

CONDITIONED LEG MOVEMENT IN CAT USING THE STIMULUS  
PARAMETERS OF A NEUROPHYSIOLOGICAL EXPERIMENT

By

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## INTRODUCTION

Pavlov's salivatory conditioning in dogs has been the prototype for classical conditioning experiments. In such conditioning the essential factors are (1) an unconditioned stimulus (US) which reliably elicits an unconditioned response, (2) a conditioned stimulus (CS) which does not produce the same response as the US, and (3) the paired presentation of these two stimuli with the US following the CS; this results in the CS eliciting a response which is some fractional part of the unconditioned response, i.e. a conditioned response.

In considering the nature of the unconditioned response, it is important to realize that even though an unconditioned stimulus such as food elicits a profound and obvious salivation response, which the experimenter chooses to measure, it also elicits a number of other responses of varying magnitude and duration of which either the experimenter is unaware or chooses to ignore. In a like manner, the CS also produces many different types of responses. Some may be overt such as an orienting response, and other internal such as an evoked potential.

Germana (1968) has reviewed a number of studies which have reported "physiological responses" occurring to a CS during the acquisition trials of conditioning. He included such things as heart rate, blood pressure, and pupil dilation in his list of "physiological responses" along with EEG and GSR. He noted that such physiological activity tended to increase in magnitude and peak concomitantly with the first correct conditioned responses that were made in the conditioning procedure. He also noted that as the subject became more adept at performing the conditioned response the other physiological responses diminished.

The role of such physiological changes in the learning process is a controversial subject. The problem is that these changes may be associated with overt responses, but possibly not essential for behavioral learning to occur.

A similar limitation in interpretation is characteristic of neurophysiological conditioning experiments involving data collection from an animal that is paralyzed. In such studies the definition of a conditioned response is usually some unique change in neural activity that occurs as a result of pairing a CS and US. The conditioned neural response is usually expected to extinguish if the CS is presented alone following conditioning and the conditioned response must not develop if the CS and US are randomly associated (Morrell, 1961). In such respects the conditioned neural response is analogous to conditioned behavioral responses.

However, there is some doubt whether behavioral conditioning would occur using stimulus parameters common to many neurophysiological studies. Neurophysiological studies frequently use very weak and discrete stimuli, and the CS and US typically do not overlap in time, i.e., a trace conditioning paradigm. Therefore, in some cases it is undetermined if the conditioning seen at the neurological level would be reflected at the behavioral level. This was true for a study reported by O'Brien and Fox (1968).

While recording from cells in the somatomotor cortex of cat during the presentation of a light flash CS and a paw shock US they found that individual cortical neurons change their response rates in a unique way during conditioning. Their cats were immobilized with D-tubo-curare or Flaxedil, thus precluding a skeletal response. With the stimulus parameters used by O'Brien and Fox, the only conditioned behavior that one could predict, a priori, would be a leg movement response. However, their stimulus



parameters were more typical of neurophysiological research than of those used in behavioral studies. Inasmuch as their stimulus intensities were low (a light flash CS paired with a US shock of 5 volts or less), and a trace conditioning paradigm was used, even the development of a conditioned leg movement was questionable.

The fact that the development of an overt response was questionable using O'Brien and Fox's stimulus characteristics plus the literature implicating many physiological responses during conditioning raised two questions: (1) Could a leg movement response be conditioned using stimulus parameters comparable to those of O'Brien and Fox (1968)? and (2) Given that an overt response developed using such conditioning procedures, would the changes in cell response rates reported by O'Brien and Fox be more closely correlated with the development of the leg response or with other physiological responses as outlined by Germana (1968)? This thesis is the report of a study attempting to answer these questions.

#### Conditioning of Neuro-electrical Activity

For some time it has been observed that electrical potentials originating in neural tissue change in predictable sequences of altering forms and latencies when stimuli are presented in a conditioning paradigm. Furthermore, these changes frequently parallel observed or expected learning behavior of the subject. This has been true for electrical potentials recorded through gross surface electrodes (Durup & Fessard, 1935; Jasper & Shagass, 1941a, 1941b; Cruikshank, 1937; Livanov & Poliakov, 1945), through macrodepth electrodes (Morrell & Jasper, 1956; Chow, Dement & John, 1957; Morrell, Naquet & Gastaut, 1957; Hoshii & Hockaday, 1958), and through microelectrodes (Morrell, 1960; O'Brien & Fox, 1968). A great many different types of conditioning have been employed in these and other studies.

For instance, Durup and Fessard (1935), Jasper and Shagass (1941a, 1941b), and Cruikshank (1937) utilized a classical conditioning paradigm as did Morrell and Jasper (1956) and Morrell et al. (1957). Also employing classical conditioning procedures while monitoring single cell activity have been Bures and Buresova (1965b, 1967), Gerbrandt, Skrebitsky, Buresova and Bures (1968) Kamikowa, McIllwain, and Adey (1964), and O'Brien and Fox (1969). These studies used either cat, rabbit, monkey, or human subjects, and the data were interpreted in terms of possible relevance to conditioned behavioral responses. Other workers have used *Aplysia* because its relatively less complex nervous system can serve as a model for exploring the various plastic capacities of neural tissues and systems (Kandel & Tauc, 1964, 1965a, 1965b). In each case, however, the implicated significance of these studies is an attempt to describe and identify the mechanisms subserving the production of a conditioned response of an awake mobile organism.

Durup and Fessard (1935) were the first to report conditioning of electrocortical potentials. Their discovery was that blocking of alpha activity occurred preceding a light stimulus in subjects who could hear the shutter movement of a camera that was being used to photograph the EEG data before and after the onset of the light stimulus. Typically alpha blocking is a phenomenon which always follows a light stimulus onset, a response analogous to a behavioral UCR. The shifting of the EEG desynchronization in time such that it preceded the light stimulus was a phenomenon directly comparable to Pavlov's conditioned salivation response in dogs. It wasn't long before Durup and Fessard's (1935) observations were verified in other laboratories (Jasper & Shagass, 1941a, 1941b).

More recently, Morrell and his associates have studied the development of electrocortical conditioned responses. In a study using normal animals, Morrell et al. (1957) paired an auditory CS with a flicker light US. Cats and rabbits were suspended in a hammock during presentation of the conditioning trials, but were not required to make an overt unconditioned or conditioned response. The development of an evoked potential wave the same frequency as that of the US light flicker frequency in the area of the occipital lobes during the CS-US interval was ruled a conditioned response. However, there were other evoked potential changes in other cortical regions that were characteristically associated with the development of the CR. For instance, after a "relatively long period of trials (17-60) the onset of tone induced a clear cortical evoked potential", a "generalized response", which appeared first in all cortical regions simultaneously, but which gave way with further trials to the more discrete and unique response localized at the occipital leads. Although Morrell et al. (1957) describe in detail the stage of CR development, they neglected specifics on the total number of trials to establish these various stages.

Morrell reported, in 1960 at the Moscow Colloquium on Electroencephalography of Higher Nervous Activity, that his research with Jasper (Morrell & Jasper, 1956) had revealed development of the conditioned response first in surface layers of the cortex and later in deeper layers. Their procedure in this study was to produce an epileptogenic lesion in the occipital regions of the cortex which would facilitate after-discharges (which served as the unconditioned response) to a flickering light. The flickering light served as the US and a tone as the CS. The CS was presented for 2-3 sec. The US was then turned on and both stimuli continued together for 3 or 4 sec.

It is impossible to predict at which "stage" of development of the electrocortically evoked CR a behavioral event might have occurred which could have been identified as a CR. Likewise, the total number of trials required to establish such a conditioned response was not reported. However, there was a pattern in the development of the evoked responses that in each case was time locked to the sequence of conditioning trials.

By summarizing both of Morrell's reports we find that (1) during early stages of conditioning neural events there is a generalized state of activation that is observed in all areas of the cortex, and in the projection area of the US the activity seems to be found more in the surface layers of the cortex than in deeper levels, (2) with repeated presentation of the conditioning trials the generalized state of activity diminishes leaving a discrete response found only in the US projection area. The activity associated with this discrete response is located in the deeper layers of the cortex.

In the foregoing studies, the subjects were not required to learn an overt response. However, there has been considerable research done on recording neural electrical activity during acquisition of a learned behavioral response and these experiments provide additional information on neural activity and learning.

#### Neuro-electrical Activity During Conditioning of an Overt Response

As was pointed out in the previous section, conditioning of neural events exclusive of behavioral conditioning showed first a generalized activation of all cortical areas. This generalized activation would then diminish in a similar fashion to those physiological responses reported by Germana (1968) when the CR became stabilized within the region of the US projection area. The above studies by Morrell et al. (1957), Morrell

(1960), and Durup and Fessard (1935) were only concerned with neural activity in sensory projection areas. The field now to be reviewed, that of recording from neural tissue during the acquisition of an overt CR, provides a description of motor, sensory-motor and parietal cortical activities as well. O'Brien and Fox (1968) sampled cells in the post-cruciate area, i.e. a sensory-motor region of the cat's cortex.

In 1958, Rusinov and Rabinovich reviewed Russian research on EEG correlates of learned behavioral responses. They reported that Livanov, in a series of articles between 1947 and 1955, described the electrical potentials monitored from various regions of the cortex during acquisition of a "defensive conditioned reflex". The general experimental procedures in these experiments was a flickering light (3/sec.) CS followed by a US of a peripheral electrical stimulation having a rhythmic pattern the same as the CS. Livanov noted that initially the occipital cortex responded to the CS with evoked potentials having the same frequency as the photic stimulus. As the CR developed these potentials disappeared. In contrast, the motor areas showed no initial response to the flickering light, but such responses became apparent as conditioning progressed. The parietal cortex activity was different from both the striate and motor cortices. It did not reflect the CS frequency until a number of conditioning trials had been given. Its response was transitory, disappearing as the motor cortex began to respond to the CS.

In the words of Rusinov and Rabinovich (1968), "At the beginning of the formation of the CR the changes in electrical activity are clearly expressed both in the centers (projection areas) of the CS and in the centers of the UCS. As the development of the CR and its fixation advance, the centers of the CS are inhibited while the centers of the UCS (in this case

the sensory-motor region of the rabbits cerebral cortex) react to the presence of the CS more and more intensely".

Dumenko in 1955 while working in Livanov's laboratory, paired a complex CS of both sound and light with an electric shock to the skin of a rabbit. She found that initially there was an increase in activity in both the auditory and optic areas with no activity response occurring in the motor areas. Later on, the increase in activity of the cortical centers of the auditory and the skin disappeared and by the time the conditioned motor reflex appeared the cortical reactivity to the CS was limited to the optic and motor areas.

Collaborative evidence for Morrell's (1960) observations of conditioning in shallow cortical layers followed by CRs in the deeper levels of the cortex was reported by Knipst (1955). Rusinov and Rabinovich claim she demonstrated that the rhythm of the stimulus is first reproduced in the upper layers of the cortex and then in the lower. By the time the conditioned motor reflex appears, the oscillations of the electrical activity of the upper and deeper levels of the cortex become synchronous.

Beck et al. (1958) conducted an experiment that in many ways was similar to that of O'Brien and Fox (1968) with the following exceptions: Their cats were awake and capable of making a leg flexion response and they recorded EEG from permanently fixated surface electrodes. In addition a 2 sec. tone overlapped the 20-45v US shock to the toes. The subjects were given daily training sessions of 25-50 conditioning trials against the massed trials given in O'Brien and Fox's (1968) acute preparation experiment.

In spite of these differences, Beck et al.'s (1958) experiment is perhaps the most similar study to compare to O'Brien and Fox's (1968) research. They found that a conditioned, low-voltage-fast response appeared in the post-cruciate areas as early as 17 trials, with a respiratory and leg flexion CR following at trials 199 and 326 respectively. They continued to condition their cats until 80% of the CSs elicited a conditioned response. It took the EEG from 83 to 144 trials to reach such a criterion whereas the behavioral CR did not reach that level of performance until after 167 to 380 trials.

From the work of Beck et al. (1958) the greatest detail has been reported correlating the development of a neural CR and a behavioral CR across trials. The neural CR precedes any behavioral indication of conditioning and this can be as large a difference as 180 trials.

#### Aim of the Present Research

As pointed out in the section on neuro-electrical conditioning, the activity in many parts of the brain changes systematically with sequential conditioning trials. In this respect then it was not surprising that O'Brien and Fox (1968) found that even individual cells show patterns of response changes during conditioning.

However, the literature on neural activity concomitant with the acquisition of a conditioned behavioral response indicates that the activity changes of different brain areas vary temporally with respect to the appearance of the conditioned behavioral response and to increasing levels of proficiency of that response. Specifically, the projection area of the CS, then the parietal areas, and lastly the motor and somatomotor areas respond to the presence of the CS as conditioning trials are conducted. The behavioral response does not appear until the CS evokes some response

in the somatomotor and motor areas. Further, as the conditioning progresses there is a synchronizing of activity between cortical layers in the motor cortex.

In the O'Brien and Fox (1968) study the activity of neurons in the somatomotor area of the cat cortex were observed to change their response frequencies during the paired presentation of a light flash and a weak paw shock. The aim of this study was to determine if a conditioned behavioral response would also develop using similar stimulus parameters when the subject was restrained with a mechanical device rather than by drug injection. Providing that a leg movement response could be developed, its quality and developmental characteristics would then be compared to those of the cortical neurons observed by O'Brien and Fox (1968).



## METHODS

### Subjects

Six fully grown male cats served as subjects and each was submitted to the same experimental procedures. The animals were numbered 1, 2, 3, 4, 5, and 6 for identification purposes. They were housed in individual cages and maintained on an ad lib eating and drinking schedule.

### Apparatus and Preparation

A special restraining device was built which held the subject in a natural reclining position with the front limbs extended and head held erect. The subject rested on a hard aluminum platform cut to the shape of the resting body parts. The aluminum platform was bolted to a rack made of aluminum rods and the platform's position, relative to the strain gauge for measuring leg movement, could be adjusted. Attached to the platform were splints made of orthoplast plastic molded to fit around the body, limbs, and neck of the animal. These splints were flexible enough to allow bending as the cat was placed in them. Then adhesive tape was wrapped around them fixing them solidly to the aluminum platform. Functionally the device served as a full-body cast which could be adjusted to varying sizes of cats.

Before being placed in the restrainer a length of 3 inch diameter orthopedic-stockenette was pulled over the subject's body. Holes were made in the stockenette appropriate for allowing extension of the limbs. In each case it was found that the snug fitting stockenette had a quieting effect on the animal which was beneficial when it came to placing it in the restrainer.

The subject was then placed on the aluminum platform and the splints were placed over the limbs and body and wrapped with adhesive tape. Because the position assumed by the subject was a natural one, the experimenter found no difficulty in restraining the animal without assistance, and without administering tranquilizing or anesthetizing drugs. The subject was allowed from 1/2 hour to 1 1/2 hours for adaptation in the restrainer before stimulus presentation commenced. During the period of restrainer acclimation and stimulus presentation the subject was in a soundproof, electrically shielded room.

#### Stimulus Instrumentation and Presentation

Two devices initiated timed events. The intertrial interval, with a mean of 15 sec. and a range of  $\pm 6$  sec., was programmed on a moving film with holes punched in it at appropriate distances to allow closure of a microswitch and passage of a 12v dc pulse at random intervals around the mean. This pulse marked time zero (T<sub>0</sub>) for all other timed events.

The second instrument for timing was a Devices Digitimer with accuracy within 0.1 msec. The Devices Digitimer plus complimentary pulse generators by Devices was used to deliver accuating pulses to the Grass PS2 photic stimulator for generation of the CS light flash and to a Devices Mark IV stimulator for generation of the US electrical stimulus.

The CS was held constant through all procedures and was a single light flash of a few msec. duration and an intensity setting of 8 on the Grass Photic Stimulator.

There were two levels of US intensity. The lower intensity (Low US) was 20% of 90v as read from the Devices Mark IV Stimulator dial. The higher intensity (High US) varied across subjects and its level was determined by

increasing the voltage until the animal showed obvious behavioral signs of feeling pain during its presentation. The duration of Low US and High US were the same; 5 pulses of 0.2 - 0.5 msec. duration at a frequency of 250/sec. The US shocks were delivered to the left hind paw of the cat through subcutaneously placed hypodermic needles.

### Procedure

Initially, there was some question as to whether a behavioral conditioned response would develop using the low voltage US. It was reasoned, however, that if the subject was exposed to a conditioning procedure using the low intensity US, it should facilitate the development of a CR during a subsequent conditioning procedure even if no behavioral response resulted during the former conditioning. The experimental design reflects this reasoning and is represented schematically as follows:

<u>Group</u>	<u>Procedural Sequence</u>		
I	Low US	High US	Pseudoconditioning
II	Pseudoconditioning	High US	Low US

Since, a priori, the development of a CR to the Low US intensity was questionable, this design would allow a comparison of the conditioned response to a High US under two conditions: (1) the development of the subject's CR to a High US following previous conditioning to a Low US; and (2) the CR developed to a High US following pseudoconditioning with equal exposure to the CS and High US stimuli, but in an unpaired manner.

The two conditioning procedures, Low US and High US, were identical except for US intensity. They consisted of 75 presentations of CS alone (Habituation) followed by 225 trials which paired the CS with the US in a

trace conditioning manner with a CS-US interval of 0.5 sec. Seventy-five CS only (Extinction) trials finished the experimental procedure. About 90 minutes of actual time was required for the habituation, pairing and extinction sequence.

The same amount of time was required for the pseudoconditioning sequence with the habituation and extinction trials being the same as above. In the case of the pseudoconditioning, 225 CSs and USs each were presented in random order with a fixed intertrial interval of 7.5 sec. The High US intensity was used for the US level during pseudoconditioning.

#### Data Collection and Analyses

A Grass Model FT10 strain gauge for isotonic forces was used to convert leg movements into electrical potentials. Signals were fed into a Grass low-level DC pre-amplifier model TP1A and then lead to a polygraph DC driver amplifier in the Grass Model 5D polygraph. A sample of the record acquired appears in Figure 8. Sensitivity settings were at 0.1 or 0.5 mv/cm.

The polygraph record was scored by counting all pen deflections greater than 1 mm. as a response. If the deflections occurred during the CS-US interval, it was labeled CR. A time equal to the CS-US interval was selected during the intertrial interval to sample spontaneous or base line responding. If during that period a response occurred, it was labeled R<sup>1</sup>. The CRs and R were totalled for each 25 trial block and the total Rs were subtracted from the total CRs. This difference score was then divided by 25 and multiplied by 100 to give a percent value. The formula for calculating

<sup>1</sup>For a more thorough description of the scoring of base line activity along with the conditioned responses see Appendix B, Figure 7.

percent of conditioning was:

$$\frac{CR-R}{25} \times 100 = \text{percent conditioning}$$

The percent CR score is used as the ordinate value for many of the graphs in the Results section. The statistical calculations were based on a score measured as the difference between the total number of CRs and base line responding for habituation and conditioning trials. The difference score for conditioning was adjusted to equate it with habituation by dividing it by 3 (there were 3 times as many conditioning trials as habituation). Finally, the difference score for habituation was subtracted from the adjusted difference score for conditioning for each subject, and these differences became the values used in a single-factor with repeated measures analyses of variance (Table 2). The analyses compared the difference between habituation and conditioning trials for three procedures or across three levels: conditioning with Low US, conditioning with High US, and pseudoconditioning with High US.

## RESULTS

### Mean Response Curves

When the percent CR scores are pooled for all subjects according to the US intensity used in conditioning, the response curves found in Figure 1 are generated. Both a high and low US intensity appears to produce a conditioning effect. This is demonstrated by the increased level of the mean percent CR during stimulus pairing above the habituated level. This did not occur during the pseudoconditioning phase of the experiment. When the CS and US were randomly associated, as in the pseudoconditioning procedure, the percent CR fluctuated around the zero percent CR level.

The highest level of mean percent CR realized under low intensity US conditioning was 12% and was reached during the 75-100 trial sequence. From trials 125 to 175 of CS-US pairing the level of conditioning was maintained between the 10 and 8 mean percent CR level. The habituated level during the last 25 trials of habituation was a mean percent CR value of 2. The mean percent CR value was thus six times larger as a result of conditioning. The first 25 trials of extinction showed a mean percent CR level of 30, a level not achieved during the stimulus pairing procedure. This high mean percent CR level was not maintained and by the 25-50 extinction trial sequence its level had dropped to about the pre-conditioning level of habituation.

When the subjects were exposed to conditioning using a high level of US intensity, an increase in mean percent CR during the paired CS-US trials resulted. The plotted representation of these results is found in the middle section of Figure 1 and is marked by a maximum mean percent CR

level of 22 which occurs during the 25-50 trial sequence. Following this maximum level of mean percent CR, the curve drops to a level just above the habituated level and remains there during virtually the rest of the conditioning procedure.

The pseudoconditioning procedure of presenting the CS and US in a random order failed to produce a higher mean percent CR level than did CS alone. These results are represented by the bottom graph in Figure 1. The habituated level of mean percent CR in this experiment was zero, and the mean percent CR during the random CS-US presentation did not deviate markedly from that value. There were some of the 25 trial sequences which actually produced a negative mean percent CR value. This was possible when there were more responses scored during the intertrial interval control period than were scored during the half second interval just following the CS event.

#### Statistical Analyses

An analyses of variance comparing scores for conditioning with High US, low intensity US, and a pseudoconditioning procedure yielded significant results at the .05 level of confidence (Table 2). Further analyses with a Newman-Keuls for repeated measures identified the source of significance found in the analyses of variance (Table 4). It was shown that the Low US and High US conditioning levels were both significantly different from pseudoconditioning but were not different from one another. Thus, it was shown that both US intensities produced conditioning when the CS and US were paired but the degree of conditioning did not differ with respect to the intensity of the US shock.

#### Orders Effect

An orders effect can be seen when the mean percent CR curves are plotted for previous and no previous conditioning procedures. These curves

are pictured in Figure 2.

Four subjects received low US conditioning followed by High US conditioning. Their mean percent CR curve is the bottom graph in Figure 2. They show a conditioning effect within the first 25 trials of conditioning with a mean percent CR value of 22 for that first 25 trial block. Their mean percent CR increases further during the next 25 trials to a maximum level of 37 percent. All subsequent trial block scores are low and approach the habituated level.

In contrast, two subjects received conditioning with the high intensity US first with low US and pseudoconditioning following. Their mean percent CR curve is the middle graph in Figure 2. These subjects did not develop a substantial mean percent CR value until the 100-125 trial sequence during conditioning and the maximum percent CR value was not reached until the last 25 trial sequence of conditioning.

A comparison of these two curves, part B and C of Figure 2, describes the orders effect. It is that previous conditioning facilitates the rate of development of the conditioned response, but does not increase the magnitude of the CR, as reflected by the percent CR scores.

#### Effects of US Intensity

Parts A and B of Figure 2 depict the response curves under Low and High US conditions for subjects with no previous conditioning experience. The development of the CR under Low US procedures is marked by a gradual increase in mean percent CR across the first 100 trials of conditioning. The curve then dips to a level comparable to the habituated state for 25 trials before increasing to its highest mean percent CR level at 150 trials. The mean percent CR value then decreases and increases a second



time before the end of the 225 conditioning trials. In summary, it can be said that the response curve under the Low US conditions is bimodal in shape with the modal mean percent CR values being 18 and 22 respectively.

Conditioning with a high intensity US produces an overall lower response curve when plotting mean percent CR across trials (Figure 2, part B). The mean percent CR value increases slightly between 25 and 50 trials of conditioning, but it virtually remains at the habituated level for the first 100 trials before increasing to a level comparable to that of the low US curve. Further, that level is not maintained but falls quickly back to a base line level for 75 more trials. The final 25 trial series in conditioning shows a dramatic increase and that level is even preserved for the first 50 extinction trials.

#### Spontaneous Recovery

Figure 3 illustrates a trend towards spontaneous recovery and was generated by summing the difference scores for each of the three 25 trial blocks of habituation for all subjects. Then the data were divided into two groups, those of subjects having no previous conditioning and those who had had some previous conditioning. A student t test of paired observations showed a nonsignificant difference between the mean responses for these two groups of subjects for the first 25 trial block of habituation. In spite of this lack of statistical significance, Figure 3 illustrates a trend in the direction which would be indicative of spontaneous recovery.

#### Low US Conditioning and Individual Response Curves

In Figure 4 the development of the conditioned response is graphed for each subject during low US conditioning and shows that the magnitude of the CR is weak in most animals and is quite variable in all subjects.

Cat number 4 showed the earliest signs of conditioning by surpassing the habituated response level during the 25-50 series of conditioning. In contrast, the latest initial appearance of a CR is at 175 trials for subject 3. Subject 5's response curve has several peaks at 100, 150, and at 225 trials with the 125-150 trial series being the highest.

The first peaks in the response curves are at 75 trials for animals 5 and 6, 100 trials for subjects 1 and 4, 125 trials for subject 2, and 175 trials for subject 3. The Low US curve of Figure 1 is the average of all the curves in Figure 4 and shows a bimodal curve as representing that average. Figures 9 through 14 show the response curves of each subject for the three different procedures; Low US conditioning, High US conditioning, and pseudoconditioning.

Subject number 1 (Figure 9) shows a great degree of variability in its percent CR scores across pseudoconditioning trials with fluctuations ranging from -20 percent CR to above a +20 percent CR value. The response variability is noticeably attenuated in the subsequent High US and Low US curves. No trend toward conditioning exists in the High US data whereas the Low US curve indicates a brief increase in percent CR after 75 conditioning trials, but the effect does not persist.

The percent CR plotted for pseudoconditioning for subject number 2 (Figure 10) never rises above zero. In contrast High US conditioning shows an upward trend in percent CR after 50 conditioning trials and a 30 percent CR is reached at 125 trials. The curve then falls to a base line level for 50 trials before increasing to its maximum level of 45 percent CR during the final 25 trial block. Low US conditioning followed High US conditioning, however, virtually no CR developed during its administration.

In Figure 11 subject 3 is shown as developing a CR late in the Low US conditioning procedure, but High US and Pseudoconditioning developed no CR.

Subject 4 (Figure 12) has the highest percent CR values and shows the best conditioning of all subjects. The CR develops gradually over the first 100 trials of conditioning and a maximum percent CR of 55 is scored for the 125-150 trial block. High US conditioning follows and shows a savings effect. Conditioning occurs quickly, within 50 trials, and reaches a percent CR level of 70%. Again pseudoconditioning produced no development of a conditioning response.

Subject 5 (Figure 13) shows some conditioning to the Low US procedure, but the response is quite variable. Like subject 4 a savings effect is demonstrated by the appearance of a high percent CR in early trials of High US conditioning. Pseudoconditioning evoked no responding above a base line level.

Finally Figure 14 shows substantial conditioning in both the Low and High US situations with the saving effect again being demonstrated.

## DISCUSSION

The results of this experiment put in evidence data confirming that in at least one situation stimulus parameters typical of many neurological experimentations are sufficient to produce a conditioned behavioral response. Specifically under conditions and experimental procedures approximating O'Brien and Fox's (1968) report of conditioned unit activity, conditioned leg movement was observed. Thus, the primary question posed by this thesis has been answered in the affirmative.

Percent CR Magnitude

It was found that all subjects had a relatively low percent CR. The percent CR score is in essence a probability score and deserves further discussion. For each 25 trial block the number of responses during the CS-US intervals was counted along with the number of responses occurring during equally large time segments sampled from the intertrial intervals. The intertrial interval responses were subtracted from the CS-US interval responses to give the number of CRs above the spontaneous level. With the best estimate of the spontaneous level thus removed, the remaining CRs were divided by 25 and multiplied by 100 to yield the percent CR scores. The percent CR score in effect then is the percent of CRs greater than the spontaneous level per 25 trial block.

Subject 4, having the highest percent CR score of all animals, reached the 50% CR level after 100 trials of Low US conditioning. Most of the other subjects developed lower magnitude percent CR scores, and in all cases the level of conditioning appeared to fluctuate across trials.

There are several possible explanations for these low percent CR scores. It could be that under the conditions of a trace conditioning paradigm lower

Levels of conditioning occur. Beck et al. (1958) were able to train their cat subjects to an 80% criterion level, however, they used spaced as opposed to massed trials and in some instances the 80% level of responding was not reached until after the 600th conditioning trial. Also the CS and US were overlapped in their study. Another possible reason for a low level of responding in this study could be the short CS-US interval of 0.5 seconds. In his book, Learning: Processes, Melvin Marx (1969) has listed the optimum interstimulus intervals for various organisms and responses. Typically 0.5 seconds is the optimal interstimulus interval for eyelid and finger withdrawal responses in humans and he even lists 0.5 seconds as the optimal interval for leg flexion in the cat, but this was not with a trace conditioning procedure. In the case of body movement type responses an interstimulus interval of 2 to 3 seconds is more effective. Perhaps a longer CS-US interval would have provided a more reliable response in this study had all other conditions remained unchanged. Still further, the semi-reclined position of the subject in addition to the resistance offered to leg movement by the strain gauge device may have inhibited responding.

The readjusting of the CS-US interval, the US intensity, and converting from a trace to a delayed stimulus pairing procedure would be expected to increase the reliability of a leg movement CR. However, it was not the intent of this study to achieve an optimal procedure to facilitate a behavioral response. Rather, it was its intent to determine if, in fact, any conditioning would develop and be observed at the behavioral level using stimulus parameters somewhat atypical to behavioral conditioning, but common to many neurophysiological experiments of conditioning.

The higher intensity US, which evoked behavior indicating that it was painful or noxious, did not facilitate conditioning. In fact, the B curve

in Figure 2 could be interpreted as demonstrating a suppression of the CR during the conditioning trials. The percent CR increases from trial 25 to 50, but then decreases to the base line level for 50 subsequent trials. Around 125 trials into conditioning it again increases, but only to fall back to the habituated level for 75 trials. The most interesting phenomenon related to this High-US procedure is that at the beginning of extinction, in the absence of the noxious US, the CR reaches its highest level and is sustained over more trials than during any period of the conditioning sequence. A similar phenomenon was observed by Pavlov who postulated that if the US was of a very high intensity that it could inhibit the conditioned response.

#### Response Variability

A universal characteristic of the conditioned response was its extreme variability. Typically the percent CR would increase progressively for the first 75 to 150 trials after which it would oscillate up and down in a seemingly unpredictable pattern. The average response curve for all subjects with no previous conditioning takes on a bimodal form which is indicative of some pattern, but such a pattern is only seen when the data are pooled. The variability of a conditioned response, being present for a series of trials and then disappearing for a time before reappearing, is quite typical of neurological observations of conditioned neural events (Morrell, 1961). It is less frequently reported by behavioral researchers possibly because in most cases it is the acquisition of the response that is of interest to them. Therefore, usually fewer than 225 trials are conducted and reported. The behavior is recorded until it reaches a certain criterion level and then the experiment is terminated. If a similar procedure had been used in this experiment, the CRs would have been recorded until they

reached, for example, 50% CR level. If that had been done only subject 4 would have successfully been conditioned. It was common for the percent CR to increase progressively in all subjects for some given number of trials at the beginning of conditioning and it is felt that the variation in percent CR across trials is mostly a function of the fact that so many trials were massed in a single session.

#### Behavioral vs. Neuronal Response Characteristics

One of the original objectives of this research was to generate a behavioral response curve that could be compared to cellular response curves reported by O'Brien and Fox (1968). Such a comparison can be made for several aspects of the conditioned response: First of all the time course of the development of the CR in both cases might provide further insight into the role of the cortical neurons of the post-cruciate area during the conditioning process. A close correlation between the leg flexion and neuronal developmental pattern might suggest that the function of the cells was associated more with motor movements of the behavioral CR than with other physiological responses referred to by Germana (1967). A low correlation or a large discrepancy between the response curves would be harder to interpret. Two possible explanations could be (1) that the cellular activity was in some way mediating other physiological responses known to be associated with the acquisition of a behavioral CR, or (2) the neural activity could be part of the learning process associating the CS to the US or the CS to the UCR, which must occur for acquisition of a CR. Related to this last possibility is the data of Morrell, et al. (1957) and Beck et al. (1968) wherein it was observed that some neural changes precede behavioral manifestations of conditioning.

The behavioral and cellular CRs can also be compared on the basis of savings and spontaneous recovery, two phenomena known to accompany classical conditioning.

#### Development of the Conditioned Response

Figure 5 compares the behavioral and cellular response curves for both previous and no-previous conditioning. The cellular response curves are taken from the O'Brien and Fox (1968) report which expressed the response magnitude (on the ordinate) in percent CR as has this study.

Under both the no-previous and previous conditioning circumstances there is a striking similarity between the response curves generated from data collected by O'Brien and Fox on the cellular level and those collected in this study at the behavioral level. It is tempting to suggest that the somatomotor neurons sampled by O'Brien and Fox are closely tied functionally to the motor movements of the behavioral CR. Such a conclusion would not be substantiated by Beck et al.'s (1958) results which showed CR development (at an 80% criteria level) in the post-cruciate area as early as 42 trials with 5 out of 7 subjects reaching that particular level of conditioning in less than 100 trials. The corresponding conditioned leg flexion responses in Beck et al.'s (1958) study were not realized until after 170 trials--a lag of some 60 trials on the average. The response curves in Figure 5 have only a 15 trial lag between the maximum cellular percent CR and the maximum behavioral percent CR.

The correlation between conditioned neuron responses and behavioral response development would, therefore, require further study before its meaning could be elucidated. In fact, further study would be necessary to clarify the meaning of the discrepancy between Beck et al.'s (1958) observations and the comparisons made here between O'Brien and Fox's data and the behavioral data of this study.



Somewhat more can be said about the bimodal curve representing the average CR for all subjects (Figure 2, part A). In our laboratory we have proceeded to replicate O'Brien and Fox's (1968) results and have collected some cell data under similar experimental procedures used in their study and in the present experiment. When the response curves of cells having received no previous conditioning are averaged they generate a bimodal curve analogous to that of the behavioral responses reported here. It should be emphasized that the cell response curve is a probability curve and so is the percent CR curve. The cell's response was measured by its frequency of responding and a CR was based on a probability of that frequency changing to a new level immediately following the CS presentation, as a result of a CS-US pairing. Therefore, it appears that the probability of cellular frequency of response changing and the probability of a behavioral CR being executed follows analogous forms of development across trials. No hypothesis can be suggested at this time to account for this unique form of response probability across trials.

#### Savings Effect

It was also noted in the results that previous conditioning affected the development of the CR during subsequent conditioning sessions. This savings effect was such that the percent CR increased more quickly across trials during subsequential conditioning. In fact, the average response curve for subjects having experienced previous conditioning rises to its initial peak by the end of 50 trials into the conditioning session. This was about 50 to 75 trials earlier than the initial peak of the average response curve when no previous conditioning had been effectuated. A similar savings effect was reported by O'Brien and Fox (1968). For an average of seven neurons the response curve for no previous conditioning

peaked at 120 trials of conditioning. When previous conditioning sessions had occurred the response curve peaked initially at 45 trials, a shift of 75 trials in CR development and almost identical to the behavioral results.

### Spontaneous Recovery

Another phenomenon characteristic of conditioned responses in general is spontaneous recovery. The Pavlovian concept is "the return in strength of a conditioned response, whether partial or complete, brought about by lapse of time following its diminution by extinction" (Kimble, 1961). The experimental design for the present experiment allowed for a test of spontaneous recovery. Subjects 3, 4, 5, and 6 all received two sessions of conditioning and extinction before being submitted to pseudoconditioning procedures. The pseudoconditioning procedure was marked by an initial 75 trials of CS alone before the random association of CS and US occurred during the pseudoconditioning trials. By scoring the number of CRs during that initial 75 trials of CS only, it was found that for those subjects having been previously conditioned there was a larger number of CRs when compared to the number of CRs by subjects having no previous conditioning. Although the effect was not statistically significant, the trend was in the direction of spontaneous recovery.

Similarly, O'Brien and Fox (1968) reported a significant spontaneous recovery effect in their cell data. It is a recognized fact from the work of Beck et al. (1958) and others (Rusinov and Robinovich, 1958) that frequently neural activity exhibits prominent effects before it is expressed behaviorally. It may be in this instance that the significant effect that O'Brien and Fox (1968) reported was not sufficient to produce a significant behavioral response.

## SUMMARY AND CONCLUSIONS

Six male cats were submitted to two conditioning procedures; one involved conditioning with an 18v US, and the other a higher US voltage varying from 45v to 81v. During these procedures the subjects were restrained with the aid of an adjustable full-body cast. The CS was a light flash and the US was delivered to the left hindpaw. Conditioned movements of the left hindleg were measured with a strain gauge and compared to (1) leg movements during a pseudoconditioning procedure and, (2) conditioned cell activity as reported by O'Brien and Fox (1968).

The results demonstrated:

(1) That an overt leg movement could be conditioned using the same stimulus parameters as those of a neurophysiological study, O'Brien and Fox, 1968.

(2) A higher intensity US did not produce a more reliable CR. In fact, no difference was observed in percent CR developed between the two levels of US intensity.

(3) An orders effect was manifest by an earlier development of a CR when subjects had received previous conditioning experience.

(4) The time course of development of the conditioned leg movement was found to be very similar to that of conditioned cell activity for both previous and no previous conditioning situations as reported by O'Brien and Fox (1968).

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## APPENDIX A

## The Restraining Device

In the experiments by O'Brien and Fox (1968) the subjects were totally paralyzed with Flaxedil during the presentation of the experimental stimuli. In order to simulate those conditions in this experiment, it became necessary to devise a restrainer that would prohibit all movement save leg activity which would serve as the conditioned response.

A picture of the restraining device used in this experiment is shown in Figure 6. It was constructed in such a way as to hold the subject in a semi-reclining position. This position is identical to that assumed by the subjects of O'Brien and Fox's (1968) experiment and had the added advantage of being a quite natural one for the subjects.

A subject under general anaesthetic served as a model from which measurements and fittings were made. The model subject, weighing 2.7 kg. was placed in a stereotaxic apparatus and positioned in a manner identical to subjects that were used for cellular recording. An outline was made of those portions of its anatomy which rested on the top of the table and a hard aluminum sheet of 1/4" thickness was cut to fit the outline.

Holes 1/8" in diameter were drilled along the sides of the aluminum base in order to affix sections of orthopedic plastic that were used as restraining splints. The splints were attached with 9/16" long 2/56 bolts and wing nuts. The orthopedic plastic carries the brand name Ortho-plast and is available at most orthopedic shops. Its property of becoming pliable when heated rendered it an ideal material to use to make the restraining splints. The Ortho-plast was heated under hot water or under an

ordinary heating pad and could be molded while in its flexible state with bare hands.

Seven different splints were attached to the aluminum base. Two splints held the forelimbs in an extended position. A third splint supported the ventro-anterior portion of the thorax, the ventral aspect of the neck and the lower mandible. A fourth splint extended from behind the ears to the posterior edge of the ribs and completely covered the dorsal side of the neck and thorax. The fifth splint supported the remainder of the torso by wrapping over the back and the left side of the abdomen. The right side of the abdomen rested on the aluminum base. An extended part of the fifth splint also held the thigh of the left hindlimb. The sixth and seventh splints secured the right hindlimb to the aluminum base. All splints were padded with orthopedic moleskin and a 1/2" thick foam rubber pad was cemented to the aluminum base to promote comfort and eliminate pressure points.

Two extension couplings were machined flat on one side, and two holes were drilled and tapped in them to allow them to be attached to the underside of the aluminum base. Flexaframe rods, no. 60090, were passed through the extension couplings and affixed to a rectangular frame made of flexaframe connectors. The restrainer's position was, therefore, adjustable with respect to the rectangular frame which also held the strain gauge used to convert leg movements into electrical potentials.

Placing of the subject into the restrainer was managed by holding the cat by the skin at the back of its neck and laying its body on the aluminum base. The hindquarters were rotated 90° to the left and the head was held erect. This is a natural position for a cat and hence little or no struggling

resulted. While still holding the back of the neck with one hand the forelimb splints were secured around the extended forelimbs and wrapped with adhesive tape. The splint which supported the ventral aspect of the neck and lower mandible had previously been bolted to the aluminum base and thus served as a rest for the subject's head during this procedure. With the subject's forelimbs bound and its chin resting on the ventral splint the dorsal splint was put into place as the experimenter released the initial grip with which he held the back of the subject's neck. The dorsal splint was held tightly in place as it was wrapped with adhesive tape.

At this point the head and forelimbs are encased in the splints and by holding the hindlimbs with one hand the subject cannot escape. The subject has thus been captured and restrained before any unnatural manipulation could signal the need for struggling. The torso is then wrapped with the final body splint and the right hindleg firmly fitted into its splints and taped to the aluminum base. The left hindleg was finally taped to the strain gauge and the subject was ready for the experimental procedures.

It required only 5-10 minutes for this procedure and 11 out of 12 naive cats tried could be placed into the restrainer by an unassisted experimenter. Body weights of cats restrained in this device ranged from 1.8 to 4 kgs.

Struggling would occur occasionally, about every 20 to 30 minutes in most instances, however, subjects resorted to vocal expression of dissatisfaction and annoyance on a more frequent basis.



## APPENDIX B

## The Raw Data and the Scoring of a Response

The subject's left hindleg was taped to a pedal which was connected to a Grass Model FT10 strain gauge by a spring. Both extension and flexion movements could actuate an electrical potential shift through the strain gauge. The strain gauge was connected to a Grass low-level DC pre-amplifier, Model TP1A and a Grass Model 5D ink writing polygraph. The driver amplifier was calibrated and the model TP1A pre-amplifier sensitivity was set at either 0.1 or .05 mv/cm. The sensitivity was adjusted to minimize noise activity, and still allow adequate detection of leg movement. Noise activity was mostly due to leg movement accompanying breathing, purring, and other such physiological and behavioral activities.

Figures 7 and 8 are xerox copies of the original polygraph record for 8 trials taken from subject number 4 during conditioning with the low intensity US. The trial number is marked above the top line and includes 75 habituation trials. Therefore, trial number 167 represents 92 trials of conditioning, and trial 228 represents 153 conditioning trials, etc. The top line in both figures marks the occurrence of the CS and US with the CS-US interval being 0.5 seconds.

The polygraph paper drive was programmed to start 1 second before the onset of each CS and to run for 2 seconds per trial, therefore, the length of the intertrial interval is not accurately represented in the polygraph record and this accounts for the equal distance between trials in Figures 7 and 8.

The method of scoring the data is represented on trial 168 in Figure 7. The CS and US event marks on the top line served to establish the boundaries

of the CS-US interval on the bottom leg movement trace of the polygraph record. A straight line was drawn between the points of intersection of the extended CS and US marks and the leg response trace and served as the base line from which the magnitude of the deflections of the leg movement trace could be measured. A positive deflection equal to or greater than 1 mm was scored as a conditioned response and a CR was placed on the record under that trial.

A control interval approaching 0.5 sec. in length was identified on each trial by the first whole three-line segment preceeding the onset of the CS. A straight line was drawn between the points of intersections of the two lines on the polygraph paper and the leg response trace, and if a leg response caused a deflection during that interval which was equal to or greater than 1 mm it was scored a spontaneous or random response and an R was placed under it.

The number of Rs and CRs were counted for each 25 trial sequence and a CR-R difference score constituted the numbers used in the statistical analyses of variance and the percent CR value used in the various graphs contained in this thesis.

Table 1 Basic data

Subjects	Low US Paired			High US Paired			High US Unpaired Pseudoconditioning			Total
	Hab	Cond	Ext	Hab	Cond	Ext	Hab	Cond	Ext	
1	0	-1.3	0	-3	0.0	4	3	-3.3	1	0.4
2	0	1.3	6	4	9.0	28	0	- .7	0	47.6
3	0	3.3	10	0	.7	0	4	.3	2	20.3
4	3	15.7	8	17	15.0	7	1	-1.7	2	67.0
5	0	10	0	2	5.0	-1	0	- .7	1	7.3
6	-2	6.7	4	1	18.3	3	6	2.0	1	40.0
Total	1	26.7	28	21	48.0	41	14	-4.1	7	182.6

The unsummed values in this table are difference scores, CR-R, with the values under conditioning having been adjusted to compare them with the habituation and extinction values.

Table 2 Data Table for Analyses of Variance

Subject	Low US	High US	Pseudoconditioning	Total
1	-1.3	3.0	-6.3	-4.6
2	1.3	5.0	-0.7	5.6
3	3.3	.7	-3.7	0.3
4	12.7	-2.0	-2.7	8.0
5	1.0	3.0	-0.7	3.3
6	8.7	17.3	-4.0	22.0
<b>Total</b>	<b>25.7</b>	<b>27.0</b>	<b>-18.1</b>	<b>34.60</b>

The number in each cell represents an adjusted conditioning difference score minus the habituation difference score.

Table 3 Summary of Analyses of Variance for Conditioning Low US x Conditioning High US x Pseudoconditioning

Source	SS	df	MS	F
<u>Between Subjects</u>	181.33	5		
<u>Within Subjects</u>	428.85	12		
Treatments	219.68	2	109.84	5.25*
Residual	209.17	10	20.90	

\*p .05 ( $F_{.95} = 4.10, df = 2, 10$ )

Table 4 Newman-Keuls Test for Differences Between Treatments

Treatment		Pseudo-Conditioning	Low US	High US
	Totals	-18.1	25.7	27.0
Pseudoconditioning	-18.1		43.8*	45.1*
Low US	25.7			1.3
High US	27.0			
		$r_2$ 3.15	$r_3$ 3.88	
	$\sqrt{\bar{n} (MS_{res})} \times q . 95 (r, 10)$	35.27	43.5	

$$\sqrt{\bar{n} (MS_{res})} = \sqrt{6(209)} = 11.2$$

Figure 1 The mean percent CR response curves when subjects received low US and high US intensity conditioning and high US intensity pseudoconditioning.

Low US signifies conditioning with a US intensity of 18v. High US signifies conditioning with a US intensity capable of producing pain. The abscissa is divided into three segments: HAB = habituation, 75 trials or three 25 trial blocks of CS alone; CONDITIONING = paired CS and US for the Low US and High US curves and random CS and US presentation for the High US pseudoconditioning curve. There were 225 such pairings or presentations or nine 25 trial blocks. EXT = extinction, 75 trials of CS alone presentations.

Mean percent CR was calculated by formula:

$$\frac{CR - R}{25} \times 100$$

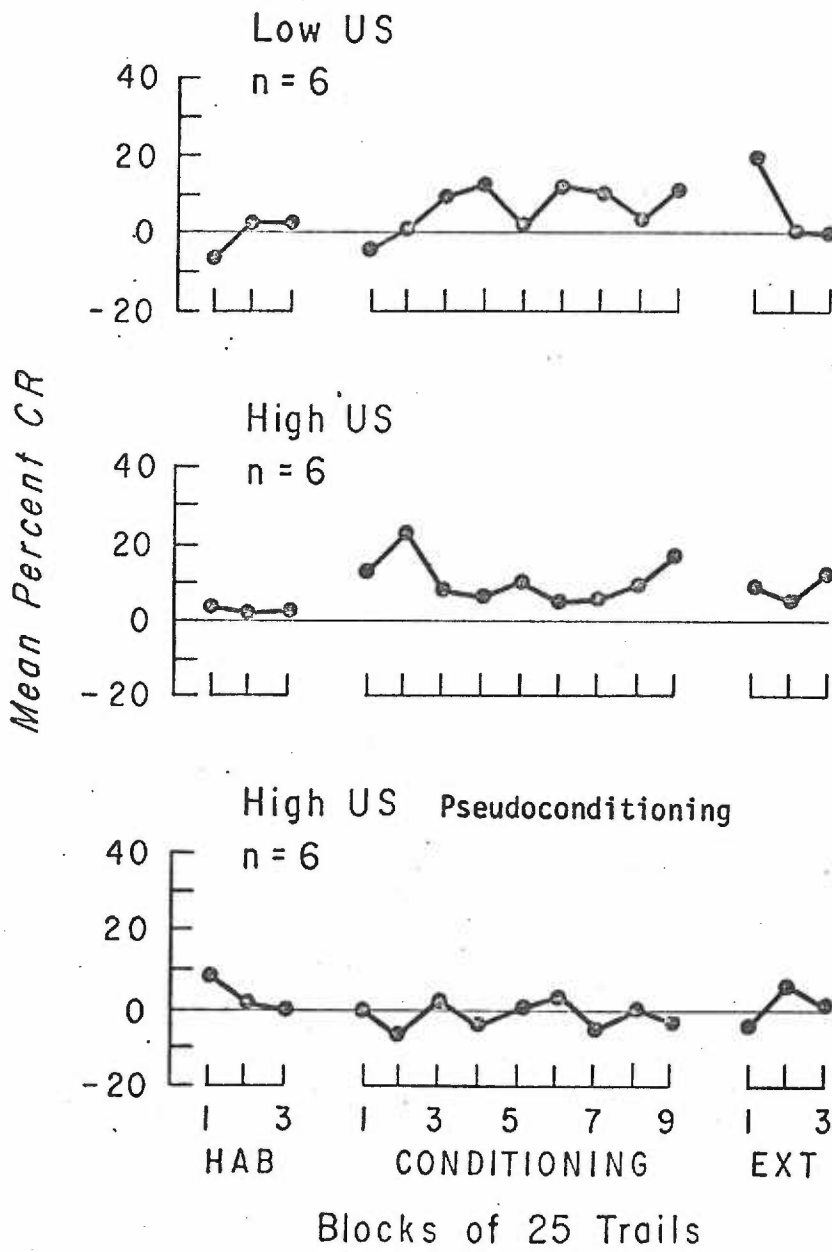




Figure 2      Response curves under the various combinations of Low and High US, and previous vs. no previous conditioning.

The mean percent CR along the ordinate was calculated by dividing the average difference score,  $\frac{CR-R}{N}$ , by 25 and multiplying the quotient by 100. This was done for every 25 trial block and each point on the graphs represent the mean percent CR for a particular 25 trial sequence. An increase in percent CR signifies an increase in the proportion of CRs to Rs, i.e., conditioning. A negative percent CR resulted when there were more random responses (R) than CRs for a given block of trials.

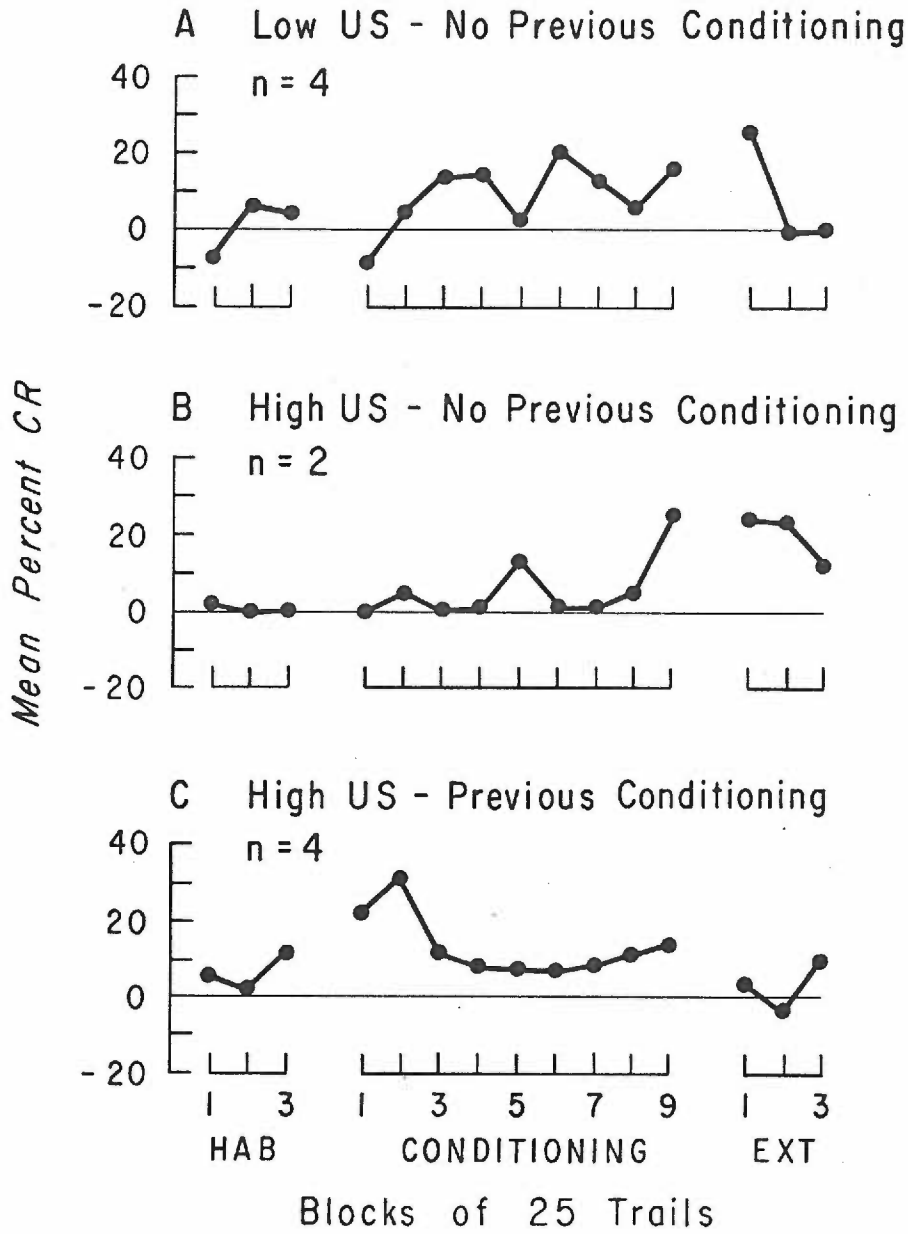


Figure 3 The mean CR scores during habituation for previous and no previous conditioning.

Mean CR, the ordinate value, was calculated by summing the difference scores (CR-R) for each of the three 25 trial blocks of habituation for all subjects. The no previous conditioning values constituted the Low US data for subjects 3, 4, 5, and 6 plus the High US habituation data for subjects 1 and 2. The previous conditioning data is a sum of the habituation trials of subjects 3, 4, 5, and 6 before pseudoconditioning and High US procedures plus Low US habituation for subjects 1 and 2.

The difference in the values of the first 25 trial block is indicative of spontaneous recovery.

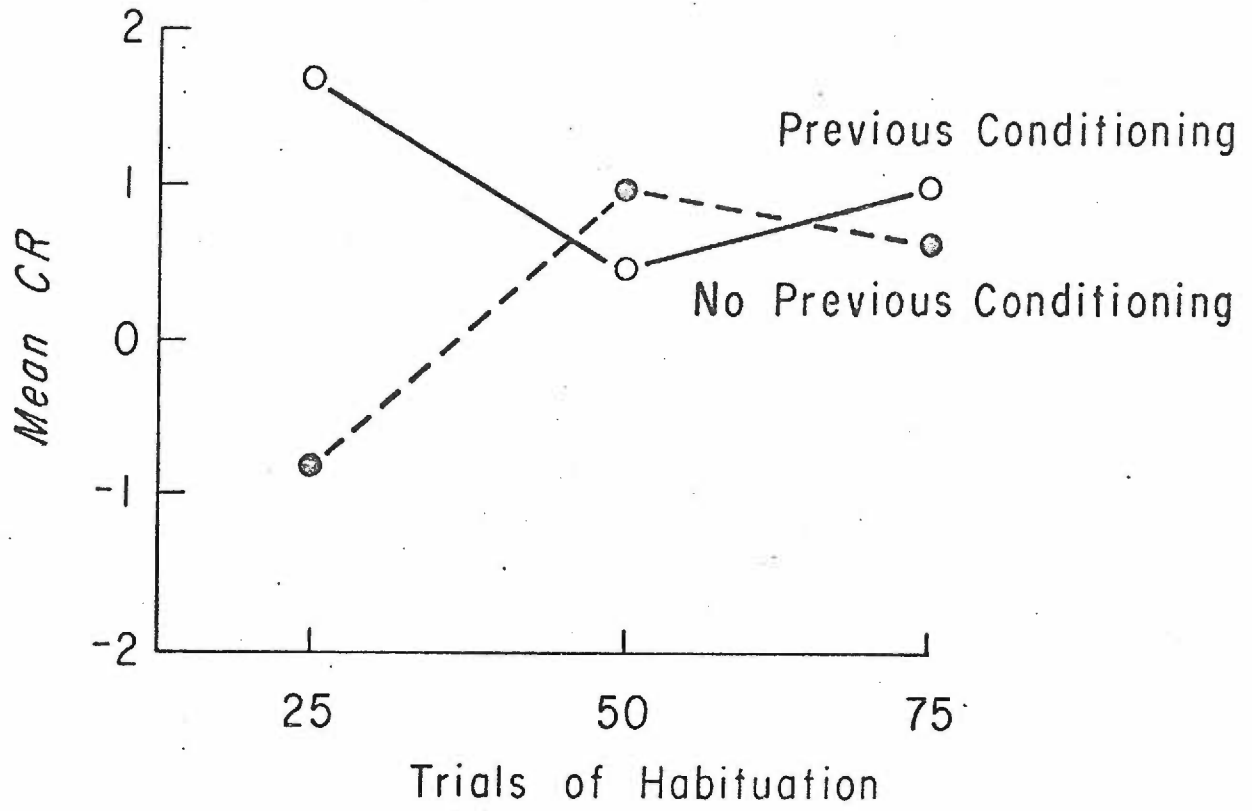
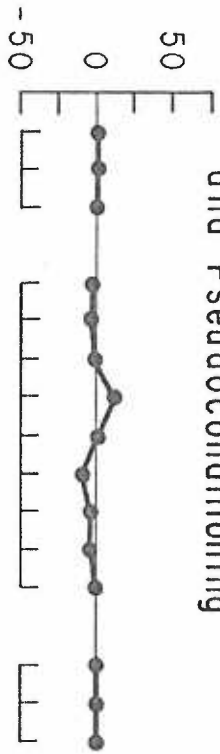


Figure 4 The percent CR response curves for all subjects receiving Low US conditioning.

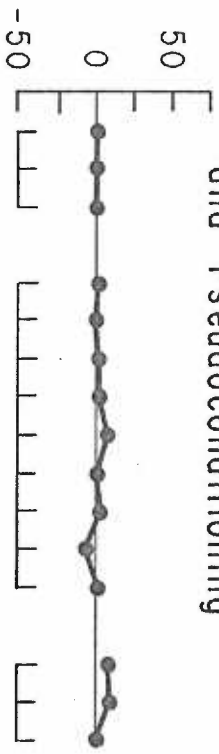
Percent CR,  $\frac{CR-R}{25} \times 100$ , was calculated for each 25 trial block and plotted for each subject. Subjects 1 and 2 had been exposed previously to High US conditioning and pseudoconditioning. For subjects 3-6 the Low US conditioning constituted their initial conditioning experience.

Percent CR

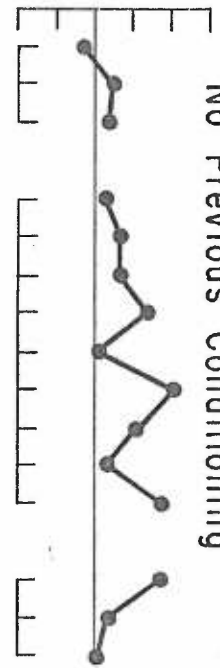
Subject 1 Previous Conditioning  
and Pseudoconditioning



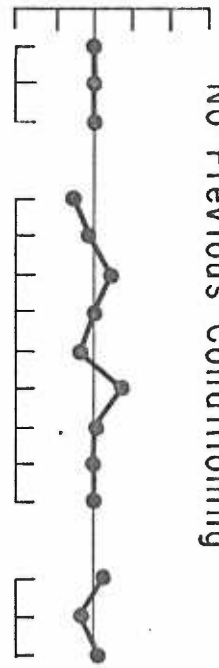
Subject 2 Previous Conditioning  
and Pseudoconditioning



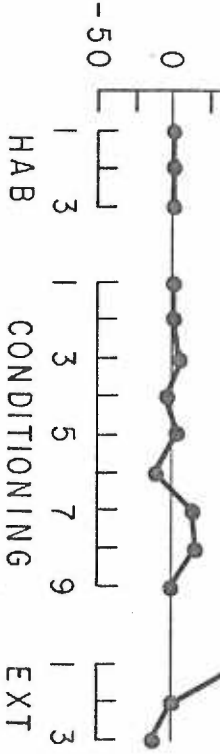
Subject 4 No Previous Conditioning



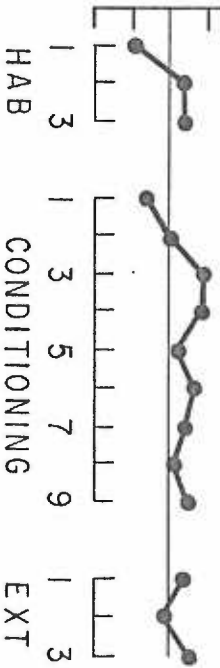
Subject 5 No Previous Conditioning



Subject 3 No Previous Conditioning



Subject 6 No Previous Conditioning



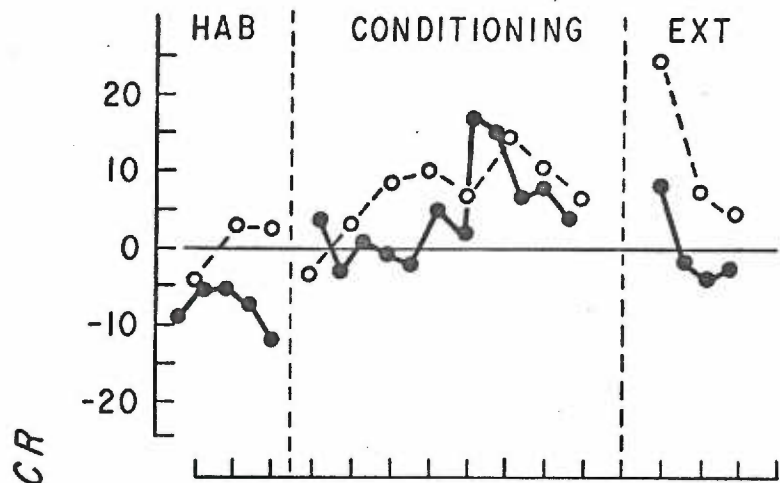
Blocks of 25 Trails

Figure 5 Percent CR response change in cells compared to percent conditioned leg movement responses.

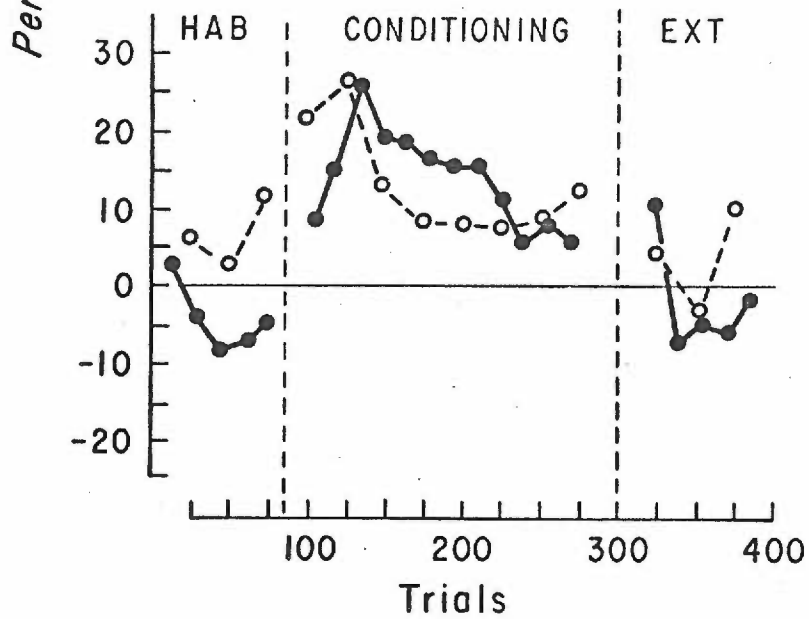
The Percent CR label on the ordinate represents the percent CR response change with respect to the cell data whereas it refers to the mean percent CR for all subjects relative to the leg response data.

The abscissa is a consecutive trials scale with the cell data being represented in blocks of 15 trials and the leg response data in blocks of 25 trials.

### No Previous Conditioning



### Previous Conditioning



o--o Leg Flexion

●—● Cells



Figure 6 The restraining device used to hold cat subjects for this study.

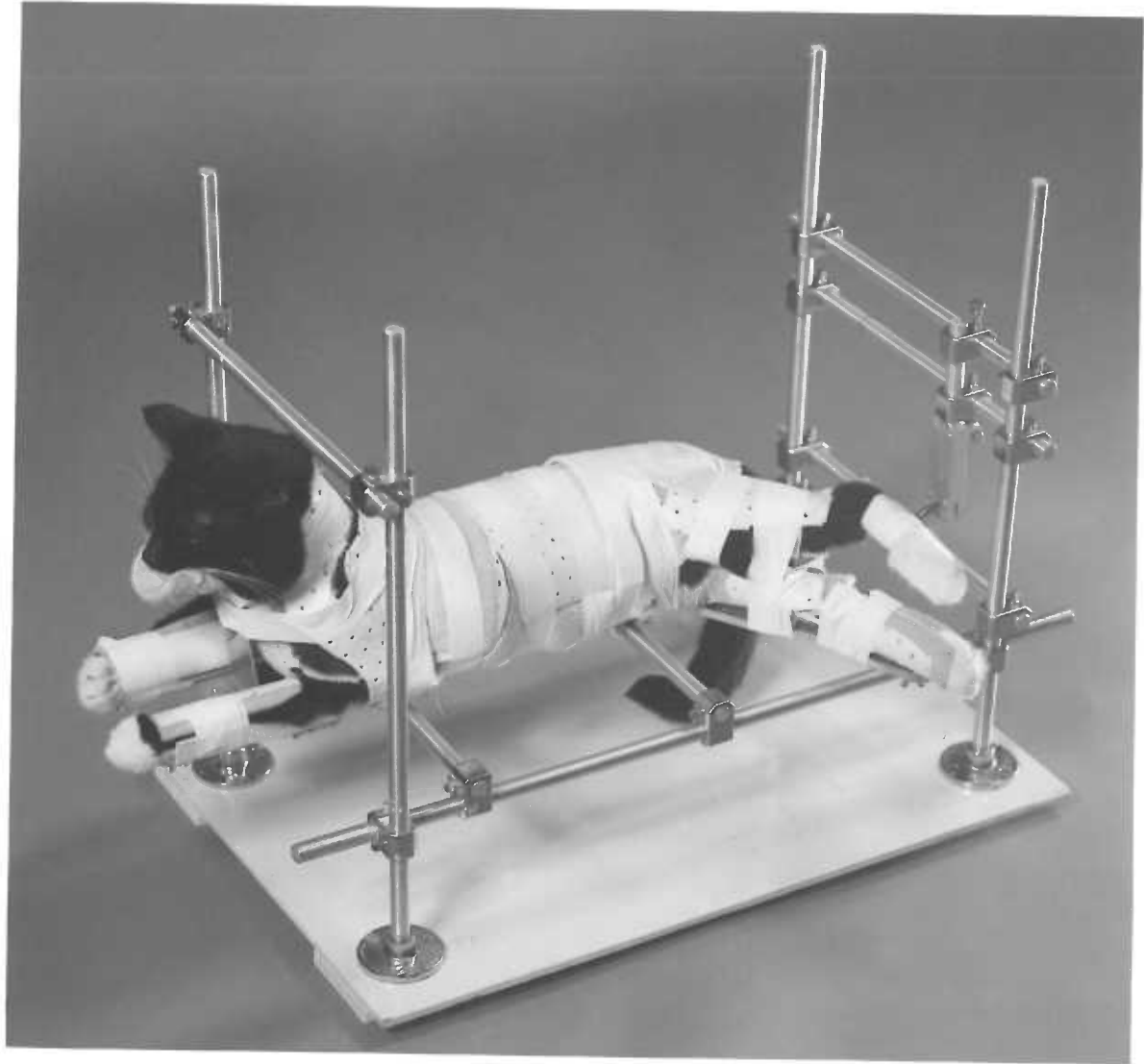


Figure 7 A reduced xerox copy of a portion of original data with important features labelled.

The top line represents the CS-US events with a pair of such stimuli constituting a trial. The two pair of broken vertical lines depicted the boundaries of the control interval and the conditioning interstimulus interval. The broken lines indicating the control interval are superimposed upon two original lines on the polygraph paper. The control interval in each trial was selected by identifying the space between the first 3 polygraph paper lines to the left of the CS marker. When a 1 mm or greater defelection in the bottom, i.e. response, line occurred it was labelled R if it fell within the control interval and CR if it fell inside the CS-US interval.

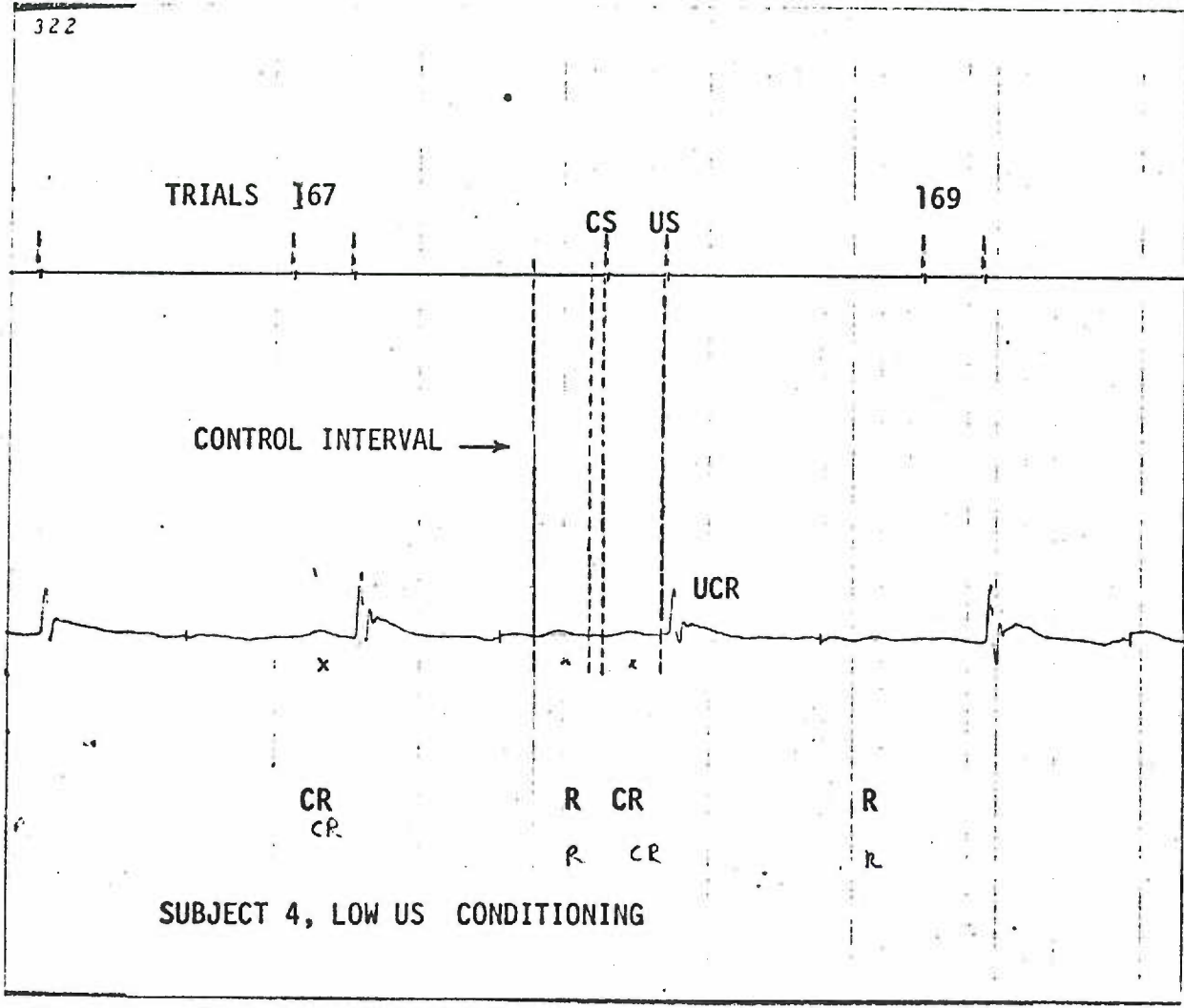


Figure 8 A xerox copy of a portion of original data (actual size).

The top line carries the CS and US event markers and the bottom trace indicates limb movements.

336

230

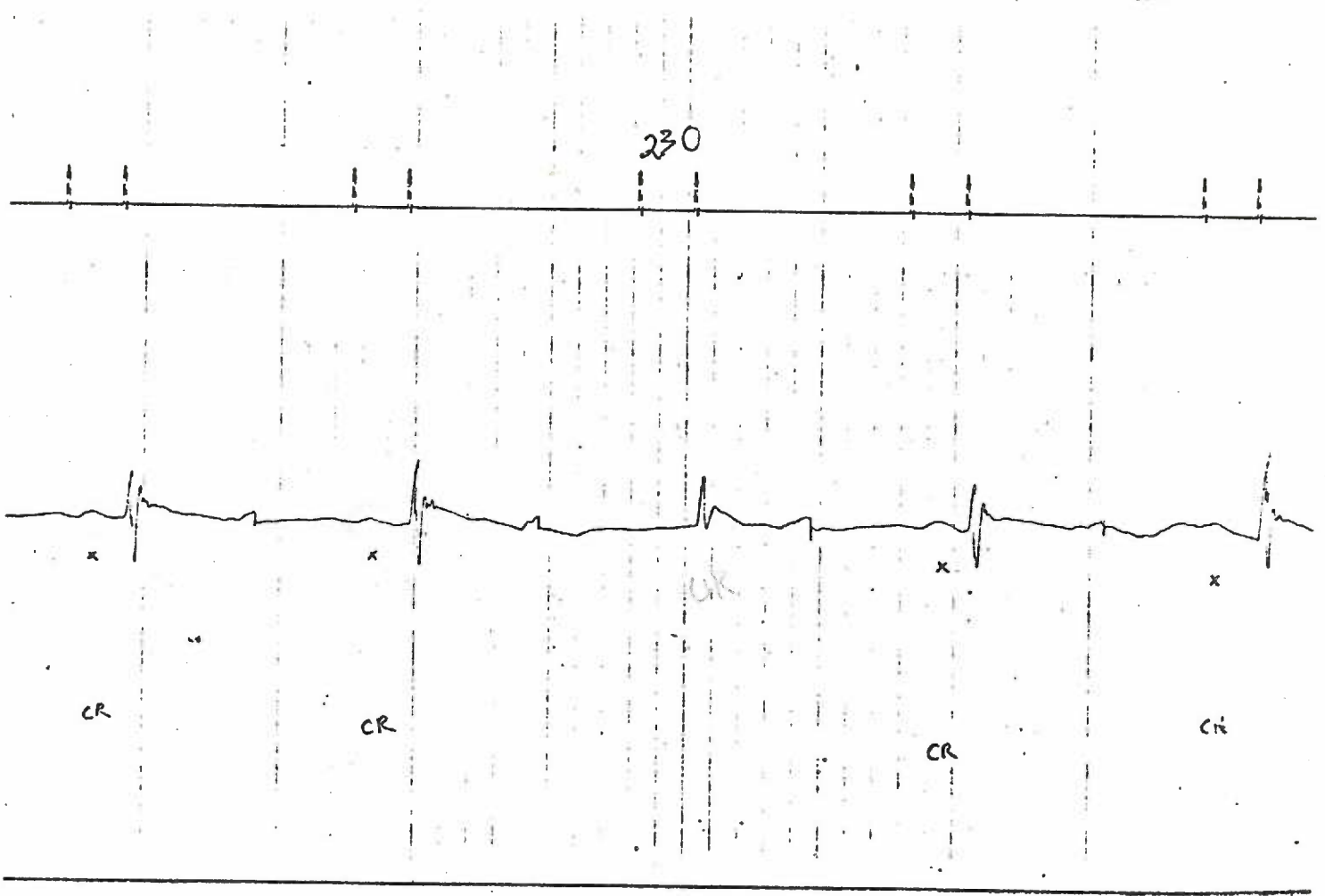


Figure 9 The response curves for subject 1.

Percent CR is the abscissa value and the data is plotted in blocks of 25 trials.

The order in which the subject was exposed to the conditioning and pseudoconditioning procedures is in the top to bottom sequence of the graphs.

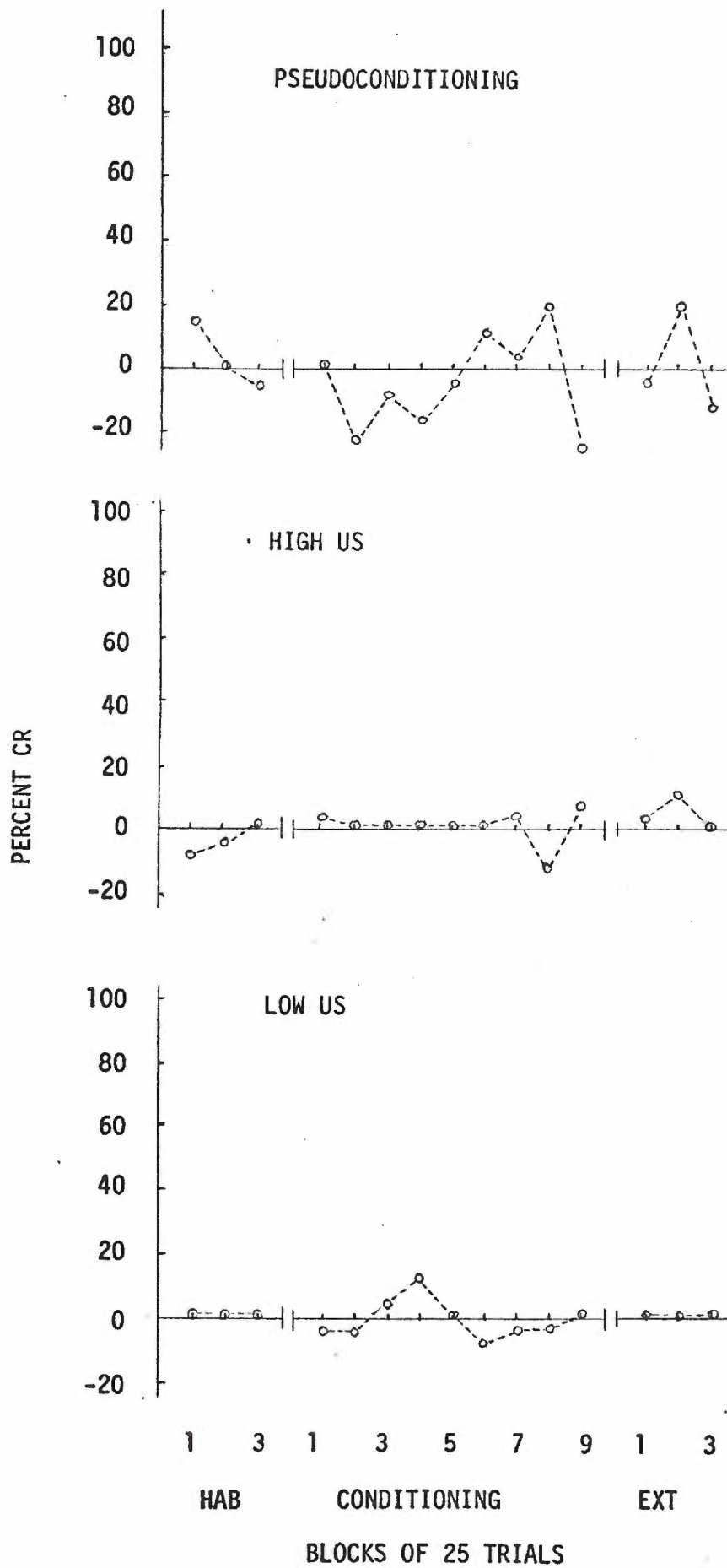




Figure 10 The response curves for subject 2.

Percent CR is the abscissa value and the data is plotted in blocks of 25 trials.

The order in which the subject was exposed to the conditioning and pseudoconditioning procedures is in the top to bottom sequence of the graphs.

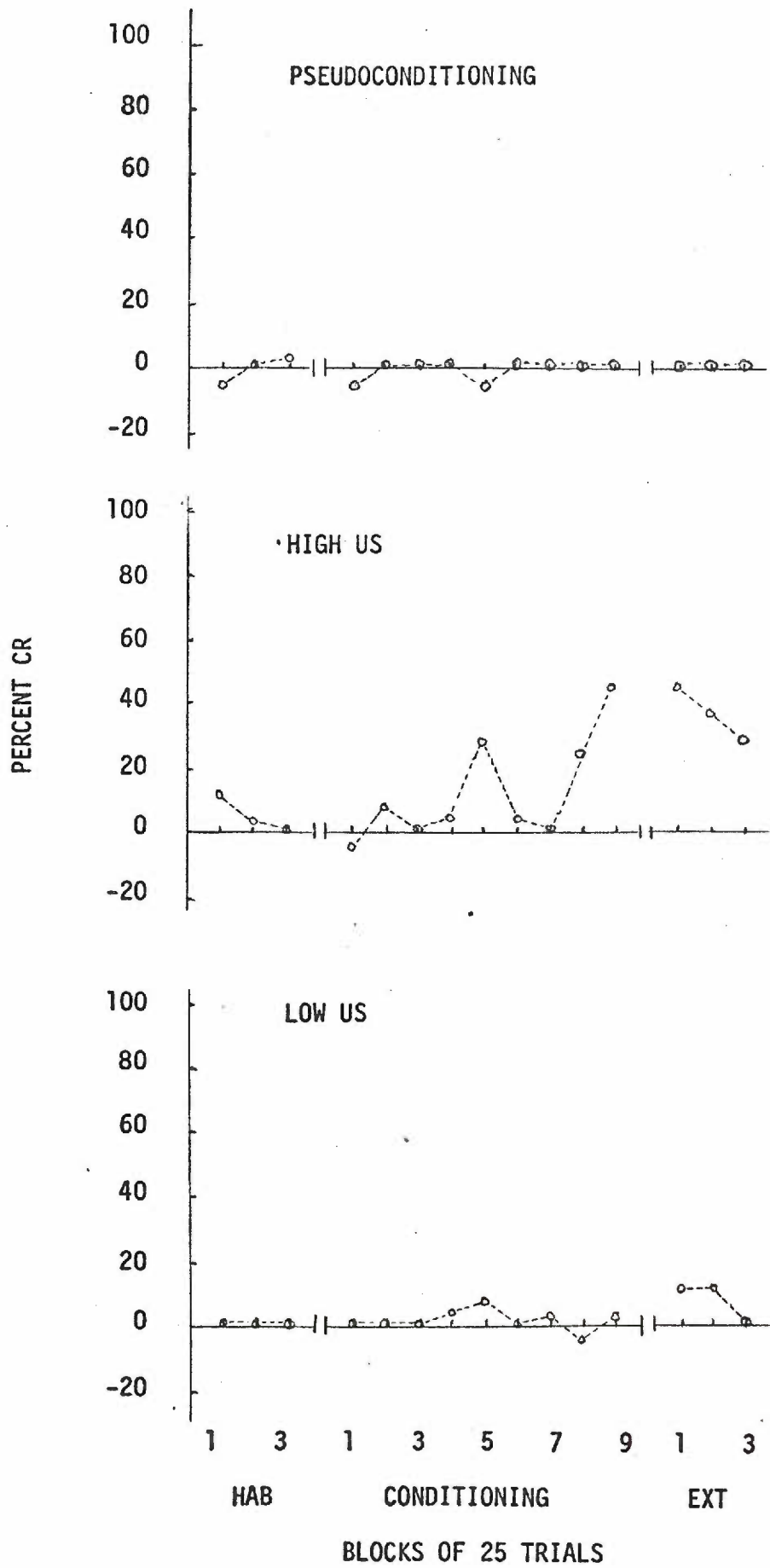


Figure 11 The response curves for subject 3.

Percent CR is the abscissa value and the data is plotted in blocks of 25 trials.

The order in which the subject was exposed to the conditioning and pseudoconditioning procedures is in the top to bottom sequence of the graphs.

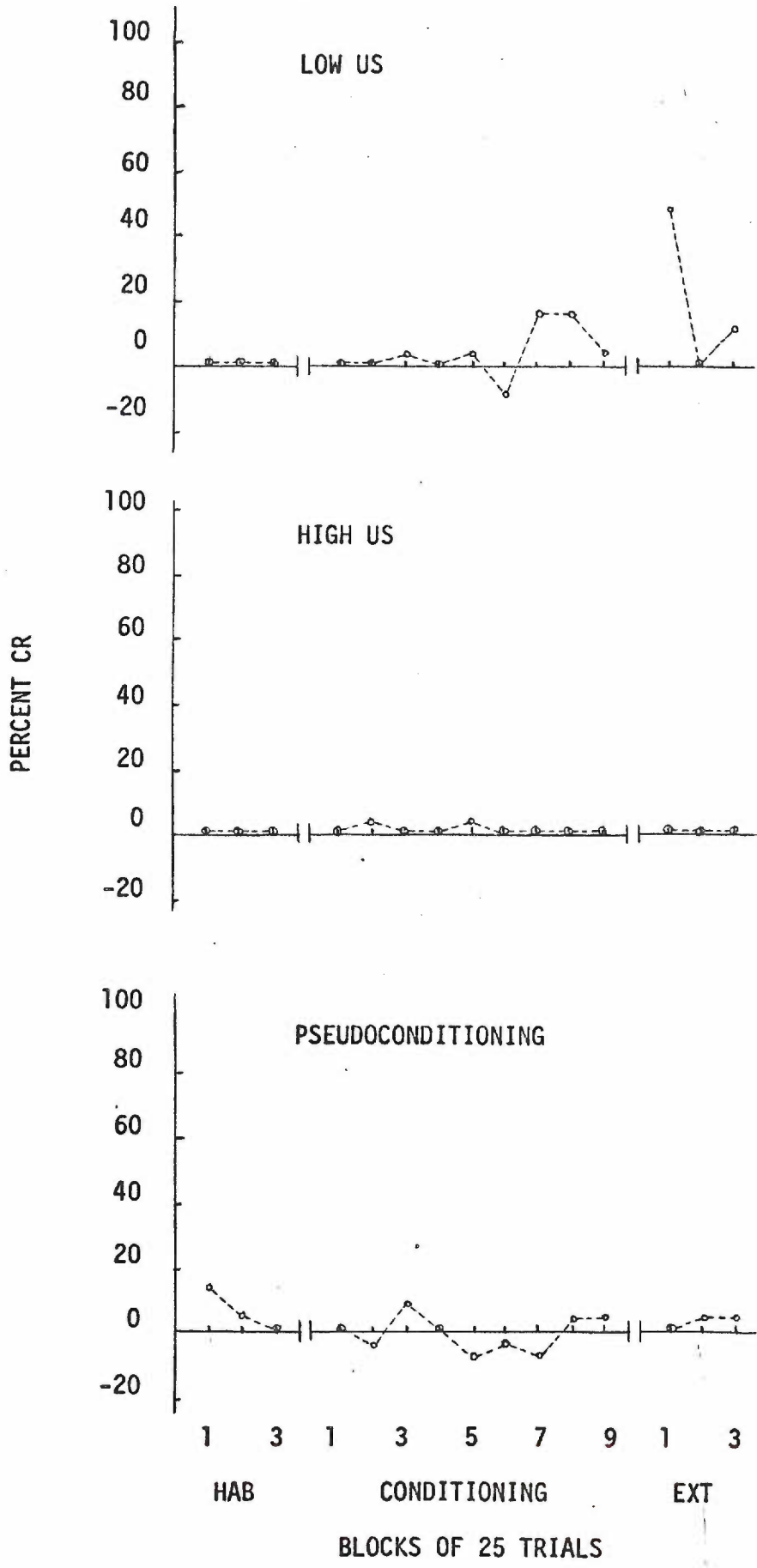


Figure 12 The response curves for subject 4.

Percent CR is the abscissa value and the data is plotted in blocks of 25 trials.

The order in which the subject was exposed to the conditioning and pseudoconditioning procedures is in the top to bottom sequence of the graphs.

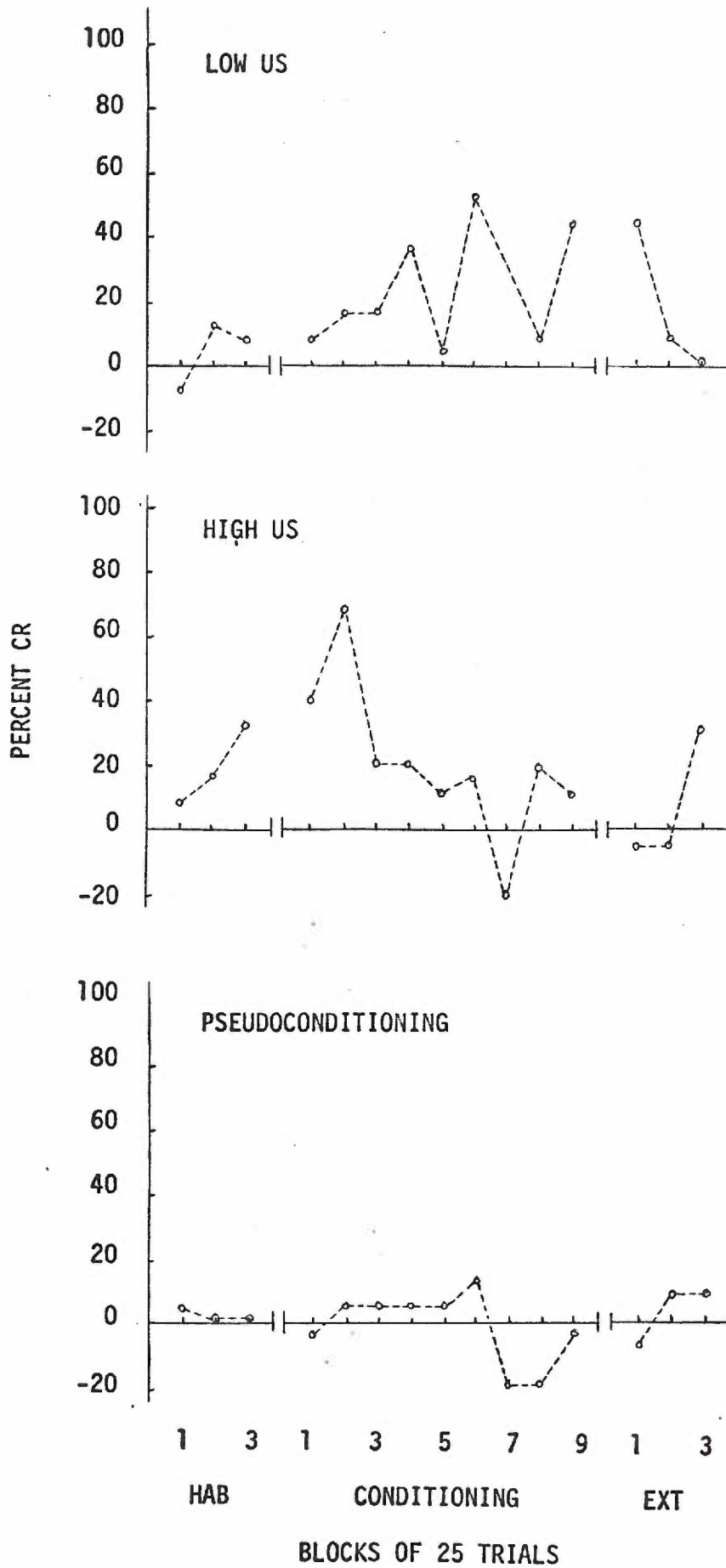


Figure 13 The response curves for subject 5.

Percent CR is the abscissa value and the data is plotted in blocks of 25 trials.

The order in which the subject was exposed to the conditioning and pseudoconditioning procedures is in the top to bottom sequence of the graphs.

PERCENT CR

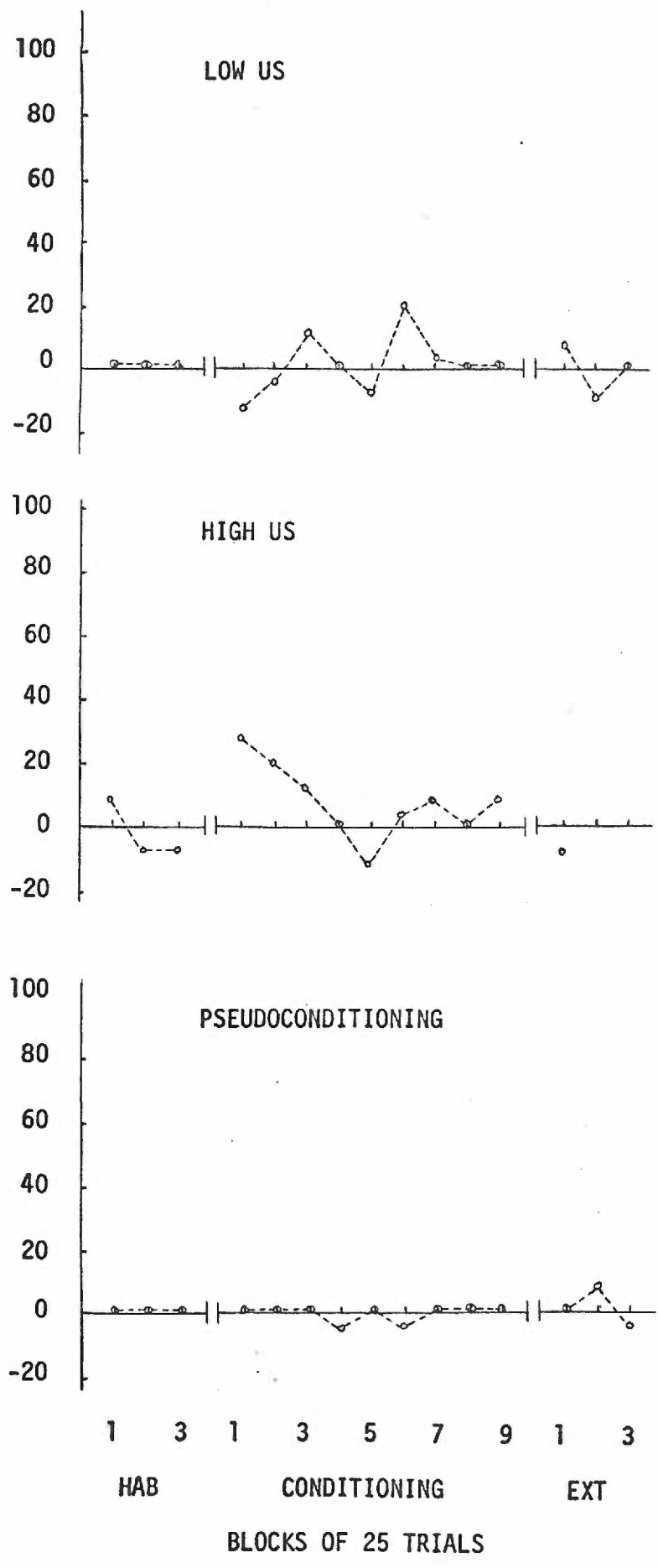




Figure 14 The response curves for subject 6.

Percent CR is the abscissa value and the data is plotted in blocks of 25 trials.

The order in which the subject was exposed to the conditioning and pseudoconditioning procedures is in the top to bottom sequence of the graphs.

