesion in the midbrain tegmentum. To make the brain lesion, current from a Grass Instrument Lesion Maker (Model LM-3), set at 85 volts for 35 seconds, was delivered through stainless steel electrodes 1 mm in diameter and insulated except for 4 mm at the tip. The distance between the centers of these electrodes was 4 mm. Location of the lesion is described below.

The electrodes through which the CS and UCS were delivered were stainless steel fish hooks, size 1/0. Trials were initiated automatically by a 35 mm punched tape, run through a Gerbrand Model 3A programmer. Presentation of the CS and UCS at interstimulus intervals greater than 10 seconds was also controlled by the tape programmer. Interstimulus intervals shorter than 10 seconds were timed by Tektronix V 162 and 161 units.

Muscle tension was measured by a Grass Model FT 10C force displacement transducer connected to the input of a Grass 5P 1 low level DC polygraph preamplifier. From there the signal was led into a Grass DC driver amplifier and written out by means of an oscillograph. Tension was calibrated for the complete system in grams.

A small plastic cannula was used to connect the femoral artery to a Statham blood pressure transducer which led into another preamplifier and amplifier combination in the polygraph consol. In a few subjects, blood pressure was recorded simultaneously with muscle tension. The <u>Ss</u> were 42 cats supplied by the Animal Care Department of the University of Oregon Medical School. Atropine (1 mg/kg) was injected intramuscularly into the right hind limb of each <u>S</u> at least 30 minutes before etherization. This effectively prevented excess fluid from accumulating in the respiratory tract. A soft plastic endotrachael tube was inserted into the trachea. A small gauze compress, moistened with ether, was clipped to the end of the endotrachael tube to maintain anesthetization.

The <u>S</u>'s head was placed in a Kopf stereotaxic apparatus and all tissue was retracted from the top of the skull. Bilateral trephine holes were made in the skull and small slits were cut in the dura. The brain lesion electrodes were positioned over these slits, straddling the sagittal sinus.

The anterior-posterior plane of the lesion was AP_O . The most medial portion of the lesion was made first with the electrodes lowered to 6 mm below H_O . After this region was lesioned the electrodes were raised and cleaned. The electrode position was moved to the right hemisphere until the left electrode was directly above the opening made by the right electrode during the first penetration. The electrodes were lowered to a position 4.5 mm below H_O and a lesion was made. The electrodes were raised 2.0 mm and another lesion was made. The process then was repeated in the left hemisphere. Histology was not done to determine the extent of the lesions, but some of the brain structures located at the level of the lesion are the superior colliculus, interpeduncular nucleus, decussation of the superior cerebellar peduncle and mesencephalic reti-

cular formation (Snider and Niemer, 1961).

The exposed area of the brain and skull were covered with saline soaked gauze compresses which were kept moist throughout the experiment to prevent stimulation of this area by the drying of the exposed tissues.

The tissues over the tenth to the twelfth thoracic vertebrae were retracted and the dorsal part of the vertebrae and tissue surrounding the cord were removed. Immediately after the spinal cord was cut, oxycel soaked with thrombin was packed into the point of transection to facilitate coagulation of the severed blood vessels. If the transection was complete the two ends of the cord retracted leaving a space of several millimeters between them. In all cases the transection was visually inspected after bleeding was reduced to ascertain whether transection was complete, and whenever there was any doubt the process of transection was repeated and rechecked. Administration of ether ceased at this time. The area of the spinal transection was covered with gauze pads kept moist for the duration of the experiment, again to prevent stimulation arising from drying of exposed tissues.

The exact location of the spinal transection varied among <u>Ss</u>, but was always located between the tenth and the twelfth thoracic vertebrae. The transection at this level avoided the blood vessels entering the cord at the level of the third lumbar vertebra, which form the major vascular supply to the caudal section of the cord.

On each hip the lateral aspect of the ischium, just below the crest, was cleared of tissue for later placement of the hip pins.

The left tibialis anterior muscle and its tendon of insertion were carefully separated from surrounding tissue. The tendon was ligated and

then cut distal to the ligature. Pins were driven into both ends of the tibia in order to fix the position of the leg.

In two control animals the right femoral artery was cannulated for recording of blood pressure.

An aluminum framework was bolted to the table. Bilaterally, the sharply tapered end of an aluminum rod was driven into the ischium just below the ischial crest and the rod was clamped to the metal frame. The left hind limb was immobilized by clamping metal bone pins to the frame. A padded clamp held the foot firmly.

The string attached to the muscle tendon was fastened to the transducer mounted on the frame. To equate resting muscle tension the transducer position was varied by a rack and pinion device. The muscle length was increased slowly in 2 mm steps during which the resting muscle tension increased in fairly uniform increments. When the change in resting muscle tension suddenly increased more than usual, the length was reduced until the muscle shortened 4 to 6 mm. The transducer was then moved away from the animal in 2 mm steps, a footshock being given 60 sec. after every step, until a length was reached at which the muscle response was of lower amplitude than the response at the previous length. The muscle was returned to that previous length and held there for the duration of the experiment. This procedure made it possible to limit the number of footshocks during this phase of the experiment to a maximum of five shocks. There was a 15 min. rest period between the end of the muscle tension adjustment and the beginning of the experimental shock series.

At the completion of the experimental series 1.0 cc Nembutal (50 mg.) was injected into the brain stem. For a few animals a CS alone

series was presented until there was no observable heart beat or respiration to eliminate the possibility that responses comparable to those of a live \underline{S} could be elicited from a dead \underline{S} .

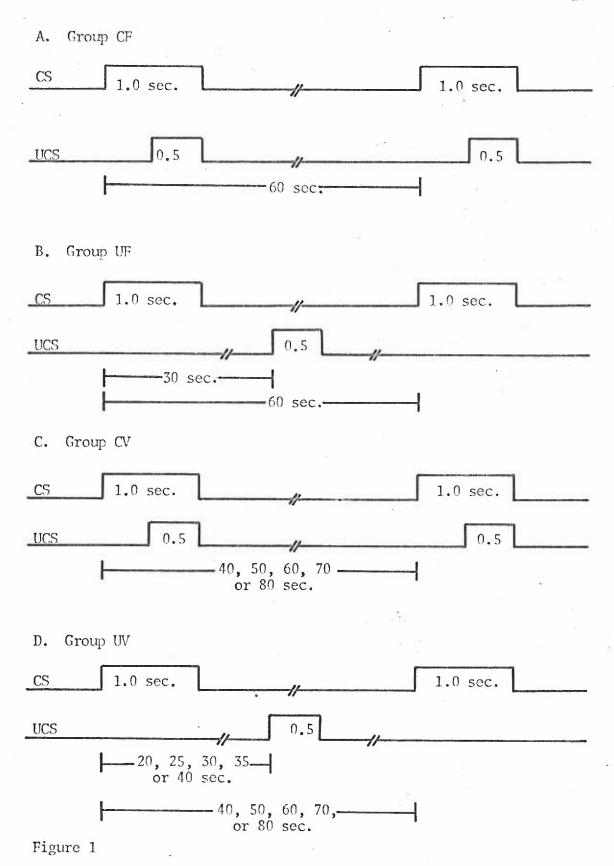
The Ss were randomly assigned to one of four groups: one conditioning group and one control group, both with fixed inter-trial intervals; one conditioning and one control group both with variable intertrial intervals. Each S received in sequence, 2 to 5 UCSs during the time that the adjustments of muscle tension were being made, 40 trials with the CS alone, 30 CSs and UCSs (paired in the two conditioning groups, unpaired in the two control groups), and 30 extinction trials with CS alone.

The CS was a 100 pps, 1.0 sec. shock delivered through electrodes in the skin over the left hip. Intensity of the CS was varied during the first 10 CS alone habituation trials and finally held at a value which resulted in an "intermediate" muscle response, i.e., the response was not the maximum possible with hip shock nor was it such a weak response that there was danger of it habituating below baseline. These 10 CS alone habituation trials were not considered part of the habituation series in the final tabulation of the data.

The UCS was a 100 pps, 0.5 sec. shock delivered through the electrodes in the footpad of the left hind leg. The intensity of the UCS was set at 50 volts, the maximum intensity from a Tektronix 161 unit. The measured output at this setting was 48 volts.

Following the habituation trials to the CS alone, the Ss in the two conditioning (C) groups were given an acquisition series of paired CS-UCS trials. The interstimulus interval from CS onset to UCS onset was 0.5 sec. The CS and UCS terminated simultaneously (Figure 1A and 1C). One of the C groups, CF, received the paired stimuli at fixed 60 sec. intertrial intervals, measured from CS onset to CS onset (Figure 1A).

- Figure 1. A diagrammatic representation of the time interval relationships used with each of the experimental groups. For each group, the CS duration was held constant at 1.0 sec. and the UCS duration was held constant at 0.5 sec.
 - A. Group CF. The interval between the onsets of any two successive CSs was equal to 60 sec. The interval between the onset of the CS and the onset of the UCS was equal to 0.5 sec.
 - B. Group UF. The interval between the onsets of any two successive CSs was equal to 60 sec. The interval between the onset of the CS and the following UCS was equal to 30 sec.
 - C. Group CV. The interval between the onsets of any two successive CSs was 40, 50, 60, 70, or 80 sec. The average interval between CS presentations was 60 sec. The interval between the onset of the CS and the onset of the UCS was equal to 0.5 sec.
 - D. Group UV. The interval between the onsets of any two successive CSs was 40, 50, 60, 70, or 80 sec. The average interval between CS presentations was 60 sec. The interval between the onset of the CS and the onset of the UCS was equal to one-half the interval between successive CSs.



In the other conditioning group, CV, intertrial interval measured from CS onset to CS onset was 40, 50, 60, 70, or 80 seconds, (Figure 1C) with an average of 60 sec.

The other two groups (U) were given unpaired CS and UCS following the CS alone series. For one group, UF, the CS onset to UCS onset interstimulus interval was fixed at 30 sec., while the CS onset to CS onset intertrial interval was held at 60 sec. (Figure 1B). In the second unpaired group, UV, intertrial interval measured from CS onset to CS onset was 40, 50, 60, 70, or 80 sec., with the UCS being presented halfway between any two CSs (Figure 1D). The pattern of intertrial intervals was the same for groups CV and UV.

The response to the CS was measured in millimeters and converted to grams tension.

RESULTS:

The responses of each S on each trial are given in Tables I, II, III, and IV of the Appendix for the UF, CF, UV, and CV groups resepctively. Trial by trail plots of the mean and median response amplitudes of each group to the CS are shown in Figures 2, 3, 4, and 5, for the UF, CF, UV, and CV groups, respectively. These figures reveal that during the habituation series group UF showed an increase in response amplitude whereas the other three groups showed a decrease in response amplitude across trials. Wilcoxin matched-pairs signed-ranks tests, using the data from the 1st and 30th trial for each S, indicated that the change across trials was significant only in the CF group. Figures 2 through 5 also indicate very little overlap between the responses of the UF group and those of the other three groups, but Mann-Whitney U tests indicated no significant differences between groups in response amplitude on the final habituation trial.

The median response to the CS (in grams tension) during the last five trials of the habituation series was taken as a control level for each S. In order to adjust for different base lines, the acquisition and extinction series were each divided into ten blocks of 3 trials each and the median response of each block was converted to a percentage measure relative to the control level for that S. Figure 6 shows the median percentages of all four groups during acquisition and extinction series, with the control level set at 100 percent. Both conditioning groups, CF and CV, and one unpaired control group, UF, show a slight increase in response amplitude across acquisition trials. Response amplitude is greater in UF than in CF. The fourth group, UV, shows no increase in response amplitude

Figure 2. The amplitude of the mean and median responses to the CS (grams tension) of Group UF for each trial of CS alone (habituation), acquisition, and extinction (N = 10).

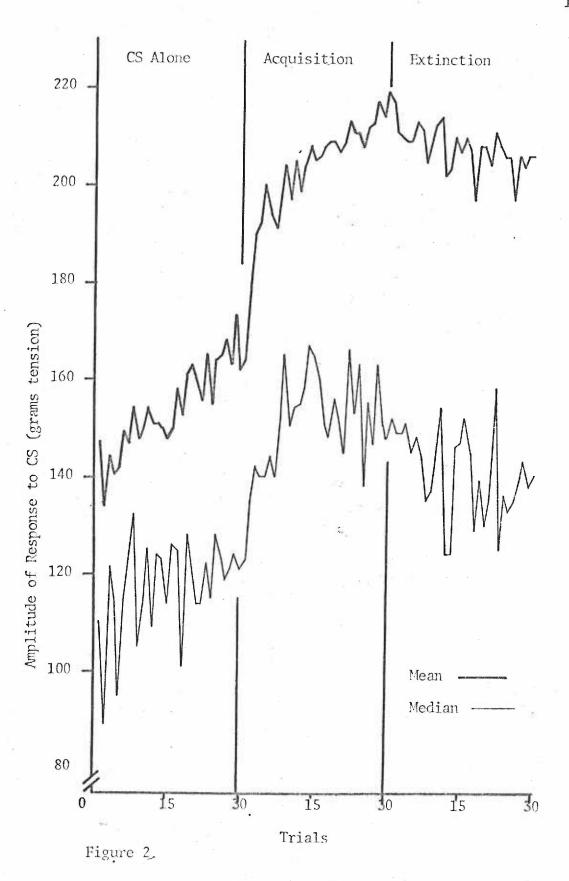


Figure 3. The amplitude of the mean and median responses to the CS (grams tension) of Group CF for each trial of CS alone (habituation), acquisition, and extinction . (N = 10)

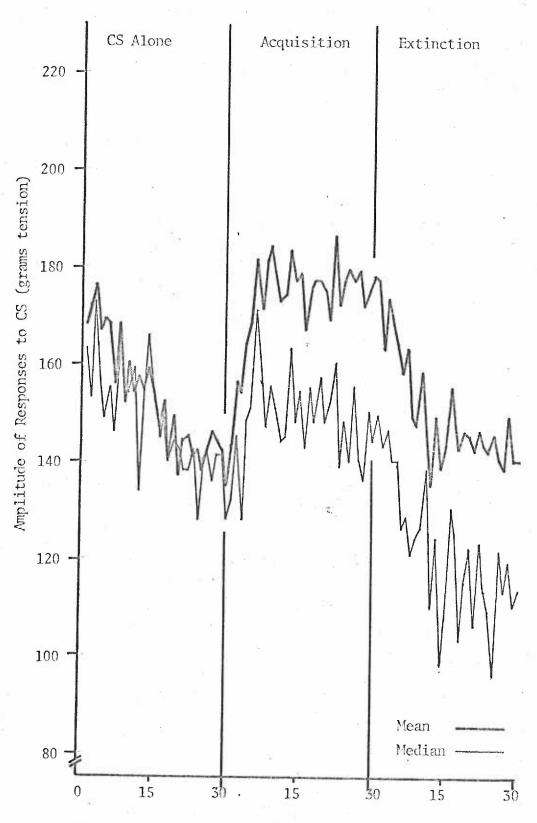


Figure 3

Figure 4. The amplitude of the mean and median responses to the CS (grams tension) of Group UV for each trial of CS alone (habituation), acquisition, and extinction. (N = 10)

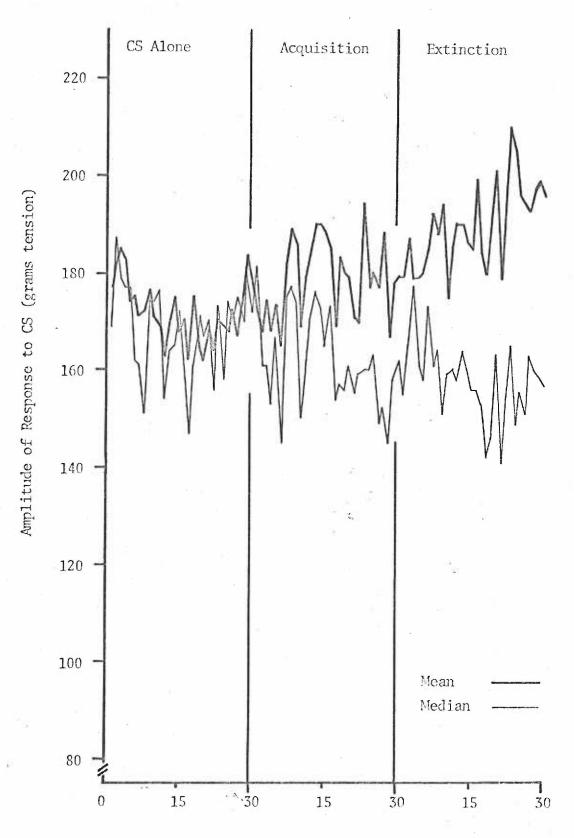


Figure 4

Figure 5. The amplitude of the mean and median responses to the CS (grams tension) of Group CV for each trial of CS alone (habituation), acquisition, and extinction. (N = 10)

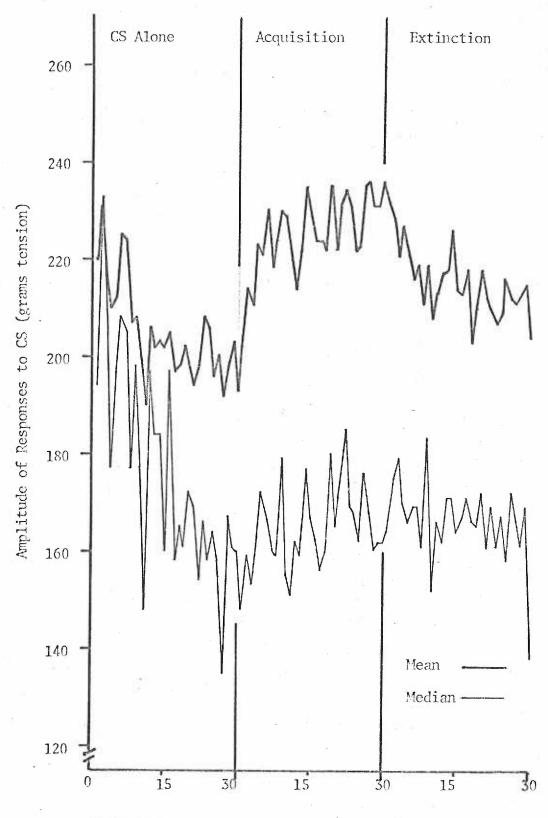
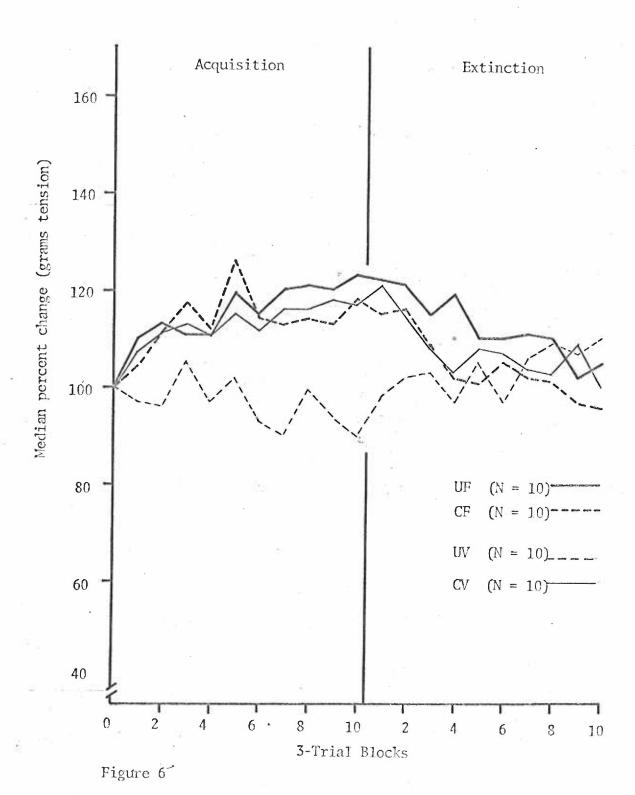


Figure 5

Figure 6. The median percent change in the response to the CS (grams tension), N = 10 for each group. For each group the median response of the final 5-trial block of the CS alone (habituation) series = 100 percent (control level.) The median response amplitude of successive 3-trial blocks in acquisition and extinction are presented as a percentage of the control level.



by the end of acquisition trials. During extinction all groups approached their original control level.

A 2 x 2 x 10 analysis of variance with the factors being conditioning vs control, fixed vs variable intertrial intervals, and trial blocks, failed to demonstrate any significant effects in either acquisition or extinction.

The variable intertrial interval groups, UV and CV, were tested separately for the presence of conditioning. Kolmogorov-Smirnov two-sample tests on the median percent scores of each block of 3 trials for both acquisition and extinction indicated a significant difference between these two groups during acquisition (p \leq .01) and no significant difference during extinction.

Since the UV and CV groups differed from the other two groups (UF and CF) only in the nature of the intertrial interval, it is possible that some feature of the variable time interval, or one of the specific time intervals (40, 50, 60, 70, or 80 seconds) acted to prevent an increase in the response amplitude of the UV group during acquisition. The median response amplitudes for all Ss in each group, arranged in order of the interval from the preceding CS, are in Tables 1 (habituation), 2 (acquisition), and 3 (extinction). In groups CF and UF the interval between any two consecutive CS presentations is always 60 seconds, but the trials, for comparison purposes, are groups as if these Ss were under the variable intertrial interval program. Mann-Whitney U tests, comparing the CF and CV, UF and UV, groups at each intertrial interval were not significant, indicating that the responses of the variable intertrial interval groups, with any particular time interval, were not different from the corresponding re-

Table 1. Median response amplitudes (grams tension) arranged in order of the magnitude of the interval from the preceding CS, CS alone habituation series.

C	CT
Group	UF
1	

		Sec	conds		
	60	60	60	60	60
Subject	•	-			
66-64	200.5	186.5	177.5	220.0	193.0
66-65	240.0	235.0	2 22.5	286.0	225.0
67-39	103.8	110.5	111.3	122.5	109.0
67-66	95.5	93.0	85.0	90.5	75.0
67-78	336.5	348.5	340.0	362.0	324.0
67-89	56.8	54.0	55.0	65.5	60.5
67-107	193.0	187.0	191.5	181.0	190.0
67-115	41.0	47.0	42.5	44.0	50.0
67-122	42.8	43.0	41.5	41.0	46.0
67-136	201.3	214.8	206.5	198.0	186.0

Group CV

	* 1	Sec	onds		
	40	50	60	70	80
Subject		2(0)		15.	
67-40 67-43 67-57 67-71 67-86 67-91 67-104	46.8 307.0 117.8 492.0 198.5 355.0	49.8 288.5 101.0 492.0 195.0 363.5 32.5	55.8 288.5 105.5 500.5 200.0 358.0 30.8	55.0 294.0 173.0 477.0 221.0 352.0 31.0	55.0 283.0 116.0 500.0 197.0 362.0 25.0
67-113 67-123 67-135	120.0 143.0 211.0	119.0 151.3 175.0	123.3 142.0 203.5	124.0 149.5 221.0	124.0 156.0 200.0

Group UF

		Sec	conds		
	60	60	60	60	60
Subject		3			
67-41 67-55 67-60 67-76 67-81 67-87 67-108 67-118 67-124 67-134	78.5 42.8 250.0 168.0 98.3 345.0 67.5 27.8 126.8 324.5	65.0 38.0 243.0 155.3 102.5 352.0 67.0 32.8 138.5 305.5	82.8 40.5 269.0 159.5 108.8 357.0 70.8 23.0 134.5 321.8	83.5 42.0 214.0 156.0 97.0 362.0 72.5 26.0 126.0 290.5	72.0 43.0 262.0 163.0 103.5 352.0 76.0 31.0 136.0 304.0

Group UV

Seconds						
	40	50 .	60	70	. 80	
Subject						
67-46	228.0	229.5	243.0	262.0	238.0	
67-49	82.8	79.3	77.0	72.0	80.5	
67-69	200.5	198.5	205.5	200.0	193.0	
67-73	333.0	340.0	335.0	328.0	338.0	
6 7-84	150.3	167.3	144.5	151.0	154.5	
67-95	52.5	50.0	47.5	67.0	65.5	
67-97	148.5	152.0	152.0	151.0	146.0	
67-120	225.5	215.0	216.5	201.5	224.5	
67-121	195.0	181.5	172.5	204.0	190.0	
67-133	80.5	75.8	106.0	87.0	93.0	

Table 2. Median response amplitudes (grams tension) arranged in order of the magnitude of the interval from the preceding CS, acquisition series.

		12 · · · ·	Group CF		
			Seconds		
	60	60	60	60	60
Subject	•	, S			
66-64 66-65 67-39 67-66 67-78 67-89 67-107 67-115 67-122 67-136	207.0 344.0 97.3 88.0 431.0 56.3 210.5 71.3 47.8 202.3	204.0 326.5 104.0 89.5 435.5 50.0 211.5 62.5 60.8 199.5	196.5 328.5 93.8 92.3 456.0 51.8 216.3 73.8 44.0	208.0 317.0 106.0 89.0 456.0 48.5 214.5 67.0 47.0 204.5	193.0 301.0 95.5 92.0 432.0 56.5 211.5 78.0 41.5 200.0

			Group CV		
			Seconds		
40	**.	50	60	70	80
			,		
36.5		47.3	46.5	55.0	48.0
399.0		378.8	379.0	372.0	379.0
74.3		78.0	82.0	70.5	82.0
574.5		578.0	578.0	571.0	571.0
180.0		176.5	164.5	176.5	176.5
457.0		454.0	466.5	452.0	462.0
41.3		38.0	39.0	47.0	38.0
155.3		153.0	150.3	149.5	147.5
144.5		160.3	144.5	143.0	147.5
219.5		210.5	204.0	214.0	221.0
	36.5 399.0 74.3 574.5 180.0 457.0 41.3 155.3 144.5	36.5 399.0 74.3 574.5 180.0 457.0 41.3 155.3 144.5	36.5 47.3 399.0 378.8 74.3 78.0 574.5 578.0 180.0 176.5 457.0 454.0 41.3 38.0 155.3 153.0 144.5 160.3	Seconds 36.5 47.3 46.5 399.0 378.8 379.0 74.3 78.0 82.0 574.5 578.0 578.0 180.0 176.5 164.5 457.0 454.0 466.5 41.3 38.0 39.0 155.3 153.0 150.3 144.5 160.3 144.5	Seconds 40 50 60 70 36.5 47.3 46.5 55.0 399.0 378.8 379.0 372.0 74.3 78.0 82.0 70.5 574.5 578.0 578.0 571.0 180.0 176.5 164.5 176.5 457.0 454.0 466.5 452.0 41.3 38.0 39.0 47.0 155.3 153.0 150.3 149.5 144.5 160.3 144.5 143.0

Table 2 (continued).

Group	UF

		Se	econds		
	60	60	60	60	60
Subject	. 12		,	XVIII 1	
67-41 67-55 67-60 67-76 67-81 67-87 67-108 67-118 67-124 67-134	33.3 33.5 442.0 183.8 154.5 455.0 110.5 24.0 148.5 451.5	54.0 36.0 443.5 178.0 156.8 460.0 110.0 24.0 150.3 438.5	61.8 45.0 453.5 186.3 142.0 462.0 114.5 23.3 151.3 460.0	40.5 29.5 419.0 186.0 139.5 464.0 103.0 22.0 153.0 456.0	48.0 33.0 432.0 173.0 153.0 445.0 107.0 17.0 149.5 464.0

Group UV

	Seconds				
	40	50	60	70	. 80
Subject					
67-46 67-49 67-69 67-73 67-84 67-95	166.5 61.8 226.5 389.0 144.5 35.0	170.0 60.0 219.5 387.5 144.3 40.0 152.0	179.8 53.8 214.0 392.5 151.3 34.3 148.5	180.0 63.5 218.0 383.0 139.5 46.0 151.0	186.0 62.0 231.0 389.0 146.0 41.0
67-120 67-121 67-133	290.5 193.5 87.3	295.0 271.0 93.0	302.0 179.5 75.5	280.0 228.0 83.0	283.0 248.0 103.0

Table 3. Median response amplitudes (grams tension) arranged in order of the magnitude of the interval from the preceding CS, extinction series.

Group	CF
-------	----

		Sec	conds		
	60	60	60	. 60	. 60
Subject	0				
66-64 66-65 67-39 67-66 67-78 67-89 67-107 67-115 67-122 67-136	138.0 270.0 83.5 84.5 331.0 63.0 210.0 65.5 36.5 194.0	154.0 275.5 82.0 89.8 327.5 60.5 208.3 67.0 38.8 185.5	131.0 254.0 92.0 85.0 324.0 62.0 214.5 58.0 50.0 191.5	149.0 270.0 104.0 94.5 329.5 59.5 211.5 75.3 36.8 177.5	141.0 250.0 89.0 95.5 331.0 60.0 211.5 61.5 49.5 179.5

Group CV

•		Sec	conds	¥0	
	40	50	60	7.0	80
Subject		•		5	
67-40 67-43 67-57 67-71 67-86 67-91 67-104 67-113 67-123 67-135	34.3 362.0 76.3 543.5 175.3 461.0 29.5 152.8 115.5	45.0 365.0 54.0 550.0 183.8 455.0 30.5 156.8 104.0 203.5	45.0 342.0 60.0 557.0 186.0 458.0 30.5 151.0 124.0 180.0	53.8 343.5 70.5 557.0 177.8 457.0 39.8 156.8 130.3 186.5	49.5 342.0 74.0 523.0 184.5 468.0 37.0 157.7 124.0 211.0

Group UF

		Sec	conds		
	60	60	60	60	60
Subject				19	
67-41 67-55 67-60 67-76 67-81 67-87 67-108 67-118 67-124 67-134	20.0 32.8 489.5 220.8 120.0 367.0 121.5 27.5 151.0 515.5	40.3 29.8 489.5 217.5 120.0 338.5 114.0 26.0 152.0 500.0	30.0 41.5 488.0 218.0 127.5 376.0 124.0 23.5 153.0 504.0	30.0 31.8 481.0 220.8 122.3 377.5 116.8 25.8 160.3 508.5	30.0 31.0 491.0 221.5 119.0 379.0 122.0 27.0 156.0 497.0

Group UV

		Seco	nds		
	40	50	60	70	80
Subjects					
67-46 67-49 67-69 67-73 67-84 67-95 67-97 67-120 67-121 67-133	152.0 44.5 269.5 403.0 157.5 53.5 149.3 348.0 264.0 67.0	149.3 53.0 276.0 397.5 146.8 48.5 147.8 334.5 226.0 69.8	170.0 53.0 310.0 405.0 157.5 41.0 149.5 342.0 241.0 62.0	159.5 54.8 276.0 404.0 149.3 62.8 147.8 360.0 245.0 73.5	156.0 56.5 283.0 405.0 156.0 55.5 154.5 331.0 238.0 73.5

sponses of the fixed intertrial interval groups.

A detailed examination of the responses on successive trials during habituation disclosed that several Ss failed to show a decrease in response amplitude, with some Ss actually showing an increase in response amplitude. Under the conditions employed in this study, i.e., repeated applications of a stimulus (the CS) which elicited a response, the expected result would be a decrease in response amplitude (Harris, 1943; Thompson and Spencer, 1966). Therefore, Ss showing an increased response amplitude did not follow the normal pattern. It could also be argued that any S which did not show a decrease in response amplitude across trials also did not follow the normal pattern. However, in the case of the present study it must be remembered that there was a series of ten trials immediately preceding the habituation series which were not counted. It is possible that the responses of some Ss habituated completely during these ten uncounted trials. Therefore, simple failure to show a decrease in response amplitude during the remaining habituation trials cannot be taken as evidence that habituation did not occur.

In order to check on the possible effects of including <u>Ss</u> showing an increase in response amplitude during habituation on the analysis of acquisition and extinction, their data were eliminated and further tests were made. An increase in response amplitude during habituation was arbitrarily defined as an increase of at least 10 percent in the medians of the last two 5-trial blocks, as compared to the median of the first 5-trial block of the habituation series. This eliminated 3 <u>Ss</u> (67-60, 67-124, and 67-134) from group UF, 4 <u>Ss</u> (66-67, 67-78, 67-115, and 67-122) from group CF, 1 <u>S</u> (67-71) from group CV and no <u>S</u> from

group UV (Table 4). The results for the remaining Ss are given in Figure 7. A Kolmogorov-Smirnov two-sample test indicated a significant difference between the CV and UV groups in both acquisition (p \leq .01) and in extinction (p \leq .01) and a significant difference (p \leq .05) between broups CF and UF in acquisition but not in extinction. Response amplitude is greater in UF than in CF.

An example of another response pattern which appeared is given in Figure 8, for a \underline{S} in the UF group. With the introduction of the UCS the response amplitude abruptly shifted, and by the end of the acquisition trials the response amplitude was at a much higher level than at the end of the habituation trials. During the extinction trials the response amplitude remained at this high level. Several \underline{S} s showed an increased response amplitude during the acquisition series which persisted throughout the extinction series. This could be considered an example of high resistance to extinction. Another possibility is that it reflected a physiological deterioration of the \underline{S} (for example, acid-base changes), which was unconnected with a specific pattern of stimulus presentation and which ultimately resulted in an increase in response amplitude.

Thompson and Spencer (1966) demonstrated that sensitization reflects a process separate from habituation and cannot be considered merely as disruption of habituation. Therefore, an increase in response amplitude during the acquisition series in this study could indicate conditioning or sensitization or the deterioration of the preparation. Increases relfecting conditioning or sensitization would be expected to decrease during the extinction phase. The increase resulting from deterioration of the preparation would not necessarily decrease during the extinction séries and might well become more pronounced. Assuming

Table 4. Subjects remaining after elimination of Ss showing an increase in median response amplitude during the CS alone habituation series.

Group UF	Group CF
67-41	 66-64
67-55	67-39
67-76	67-66
67-81	67-89
67-87	67-107
67-108	67-136
67-118	(0)

Group UV	2.93	Group CV
67-46	2	67-40
67-49		67-43
67-69		67-57
67-73		67-86
67-84		67-91
67-95		67-104
67-97		67-113
67-120		67-123
67-121		67-135
67-133		

Figure 7. The median percent change in the response to the CS (grams tension) after elimination of those Ss which failed to show habituation during the CS alone (habituation) trials. For each group, the median response of the final 5-trial block of the CS alone (habituation) seris = 100 percent (control level). The median response amplitude of successive 3-trial blocks in acquisition and extinction are presented as a percentage of the control level. Group UF, N = 6; Group CF, N = 7; Group UV, N = 10; Group CV, N = 9.

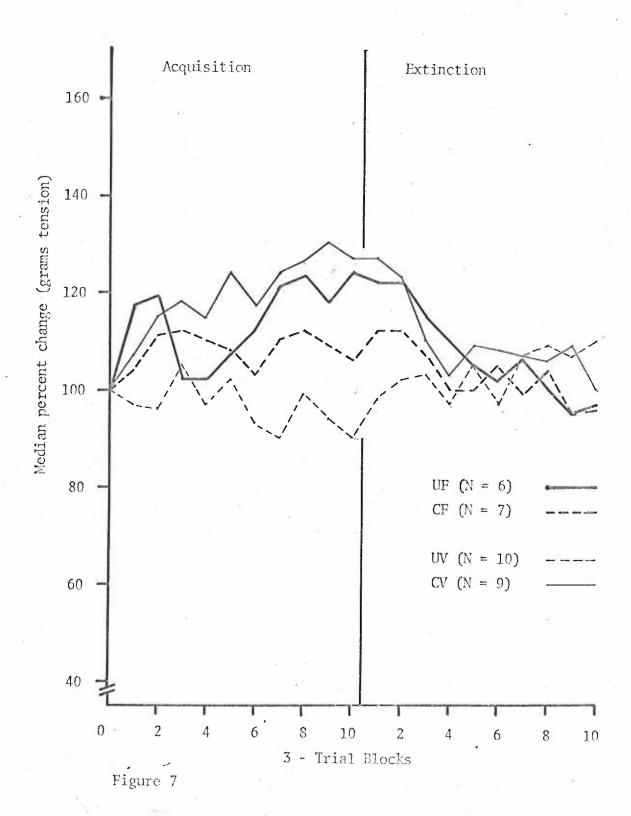


Figure 8. Amplitude of the response to the CS (grams tension) for S 67-108 from Group UF, for each trial of CS alone (habituation), acquisition, and extinction.

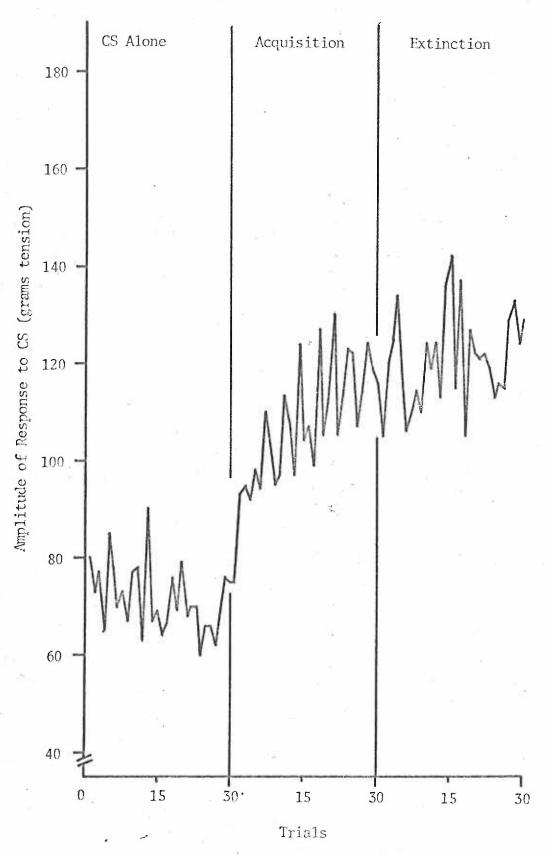


Figure 8

that the failure of some \underline{S} s to show decrease in response amplitude during extinction reflected physiological deterioration and not simply an unusually high level of resistance to extinction, \underline{S} s in which the median response during the last two blocks of 3 trials in the extinction series was at least 15 percent greater than the median response during the last 5 habituation trials were eliminated from the list of \underline{S} s which showed habituation. This removed 2 \underline{S} s (67-76 and 67-108) from group UF, 1 \underline{S} (67-89) from group CF, 3 \underline{S} s (67-43, 67-91, and 67-113) from group CV and 4 \underline{S} s (67-69, 67-73, 67-95, and 67-120) from group UV. The remaining \underline{S} s in each group are shown in Table 5. The data of these \underline{S} s are given in Figure 9. A Kolmogorov-Smirnov two-sample test indicated a significant difference between a) the fixed interval groups, CF and UF, in acquisition (p \underline{C} s.01) but not in extinction, and b) the variable interval groups, CV and UV, during acquisition (p \underline{C} s.01) and extinction (p \underline{C} s.01). Response amplitude is greater in UF than in CF.

Heart-rate data can be presented for only one \underline{S} , 67-142. Instability of the record of the second control \underline{S} made it impossible to obtain accurate heart-rate data for that \underline{S} The responses of the tibialis anterior muscle to the CS in \underline{S} 67-142 are given in Figure 10. The number of beats was tabulated for the 2.5 second period immediately preceding and following the onset of the CS (Table 6). Wilcoxin matched-pairs signed-ranks tests indicated no significant differences between these two heart-rate measures during habituation, acquisition, or extinction.

The muscle responses of \underline{S} 67-142 on each trial were tested for randomness by a one-sample runs test in habituation, acquisition, and extinction. The results were significant (p \leq .05) only for habituation

Table 5. Subjects remaining after elimination of Ss showing an increase in median response amplitude during the CS alone habituation series or an increased median response amplitude at the end of the extinction series.

Group UF			Group CF
67-41			66-64
67-55			67-39
67-81			67-66
67-87	7	,	67-107
67-118			67-136

Group UV			Group CV
67 16		3 3 3	67.40
67-46	, K		67-40
67-49			67-57
67-84			67-86
67-97			67-104
67-121			67-123
67-133			67-135

Figure 9. The median, percent change in the response to the CS (grams tension) after elimination of those Ss which failed to show habituation during the CS alone (habituation) trials and those Ss which had a response level at least 15 percent above control on the last two 3-trial blocks of extinction trials. For each group, the median response of the final 5-trial block of the CS alone (habituation) series = 100 percent (control level). The median response amplitude of successive 3-trial blocks in acquisition and extinction are presented as a percentage of the control level. Group UF, N = 5; Group CF, N = 5; Group UV, N = 6; Group CV, N = 6.

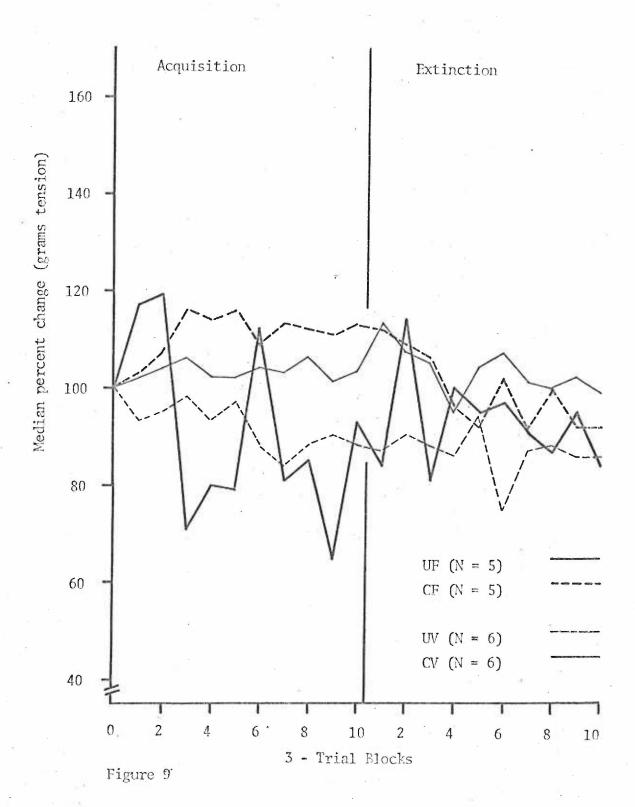


Figure 10. Amplitude of the response to the CS (grams tension) for S 67-142 for each trial of the CS alone (habituation), acquisition, and extinction. The heart rate recorded from this S 2.5 sec. before and 2.5 sec. after the CS are presented in Table 10.

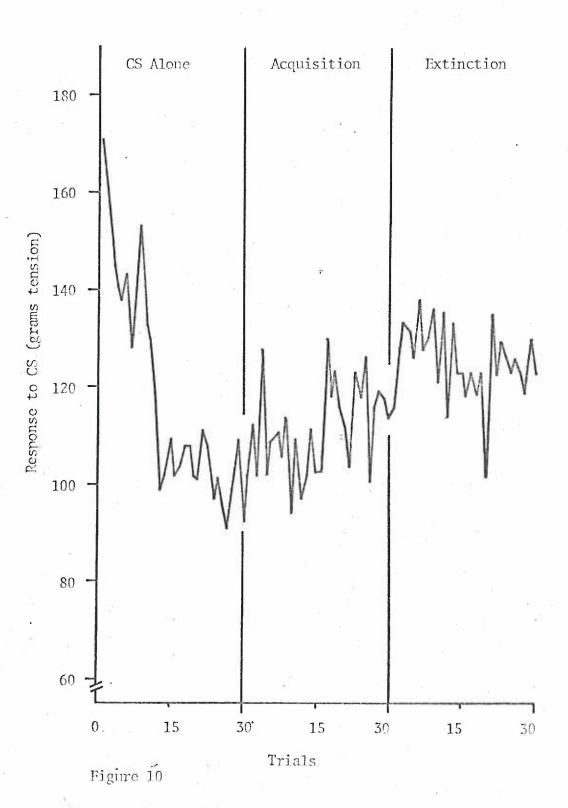


Table 16. Number of heart beats in 2.5 second period preceding CS onset and 2.5 second period following CS onset.

	CS Alon	<u>e</u> .	Acquisiti	on	Extino	tion
Trial	Pre-CS 2.5 sec	Post-CS 2.5 sec		Post-CS 2.5 sec	Pre-CS 2.5 sec	Post-CS 2.5 sec
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29	7 8 7 8 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	8 7 8 7 8 8 7 8 8 7 7 7 7 7 7 7 7 7 7 7	7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	7 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
30	7	7	7	7	7	6

trials, indicating that the distribution of scores deviated from randomness in habituation trials.

The occurrence of the CS or UCS did not produce any visible change in the blood pressure of the control \underline{S} . In addition, there was no visible change in blood pressure during the muscular contractions following the CS or UCS. The effect on blood pressure of the muscular response to the CS and UCS was not determined in the left hind leg.

DISCUSSION:

The results of the analysis of variance, using data from all <u>Ss</u> in all groups, did not provide support for the presence of spinal conditioning under the conditions of this experiment.

The large inter-S variability in the muscle response of the Ss may be attributed, in part at least, to differences in the size and the strength of the Ss. An attempt was made to control these variables by restricting the Ss in terms of size and weight and by equating the amplitude of the muscle responses before starting the experimental procedures.

When the variable intertrial interval groups (UV and CV) were tested apart from the fixed intertrial interval groups, some evidence of spinal conditioning was obtained. The analysis of the responses of the UV and CV groups indicated that they were not substantially different from the responses that occurred on identical trials under a constant intertrial interval. This would seem to indicate that any effect attributable to the variable intertrial interval procedure cannot be from any one of the particular intervals that were used. It may be that any fixed intertrial interval would produce the same results found in groups UF and CF or that any combination of variable time intervals would produce results similar to those of groups UV and CV. Fitzgerald and Thompson (1967) did find positive results of spinal conditioning. In that study, however, the CS-UCS interval and the intertrial intervals were deliberately varied between Ss, while the intervals used with any individual S were held constant.

Habituation, defined as "response decrement as a result of repeated stimulation" (Harris, 1943), has been repeatedly demonstrated in a variety of animals and with several responses (Engen and Lipsitt, 1965; Harris, 1943; Lethlean, 1965; Marsh, McCarthy, Sheatz and Galambos, 1961). The failure of some Ss in the current experiment to exhibit habituation during the pre-test CS alone trials under conditions similar to those used in previous studies is difficult to explain. Thompson and Spencer (1966) demonstrated habituation of the flexion response of the tibialis anterior muscle in the acute unanesthetized decerebrate cat with spinal transection. However, the intertrial intervals in the present study were quite long as compared to those used by Thompson and Spencer, and it may be that this difference accounts for the failure of some Ss to show habituation.

Another possible explanation is that in those <u>Ss</u> that failed to exhibit habituation there was a general deterioration of physiological functioning. This could also explain the increase in response amplitude during extinction trials which was shown by some <u>Ss</u>. It is, however, entirely possible that, under the conditions employed in this experiment, resistance to extinction is more pronounced than in intact <u>Ss</u> that are classically conditioned. It is also possible that the <u>Ss</u> with severe physiological deterioration are the same ones whose response level returned to control level during extinction trials.

Shurrager and Culler (1940) used extinction of the CR as one of their criteria for conditioning. Using 15 percent above control level as the criterion for failure to extinguish the CR eliminated 4 Ss (66-65, 67-89, 67-107, and 67-115) from group CV.

The first conditioning criterion listed by Shurrager and Culler

(1940) was that the "CR is not present upon the original presentation of the CS." One can argue that, in the present study, the CR was the original response to the CS. The position taken here, however, is that the original response pattern to the CS is a decrease in response amplitude across trials, i.e. habituation, and that a shift to a pattern of an increase in response amplitude across conditioning trials was not the same response. In Figure 9 of the present study, 45 percent of the Ss given the conditioning procedure were eliminated for failing to habituate or failing to extinguish the CR. This approximates the 50 percent Shurrager and Culler (1940) reported as failing to show conditioning. Unfortunately, Shurrager and Culler did not report how many animals failed to meet each conditioning criterion.

The 55 percent of the Ss which showed conditioning did so in 30 trials. This also agrees with Shurrager and Culler's report of conditioning in 30 to 40 trials, a point questioned by Kellogg (1947). It must be remembered that the results of analyses not involving all Ss included in this study should be considered as coming from biased samples and therefore no strong conclusion can be drawn from them.

The large inter-S variability suggests that amplitude of the muscle response employed in this study may be an inappropriate measure to use in a test of spinal conditioning because the factors influencing it cannot be adequately controlled. Therefore, it would seem desirable that another test for spinal conditioning be made, employing a variety of physiological monitors to determine the stability of the preparation and recording responses of spinal motor neurons instead of muscle twitches. In such a preparation it might be possible to analyze the effects of many conditioning factors and, hopefully, to obtain a bet-

ter understanding of what happens in the spinal neurons during classical conditioning.

The unusual pattern of stimulus presentations employed in some of the previous spinal conditioning studies was mentioned earlier in this paper. The present study employed one of the most common and simple CS-UCS patterns in order to avoid the problem of overlapping effects of several discrete stimuli which, as a unit, are termed the CS (Shurrager and Culler, 1940; Kellogg, et al., 1947).

Immobilization of the foreparts of <u>S</u> has been shown by Pinto and Bromiley (1950) to be important in any assessment of changes in responsiveness posterior to the spinal transection. In the present study immobilization of the foreparts was accomplished by a large bilateral lesion of the reticular formation which rendered the <u>S</u> comatose. This does not mean that there were no movements at all anterior to the section, for respiratory movements continued throughout the experimental period. But large movements involving repositioning of the limbs and all movements which could be considered as "struggling" were absent.

The heart-rate results from the control animal in the present study cannot be used as the basis for strong conclusion. They do seem to indicate that there was no direct effect from using electrical stimuli.

SUMMARY AND CONCLUSIONS:

In summary, the data from all four groups in this study, taken together, do not support spinal conditioning. The performance of the fixed interval control group essentially matches that of the fixed interval experimental group. The two groups in which a variable intertrial interval was used do differ in their performance across trials in a manner suggesting conditioning and extinction of the flexion reflex. Of all the Ss given the conditioning procedure, 55 percent (11 Ss) met conditioning criteria used originally by Shurrager and Culler (1940) while 45 percent (9 Ss) did not meet the criteria through failure to habituate or failure to show extinction. Conditioning in these 11 Ss occurred within 30 trials. These results agree with the results of Shurrager and Culler (1940). It is possible that certain factors in the experimental situation relating to the physiological condition of the subject might account for the response patterns of several subjects, and these factors should be tested in any future experimental work on this topic.

It is suggested that perhaps a better test of spinal conditioning would involve analysis of responses from spinal motor neurons in a preparation similar to that used in this study, with physiological monitoring to indicate the physiological stability of the preparation.

APPENDIX

- Table I. Individual response records for subjects in Group UF.
- Table II. Individual response records for subjects in Group CF.
- Table III. Individual response records for subjects in Group UV.
- Table IV. Individual response records for subjects in Group CV.

Table I. Individual response records for subjects in Group UF.

Response amplitude to the CS is in grams tension.

GROUP UF

Triel	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
1234567890 111234567890 1234567890 1234567890	32 53 53 54 55 57 57 57 57 57 57 57 57 57	4143753555555555555555555555555555555555	49.55 49.55 49.50 52.49.00 49.00 52.40.00 52.40

GROUP UF

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
123456769011234567690	21 21 21 21 21 21 21 21 21 21	429218798474934408183780542005 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	327980517.5 327980517.5 323435422912.5 42341667.5 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5.5 5.5

GROUP UF

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
1 2 3 4 5 6 7 8 9 0 1 1 1 2 1 3 1 4 5 6 7 8 9 0 1 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	227 1918 297 201 201 201 201 201 201 201 201 201 201	33333444444444444444444444444444444444	4791 4791 4797 4444 4444 445 445 445 445 445 445 445

GROUP UF

Trial	Response CS Alone	to C3 (grans Acquisition	tension) Extinction
123456789011234567890 111234567890 122222222223	17549.5 175	186 176 161 161 161 161 163 176 163 179 179 179 179 179 179 186 193 193 193 193 193 193 193 193 193 193	223 214.55 206.5 207 214.3 213 213 213 213 214.5 215.5 216.5 216.5 216.5 217 218.5 2

GROUP UF

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
123456789011234567890 111234567890 1234567890	102 70 97 102 97 103 103 103 103 103 103 103 103	107.5 107.5 107.5 107.5 109.5	133 141 129.5 1336 136 136 136 136 136 136 137 137 137 137 137 137 137 137 137 137

GROUP UF

Trial	Response	to CS (grams	tension)
	<u>CS Alona</u>	Acquisition	Extinction
123456789011234567890 111234567890 1234567890	36622569951628522285225699516285222333333333333333333333333333333333	34444444444444444444444444444444444444	471 471 471 471 471 471 471 471 471 471

GROUP UF 67-108

Trial	<u>C</u>	Response 3 Alone	to CS (grams Acquisition	tension) Extinction
12345678901234567890122222222222		77776877767769666677676766666677 555 57776877769666667767676666667776966666777	75.5 76.5 76.5	105 125 125 125 125 125 125 125 126 127 121 121 121 121 121 121 121 121 121

GROUP UF 67-118

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890	5 5 5 5 5 5 5 5 5 5 5 5 5 5	19.55 19.55 19.50	279575.5 5 55 27957007523125322597755 27957523125322597777931587

GROUP UF

Trial Response to CS (grams tension) Extinction 1 117.5 137.5 171.5 2 106 143 157.5 3 149.5 141 168 4 127.5 137.5 166 5 106 137.5 154.5 6 136 144.5 159.5 7 139.5 136 156 8 133 153 143 9 106 157.5 153 10 126 149.5 154.5 11 137.5 143 163 12 120.5 154.5 124 13 144.5 164.5 126 14 139.5 157.5 156 15 147.5 166 153 14 139.5 159.5 150 15 147.5 166 153 17 149.5 159.5 150 18 19				,
11 137.5 143 163 12 120.5 154.5 124 13 144.5 164.5 126 14 139.5 157.5 156 15 147.5 166 153 16 143 149.5 179.5 17 149.5 159.5 153 17 149.5 153 151.5 19 117.5 153 129.5 136 147.5 149.5 149.5 22 131 191.5 193 23 129.5 157.5 131 24 136 149.5 159.5 25 137.5 143 149.5 26 139.5 156 154.5 27 136 149.5 153 28 122.5 184.5 153 136 143 147.5	Trial	Response <u>CS Alone</u>	to CS (grams Acquisition	tension) Extinction
	11 12 13 14 15 16 17 19 21 22 24 25 27 28 29	106 1427. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 139. 106 106 106 106 106 106 106 106 106 106	147 147 147 147 146 157 146 157 147 157 147 157 147 157 157 157 157 157 157 157 157 157 15	15.55 15.664.96 15.563.4666

GROUP UF

٥				
	Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
	123456789011234567890 11234567890 1234567890	275 253.55 253.55 253.6	3416 3416 3416 36 4016 4016 4016 4016 4016 4016 4016 401	464 444 447 464 447 464 447 464 464 464

Table II. Individual response records for subjects in Group CF.

Response amplitude to the CS is in grams tension.

GROUP CF

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890 111234567890 222222223	223 241 223 208 237 220 191 220 193 220 188 193 163 160 175 160 175 160 175 160	175 188 160 200 235 200 210 210 210 2193 214 220 210 2193 210 208 208 208 208 208 208 208 208 208 20	204 200 173 160 160 160 160 152 131 133 145 148 149 153 145 148 149 151 148 151 153 153 153 153 153 153 153 153 153

GROUP CF 66-65

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
1234567890 111234567890 1234567890 22222223	305 305 305 305 305 305 305 305 305 305	235 250 289 3288 3277 344 3317 3317 3317 3317 3317 3317 331	352 258 359 359 370 229 239 239 239 239 239 239 239 239 239

GROUP CF

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890 111234567890 1234567890	143.5 123.5 1539.5 1269.5 1269.5 127.5 128.5 129	89 102 95.5 116 1102 1109 12109 1010 1010 1010 1010 101	102 92 1106 92 106 85 82 102 118 72 109 118 85 97 82 109 118 97 85 97 85 97 87 97 87 97 87 97 87 97 87 97 87 97 87 97 87 97 87 97 97 97 97 97 97 97 97 97 97 97 97 97

GROUP CF

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
1234567890 111234567890 1234567890	117.5 127.5 127.5 127.5 1299990.5 1029990.5 1029990.5 1029990.5 105990.5 105990.5 105990.5 105990.5	65 795. 109988 10998 10998 10998 10998 10998 10998 10998 10998 1099888 1099888 109988 109988 109988 109988 109988 109988 109988 109988 109988 109988 109988 109988 1099888 109988 109988 109988 109988 109988 109988 1099888 109988 109988 109988	104 87 90.5 107.5

GROUP CF

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
123456789011234567890 111234567890 1234567890	3495525885552222255208044188844545 3333333333333333333333333333333	33334444444444344444444444444444444444	4296 4296 4296 4391 4383 4395 4395 4395 4395 4395 4395 4395 439

GROUP CF

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
1234567890 111234567890 1234567890 1234567890	5565466555577664468565574576544 5565495555577664498975817381032.	55 55 55 55 55 55 55 55 55 55 55 55 55	64871392496.36146.089355264013728 6455656555655566666768666666666666666666

GROUP CF

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890 11111111112222222223	183 177.5 183 187.5 183 183 184 186 190 188 190 188 190 191 193 193 193 193 193 193 193 193 193	196.5 191.5 203 201.5 206.5 206.5 211.5 21	213.55 210.55 210.55 210.55 210.55 210.55 210.55 211.55 21

GROUP CF

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
1 2 3 4 5 6 7 8 9 0 1 1 1 2 1 3 1 4 5 6 7 8 9 0 1 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	555 555 555 555 555 555 555 555 555 55	42.55 557 557 557 557 557 557 557 557 557	63.55 63

GROUP CF

<u> Prial</u>	Response <u>CS Alone</u>	to CS (grams Acquisition	tension) Extinction
1234567890 11234567890 1234567890 123222222223	6444533344121.55 64445333344121.55 6444348594121.55 6445348594121.55 644534854510096958.73 6434424344	4416344694774443675555555555555555555555555555555	54949413736426161115 54949413333353434444426443333333333333333333333

GROUP CF

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
12345678901123456789012345678	190 213 243 204 204 204 204 204 204 204 204 204 204	204.5 193 200 200 190 213 200 191.5 198 2191.5 198 2191.5 199 213 200 213 200 213 200 213 200 213 200 213 200 213 200 213 200 213 200 213 200 213 200 213 200 213 200 213 200 200 200 200 200 200 200 200 200 20	193 179.5 174.5 216.5 216.5 216.5 216.5 186.5 179.5 188.1 197.5 188.1 197.5 188.1 196.5 196.5 196.5 197.5 196.5 197.5 197.5
29 30	173 164.5	220 214.5	173 136

Table III. Individual response records for subjects in Group UV.

Response amplitude to the CS is in grams tension.

GROUP UV

•	Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
	123456739011234567890	317936126492856518514848117936837986856518514848117968879868197	204 166.5 1997 1990 1990 1990 1990 1990 1990 1990	156.5 17770903030303030303030303030303030303030

GROUP UV

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890	22 126 129 107 107 107 107 107 107 107 107 107 107	5 5 5 5 5 5 5 5 5 5 5 5 5 5	555 555 5693691 5693691 5555 56936960 5755

GROUP UV

Trial	Response <u>CS Alone</u>	to CS (grams Acquisition	tension) Extinction
12345678901234567890	214 2184 2004 1076 1188 2004 1071 1188 2004 1071 1188 1190 1190 1180 1190 1190 1190 119	204 204 209 209 209 209 209 209 201 201 201 201 201 201 201 201 201 201	222225693293994 2222222222222222222222222222222

GROUP UV

Trial	Response	to CS (grams Acquisition	tension) Extinction
12345678901234567890	3290 3291 3212 3223 3221 3222 3222 3222 3222	565996623999999966623636922659965999689999999999999999999999999	403943334334444444444444444444444444444

GROUP UV

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890 111234567890 1234567890	171 173 173 175 175 175 175 175 175 175 175 175 175	1579.55.55.55.55.55.55.55.55.55.55.55.55.55	13470 1470 1470 15379 15379 15379 15379 15379 15379 15379 15379 1539 1539 1539 1539 1539 1539 1539 153

GROUP UV

6	7	-	0	15
\sim	-		/	-

Trial	 Response CS Alone	to CS (grams Acquisition	tension) Extinction
12345678901234567890	5 5 5 5 5 5 5 5 5 5 5 5 5 5	5 5 5 5 5 5 5 5 5 5 5 5 5 5	5 920946868249754631663855801513 5665533545544556444555466355855855

GROUP UV

Trial	Response CS Alone	to CS (grams	
A. Ja. Ja (24 - 1-	OD ALLOHE	Acquisition	Extinction
1234567890112345678901222222222222222222222222222222222222	1466337.55 5.55 1456337.96 5.5 5.55 1456337.96 9.34.96 9.34.96 9.34.96 9.34.96 9.34.96 9.34.96 9.34.96 9.34.96 9.34.96 9.36 9.36 9.36 9.36 9.36 9.36 9.36 9	1456 1456 1456 1456 1456 1555 1555 1555	154 154 154 154 154 154 154 154
30	161	157.5	139.5

GROUP UV

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
123456789011234567890 111234567890 1234567890	17.55 5 2248 2320 2030 220300 22030 2200000 22000000	216.5 250.5 273.5 276.5 276.5 276.5 276.5 276.5 276.5 277.5	518 518 518 518 518 518 518 518 518 518

GROUP UV

Trial	Response	to CS (grams	tension)
	CS:Alone	Acquisition	Extinction
123456789011234567890 111234567890 1234567890	163 218 218 219 190 190 193 193 193 193 193 193 193 193 193 193	255 180 250 250 250 250 250 250 250 250 250 25	193 193 193 215 241 241 241 241 241 241 241 241 241 241

GROUP UV 67-133

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890123 11111111122223	94 1199 1199 1199 1189 1189 1189 1189 11	97.5 1054 1054 11054 11054 11054 11054 11054 11054 11054 1105 1105	33 843 70 72.6 70 72.6 70 70 70 70 70 70 70 70 70 70 70 70 70
22 23 24 25 26 27 28 29 30	58.5 108.5 105 103 73.5 117 98	65 87 60 73.5 84.5 83 73.5 70	75 64 98 77 68 50 50

Table IV. Individual response records for subjects in Group CV.

Response amplitude to the CS is in grams tension.

GROUP CV

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890123456 11123456789012222222222	80.5 80.5	55 948 55 948 333 602 55 55 55 55 55 55 55 55 55 5	55 55 55 55 55 55 55 55 55 55
25 25 26 27 28 29 20 30	56.5 40.5 55 45 33.5 53	48 48 48 48 48 49 48 49 48	42.5 51 42.5 45 45 45 645 35

GROUP CV

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011234567890 111234567890 1234567890	2694 2694 2694 2694 2694 2694 2694 2694	287 314 37624 37624 37652 477 37652 477 37652 477 37653 37653 37653 37653 37653 37653 37653 37653 37653 37653 37653 37653	392599180005229624902958259333333333333333333333333333333333

GROUP CV 67-57

<u>Trial</u>	Response CS Alone	to CS (grams Acquisition	tension) Extinction
1234567890 111234567890 1234567890 1234567890	180 234 1234 1235 1355 1355 1355 1355 1355 1355 1355	1385.5 104984.5 100115 1	74.5.5.5.5.5.76.60.5.5.5.5.5.5.5.5.5.5.5.5.5.5.5.5.5.5

GROUP CV

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
1234567890 11234567890 11234567890 22224567890	44444444444444444444444444444444444444	546 5778 5778 5778 5778 5777 5777 5778 5777 5778 5777 5778 5777 5771 5771	57000 57700

GROUP CV

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
1234567890 11234567890 1234567890 1234567890	207 231 2524 231 231 231 231 231 231 231 231 231 231	156 180 166 183 190 173 176 177 159 176 163 180 176 183 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 176 180 180 180 180 180 180 180 180	166.5 190.5 181.77.5 183.6 174.5 174.5 174.5 184.5 184.5 184.5 186

GROUP CV

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
1234567890 11234567890 11234567890 12234567890 222222234567890	33333333333333333333333333333333333333	33444435396226268828448744449932596226268882844874444999	44444444444444444444444444444444444444

GROUP CV

Trial	CS Alone	to CS (grams Acquisition	tension) Extinction
1234567890 111234567890 111234567890	55 55 55 55 55 55 55 55 57 57	214434 21443 21443 21443 21443 21443 21443 21443 21444 2154 21443 21	5 5 5 5 5 5 5 5 5 5 5 5 5 5

GROUP CV

Trial	Response <u>CS Alons</u>	to CS (grams Acquisition	tension) Extinction
123456789011234567890 11234567890	124 124 127 124 127 124 124 124 124 124 125 129 124 124 129 124 124 124 124 124 124 124 124 124 124	1379.55 1379.55 1379.76 1379.76 1471.77 147	11111111111111111111111111111111111111

GROUP CV

Trial	Response CS Alone	to CS (grams Acquisition	tension) Extinction
123456789011254567890 111254567890 222222223	150 157.5 150 159.5 159.	126 133 149 154 154 163 163 163 163 163 163 163 163 163 163	140.55 110.55 12

GROUP CV 67-135

Trial	Response	to CS (grams	tension)
	CS Alone	Acquisition	Extinction
123456789011234567890 11111111111122222222222	2932452° 2932452° 2932452° 22541 226431768 227532 221220 22120 221220 221220 221220 221220 221220 221220 221220 221220 221220 221220 221220 221220 221220 221220 22120 22120 22120 22120 22120 22120 22120 22120 22120 22120 22120 22120 22120 22120 22120 221	174 174 194 197 198 198 198 195 195 195 195 195 195 195 195 195 195	2314830 2314830 2318931320 23198311893119963 119140

SYMBOLS AND ABBREVIATIONS

CS Conditioned stimulus

UCS Unconditioned stimulus

CR Conditioned response

UCR Unconditioned response

Subject

N Number of subjects in a group

 AP_0 Midpoint of the anterior-posterior plane of the stereotaxic

co-ordinates of the brain

H₀ Midpoint of the anterior-posterior, lateral, and horizontal

planes of the stereotaxic co-ordinates of the brain

 T_{10-12} Tenth to twelfth thoracic vertebrae

cc Cubic centimeter

Kg. Kilogram

mg. Milligram

mm Millimeter

pps Pulse per second

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