A NEW ELECTRODERMAL GRID TECHNIQUE

A NEW GRID TECHNIQUE FOR STUDYING THE ELECTRODERMAL CORRELATES OF CONDITIONED SUPPRESSION IN THE UNRESTRAINED RAT

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SCOPE AND CONTENTS:

Skin Conductance (SC) and Bar-pressing for a food reward were examined throughout discriminative CER training in two groups of rats. In one group of rats SC was measured through a traditional grid electrodermal measurement system. In the second group, the experimental group, SC was measured between each grid bar successively and a subdermal reference electrode, so that measurement artifacts inherent in grid systems of measurement were either reduced or eliminated in this group. Conditioned suppression of bar-pressing was accompanied by a great increase in S.C. in the experimental group. These results were attributed to peripheral and central response mechanisms regulating electrodermal activity.

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INTRODUCTION AND BACKGROUND

A widely used method for measuring electrodermal responses in unrestrained and unanaesthetized animals employs the grid bars upon which the animal is supported as measuring electrodes through which small sub-threshold measuring currents are run (Anderson, Plant and Paden, 1967; Kaplan and Kaplan, 1963; Kaplan, 1963; Roberts, 1967; Walters and Tullis, 1966). A commercially manufactured grid system has been described by Hobart and Kaplan (1964), although similar methods were used by Campbell and Teghtsoonian as early as 1958. The major advantage of these grid methods is that a wide variety of electrodermal and behavioral phenomena may be investigated without using elaborate restraining devices that might interfere with the behavior patterns observed. Another important advantage is that arousal due to such restraint is kept at a minimum.

Because of their obvious flexibility, grid methods have been employed to study the electrodermal correlates of a variety of behaviors in aversive situations. In a study of avoidance conditioning, Carran, Yeudall and Royce (1964) found that the C3H strain of mice, which performed well in avoidance training, displayed a lower tonic level of Skin Resistance (SR) when stressed with shock than did the C58 strain, which was deficient in avoidance learning. The authors concluded that the C3H strain was more fearful than the C58. Other investigators have examined electrodermal correlates of the conditioned-suppression of operant responding. For example, Anderson, Plant, and Paden (1967) first trained rats to approach a goal box to obtain food, and then examined the effect, on running behavior, of the presentation of a buzzer that had previously been paired with shock. They found that rats which had been shocked in the presence of the buzzer showed greater conditioned-suppression of the running response and had significantly lower SR levels than control rats for which the buzzer and shock had not been paired. In another conditioned-suppression experiment, Roberts (1967) investigated electrodermal correlates of the suppression of bar-pressing in the mouse. He found that conditioned-suppression was attended by a large increase in Skin Conductance (SC, the reciprocal of SR) and that the SC response and suppression were conditioned, discriminated, and extinguished at approximately the same rates. Since conditioned-suppression is usually accompanied and presumably caused by freezing behavior, the experiments by Anderson et al. (1967) and Roberts (1967) demonstrating an association between electrodermal activity and conditioned-suppression are in agreement with the results of a study by Deutsch (1968), who investigated autonomic correlates of freezing behavior. Deutsch found that freezing responses elicited by the sight of the experimenter were accompanied by increased defecation and SC. Furthermore, early handling, which is believed to reduce emotional reactivity (Denenberg, 1964), abolished or greatly attenuated freezing, defecation, and SC responses (Deutsch, 1968). The conclusion that may be drawn from these studies using grid methods of electrodermal measurement is that Ss which show behavioral manifestations of fear (efficient avoidance responding, suppression of bar-pressing, suppression of the running response, freezing behavior and defecation increases) also tend to display increased SC. In addition, procedures which are believed to reduce fear, such as experimental extinction (Roberts, 1967) or early handling (Deutsch, 1968) also attenuate or abolish electrodermal manifestations of fear.

One interpretation of the relationships observed between electrodermal responses and behavioral manifestations of fear is that electrodermal activity is regulated in some way by a central neural mechanism that controls fearful behavior. However, an alternative explanation is that these relationships are due to artifacts in the measurement of the electrodermal response. Of particular interest here is the possibility that freezing behavior which is associated with conditioned suppression leads to increases in contact-area and therefore to an increase in the recorded level of SC (Edelberg and Burch, 1962).

Freezing could lead to an increase in contact-area in two ways. First, an increase in the number of contact-sites, causing an increase in total contact-area, would occur if the animal touched additional grid bars as he froze. Since all of the grid bars are connected in parallel in traditional grid systems, touching additional bars would increase the number of paths through which current can pass, and therefore would lead to an increase in the recorded level of SC. A second and subtler way in which a contact-area increase might occur would be if S were to grasp the grid bars more firmly when freezing than when moving about. This would expose more volar surface for measurement and would therefore increase apparent SC. Since freezing behavior almost invariably accompanies fear responses in unrestrained animals, it is difficult to determine how much of a measured increase in conductance should be attributed to these contact-area artifacts and how much to a neurally controlled increase in electrodermal activity of the footpads.

Objectives of the Present Research

The purpose of the present study was to evaluate the contribution of contact-area artifacts to electrodermal correlates of conditioned suppression. The approach taken employed the use of an improved grid technique of electrodermal recording. This technique eliminated the artifact which arose from an increased number of contact-sites on the grid, while simultaneously enabling these sites to be counted. The technique was also designed to permit the evaluation of the "gripping" hypothesis by measuring Skin Potential (SP), which is highly correlated with SC and is influenced by the same effector, but is independent of contact-area (Martin and Venables, 1966; Lykken, Miller and Strahan, 1968).

An understanding of the rationale of the present study requires a brief description of the recording technique used. The contact-sites artifact artifact arises when traditional grid methods of electrodermal measurement are used because most of the grid bars are electrically active at any time, and therefore provide additional current paths when touched. Thus, to eliminate increments in SC due to an increase in the number of grid bars contacted, one must utilize a technique in which only one grid bar at a time is electrically active. The technique used in the present study accomplished this objective in the following way. A reference electrode for electrodermal recording was implanted beneath the skin of the rat. This electrode was connected into the recording circuit by means of a wire that ran beneath the skin to an Amphenol connector on the rat's head, and from there through a flexible spring to a rotating swivel mounted on the ceiling of a Skinner box. As with all grid methods, the active recording electrodes were the grid bars on which the rat stood, except that in this case each grid bar was energized individually by a commutator which swept across the grid, connecting each bar in and out of the measuring circuit sequentially. Thus, at any instant only one grid bar was activated, and conductance measurements were taken only from the area of the skin in touch with this bar. Consequently, if an animal touched additional grid bars when freezing, the recorded values of SC did not change. However, an increase in the number of grid bars contacted was detectable as an increase in the frequency with which the recording pen deflected, indicating the presence of a contact-site. In this way, two phenomena, an increase in SC and in contact-sites, were independently measured by the recording system. This recording technique was also designed to permit the measurement of SP. When

SP rather than SC was to be measured, the grid bars were connected together, thereby permitting the entire grid to serve as an active recording electrode. The measured value of SP was the potential observed between the subdermal reference electrode and the grid floor.

Electrodermal correlates of conditioned suppression were examined in this study by using both traditional and improved grid methods of electrodermal recording. It is possible to specify the outcomes expected on the basis of various hypotheses concerning the role of contact area artifacts. If the increase in SC observed during conditioned suppression is due mainly to an increase in the number of grid bars contacted, one would expect to observe increments in conductance attending conditioned suppression in control rats, for which traditional grid methods were used, but not in experimental rats for which the improved grid technique was employed. Furthermore, experimental rats should show an increase in signal frequency during conditioned suppression, indicating an increase in the number of grid bars contacted. On the other hand, if electrodermal correlates of conditioned suppression are mainly due to neural control of epidermal conductivity, one would expect to find increased SC attending conditioned suppression in both experimental and control rats, although an increase in signal frequency might still be observed in the experimental rats.

The possible contribution of the gripping artifact to electrodermal correlates of conditioned suppression may be evaluated by recording SP. An essential point here is that SP is highly correlated with SC, but is independent of contact-area (Lykken, Miller, and Strahan, 1968). Therefore, if increased contact-area resulting from an increase in strength of grip is a major determinant of the SC response attending conditioned suppression, one would expect an increase in SC during suppression, but no corresponding increase in SP. Alternatively, a demonstration of an increase in SP accompanying conditioned suppression would constitute convincing evidence against artifactual interpretations, and would lend plausibility to the view which attributes electrodermal correlates of suppression to neural control of epidermal conductivity.

METHOD

Subjects

The Ss were fifteen naive, male, hooded rats ranging from three to six months in age. The Ss were divided into two groups, consisting of ten experimental animals and five controls. For the experimental group, SC was measured by the new subdermal reference electrode technique, while for the control group SC measurements were made using the standard grid system.

Apparatus

The apparatus consisted of a Skinner box controlled automatically by programming equipment located one room away. The Skinner box had dimensions of 11 in. by 5 3/8 in. with a depth of 9 3/4 in.; a lever was located on the narrow end of the box and was 3 in. above the grid floor. These dimensions were selected so that the rat almost always maintained a lengthwise orientation in the box, perpendicular to the grid bars. This orientation made it difficult for the rat to place more than two feet on one grid bar at a time[1].

During the experimental session, the Skinner box was illuminated by a 7½ watt, 110 volt frosted lamp located above a transparent lid. The room was otherwise dark.

The two Conditioned Stimuli (CS) of 70 db SPL were produced by a Foringer multiple stimulus panel. One stimulus was a clicker of a frequency of 38.5 clicks per sec., and the other was a 1754 Hz tone. The Unconditioned Stimulus (UCS) was an electric shock of .3 sec. duration and 1.8 to 2.0 ma. intensity. Shock intensity was measured by monitoring, on an oscilloscope, the voltage developed across a 1 Kilohm resistor in series with S. Shock was applied between the subdermal reference electrode and the grid bars for both experimental and control Ss.

All Ss were fitted with subdermal reference electrodes for use in recording SC or administering shock. The electrode consisted of two Grass EEG discs which were bilaterally placed over the rib-cage while the animal was under Nembutol anaesthesia. Wires approximately 5 cm. long ran from the discs to an Amphenol connector (223-1) that was firmly attached to the skull with dental cement. During SC recording, a small electric cable, which was suspended from a swivel located on the lid of the apparatus, was attached to the connector on the rat's head. This technique was adapted from one described by de Toledo and Black (1965) for measuring heart rate.

Measuring current flowed from a 1-volt source to a commutator which connected each of the sixteen grid bars, one at a time, into the circuit approximately two times per second. From the grid bars, the current flowed through the rat to the reference electrode, and from there through a 100 Kilohm resistor back to the source. The signal developed across the 100 Kilohm resistor was proportional to S's conductance and was recorded on a Sanborn 7701A oscillograph. The S's SC was evaluated by comparing the conductance measurements to a calibration curve obtained when ten precision resistors of known value were substituted for the rat before the start of each experimental session. Although control rats had headcaps and were connected to the swivel on the lid of the apparatus, their SC was recorded by the traditional grid system described by Roberts (1967).

A simple experiment was performed in order to show how artifacts caused by the rat's contacting more than one grid bar at the same time would be eliminated by the subdermal reference electrode system. A mock electrical rat was built by soldering together on one wire the lead of each of seven 1 megohm resistors. The other ends of the resistors were connected, one by one, to the grid bars, by means of alligator clips. After each connection, conductance was recorded. The results are given in figure 1 and are compared to results obtained by Roberts (1967) using the traditional grid system. The difference between the two systems is clearly demonstrated in the figure. The broken line, representing the improved grid technique, remains constant at 1 micromho as each resistor is added, while the traditional grid method (solid line) records increasing conductance with the addition of each resistor. With the new grid technique, addition of new contact-sites increased the frequency of deflections of the recording stylus; this effect is not shown in the figure. To summarize, with the subdermal electrode system increases in contact-sites appear to increase in signal frequency, whereas with the traditional grid system they appear as increases in signal amplitude.

Procedure

From seven to fourteen days after the operation, Ss were placed on a twenty-four hour feeding schedule and reduced to approximately 75% of their normal body weight.

Following magazine training, the animals were given two-hour sessions of bar-press practice daily for seven consecutive days, under a VI-1 min. schedule of reinforcement. During the first three days of bar-press training, the Amphenol connector on the animal's head was not attached to the swivel on the lid of the apparatus, in order to allow the animal to increase the speed and eveness of bar-pressing. On the fourth day and thereafter, the animal's headcap was connected to the swivel. The seventh day of bar-press practice was a "pre-test" session during which the clicker the tone were presented four times each and SC and bar-pressing rates were recorded. CS duration was three minutes. The intertrial interval averaged twelve minutes (range: 8 min. - 15 min.).

Conditioning began on the eighth day and continued for at least ten days. The conditioning sessions were identical to the pre-test session in all respects, except that one CS (CS+) was paired with the shock, while the other CS (CS-) was not. For six of the experimental animals the clicker was the CS+ for four the tone was the CS+. The sequence of trials was: CS+, CS-, CS-, CS+, CS-, CS+, CS+, CS-. On alternate days this sequence was reversed.

On the last days of conditioning, a frequency analysis was performed for the experimental Ss to determine whether more grid bars were touched during the three minute CS-US interval than during a three minute Pre-CS control interval which immediately preceded the CS onset. This comparison was made by speeding up the chart paper in the Sanborn recorder from 10 mm/min. to 10 mm/sec. during the middle 30 seconds of the pre-CS and CS-US intervals. The faster chart speed made each pen deflection distinct from the others, enabling the number of deflections to be counted. Since the stylus deflected if S was touching a grid bar at the time that grid was sampled, the number of deflections during each cycle of the commutator was equal to the number of grid bars contacted. This procedure was followed for CS+ trials only. The days on which the frequency analysis was run for each rat are listed in Table 1.

In order to make SP measurements, the grid bars were connected together and SP was measured as the potential difference between the reference electrode and the grid. The experimental procedure was otherwise the same as for SC measurements. For reasons discussed in the Results section, SP measurements were made on only one rat, rat No.59, on day C-11.

Analysis of Data

SC levels and response amplitudes were determined in the following way. Measurements of SC were taken at the midpoint of each minute of the pre-CS and CS intervals, thereby providing three readings for each interval, or a total of six readings for each trial. The median of the three measurements taken during the pre-CS interval provided a measure of SC level prior to CS onset, whereas the median of the three measurements taken during the CS interval gave a measure of SC level during CS presentation. The difference between SC level during the CS interval and SC level during the pre-CS interval was taken as SC response amplitude. Conductance readings larger than 13.3 micromhos were discarded from this analysis, as these were almost certainly due to feces or urination shorts.

Suppression of operant behavior was quantified by dividing the number of responses made during the CS interval by the total number of responses made during the trial, i.e., during the pre-CS and CS intervals (Annau and Kamin, 1961). Thus, a ratio of .0 indicates complete suppression of bar-pressing during the CS, whereas a ratio of .5 indicates that operant behavior was unaffected by CS presentation. Ratios larger than .5 indicate facilitation of bar-pressing by the CS. Except where otherwise indicated S'S responses were summed over CS+ (or CS-) trials each day before suppression ratios were determined.

RESULTS

Conditioning of SC and Suppression

SC Level and rate of bar-pressing from the pre-test day through conditioning day 10 (C-10) are shown for the experimental rats in figure 2 and for the controls in figure 3. Each point on these graphs represents SC level or rate of bar-pressing averaged over Ss as well as over the four CS+ trials (solid lines) or the four CS- trials (dashed lines) on each day. Values are plotted for each minute of the pre-CS and CS periods and results are shown for each session.

Examination of figure 2 shows the conditioning and discrimination of SC and suppression for the experimental Ss. On the pre-test day and the first day of conditioning, both SC and bar-pressing responses to the CS+ were either very small or totally absent. By day C-2, elevation of SC and suppression of bar-pressing occurred on both CS+ and CS- trials in the experimental Ss. Yet, although responses were not yet at maximal amplitudes at this stage, already there was evidence of discrimination:

SC was a little more elevated on the CS+ than on the CS- trials; and while rate of bar-pressing was somewhat suppressed to both CSs, it was more depressed during the CS+ than during the CS-. From day C-3 onward, a clear discrimination between CS+ and CS- was seen for both SC and bar-pressing in the experimental rats.

Figure 3 shows the SC level and rate of bar-pressing for the control rats, which were tested using the traditional grid method of electrodermal measurement. On the pre-test day, they showed no SC or bar-pressing responses upon the presentation of the CSs. On day C-1, as with the experimental Ss, there was no discernable SC response or suppression of bar-pressing. On days C-2 and C-3, SC was elevated on both the CS+ and CS- trials. Bar-pressing was slightly suppressed on C-2 to the CS+ for the controls, and by C-3 was clearly suppressed with discrimination between CS+ and CS- trials being quite apparent. The bar-press response continued to be suppressed for the rest of the conditioning sessions. Although SC was elevated in the presence of the CS+ from C-2 onward, it was not until C-5 that the conditioning and discrimination of SC became clear-cut.

Thus, inspection of figure 3 suggests that the rates of conditioning and especially of discrimination of the SC response were slower than those of suppression for control Ss, whereas examination of figure 2 indicates that CS and suppression were conditioned and discriminated together for experimental Ss. The reason for the difference between the two groups is not known, but it may be due, in part, to the small number of Ss in the control group (N=5).

Detailed statistical analyses of the bar-pressing and SC data supported the conclusion that SC and suppression were conditioned and discriminated at the same rates for the experimental Ss, but at different rates for control Ss. The findings of these analyses are presented in Table 2 for experimental Ss and Table 3 for control Ss. These tables report the outcome of the t-tests performed on the amplitude of the SC responses and the suppression ratios observed from the pre-test day through the third or fifth day of conditioning. The manner in which they are read may be illustrated by taking day C-1 for the experimental Ss in Table 2 as an example. The positive value of t listed for conductance under the column CS+ indicates that SC increased when the CS+ was presented. The negative value of t for conductance under the CS- column indicates that SC decreased slightly when CS- was presented. Similarly, the negative values of t shown for bar-pressing in the CS+ and CS- columns indicate that bar-pressing decreased on both the positive and negative trials. However, none of these effects was statistically significant. The positive values of t listed in the third column (CS+ - CS-, indicate that SC responses and suppression were larger on CS+ trials than on CS- trials, but these differences were also insignificant. Thus, the results for experimental Ss on C-1 fail to show conditioning to either CS, and also show no discrimination between CS+ and CS- in either conductance or bar-pressing measures.

The interpretation of other findings in Table 2 is relatively straightforward, and serves as an amplification and quantification of the results seen for experimental Ss in figure 2. There was a slight increase in conductance and the rate of bar-pressing on the pre-test day of both the CS+ and CS-. But these increments, which are clearly visible on the graph, are not significant, except for the acceleration of bar-pressing to the CS-. The acceleration of bar-pressing which occurred on the pre-test day was opposite to the direction of bar-press changes on conditioning days, when bar-pressing decreased to the CS. By the second day of conditioning, there is a significant increase in SC and a significant decrease in rate of bar-pressing during the CS+. It is also evident that CS- had little effect during conditioning. Both response measures showed a clear discrimination between positive and negative trials by day C-2. The pattern of findings shown on conditioning day 2 persisted for the remainder of the experiment.

The results reported in Table 3 also quantify the findings reported for the controls in figure 3. There was a slight decrease in SC to the CS+ on the pre-test day, while a slight increase in SC occurred to the CS-, but neither change was significant. Bar-pressing tended to decrease to both the CS+ and the CS- on the pre-test day, but this deceleration was not significant. By the second day of conditioning, the suppression of bar-pressing to the CS+ became significant and persisted for the rest of the experiment. On the same day, C-2, a significant discrimination between the CS+ and CS- trials also developed for bar-pressing and remained for the rest of the experiment. Although SC showed elevation to the CS+ and the CS- from C-2 onward, these conductance increases did not become significant until C-5 when SC conditioning to the CS+ and discrimination between the CS+ and CS- first became reliable. _t_-tests were not performed on the following sessions because it was apparent that the SC increases and bar-pressing decreases seen on C-5 persisted for the rest of the experimental sessions.

To summarize, the data of figure 2 and table 2 indicate that SC and suppression of bar-pressing were conditioned and discriminated at the same rate in the experimental Ss. The results shown in figure 3 and table 3 reveal a disparity between the rates of conditioning and discrimination of SC and bar-pressing for control Ss, but this may have been due to a small N.

Parallel rates of acquisition of the SC response and suppression indicate a strong relationship between these two variables. The magnitude of this relationship was examined further by calculating, for each experimental and control S, the Product-Moment Correlation between the amplitude of SC responses and suppression ratios on each CS+ trial from the pre-test day through the last day of conditioning. The results of this analysis are shown in table 4. The correlations shown here are uniformly negative, indicating an increase in SC attending suppression of bar-pressing, and hover about -.40 for both experimental and control animals. Perhaps the most surprising aspect of this data, however, is that the correlations are relatively low. This result is probably attributable to a lack of variability in the suppression ratios beyond the second day of conditioning, and also to the difficulty of obtaining precise estimates of SC response amplitudes when the traditional or improved grid methods of SC recording are used. In order to minimize the contribution of these factors, correlations were calculated for the data of days C-1 through C-4 only, where the amplitudes of SC responses and suppression were changing systematically due to the conditioning procedure. The results are shown in table 5, where it may be seen that the relationship in both groups improved only slightly. The failure to observe even stronger relationships here, where the suppression ratios are variable, could mean that error in the measurement of SC response amplitudes was the primary cause of the low correlations reported in tables 4 and 5.

Since the variability of the suppression ratios appeared low and error in the measurement of SC responses relatively high, a better measure of the correlation of the two responses was one which did not take amplitude into account, but examined only the direction of the changes. This was accomplished by calculating directional correlation coefficients which measure the tendency for two variables to change in the same direction from one sampling occasion to the next (Strahan, 1966). These correlations were determined for each control and experimental S by comparing the SC response to the change in bar-pressing on every CS+ trial, starting from day C-2, when conditioning first appeared, through the end of the experiment. A plus (+) was assigned to a given trial if SC and BP changed in the same direction, either an increase or a decrease, when CS+ was presented. If, on the other hand, the two responses changed in opposite directions, with an increase in SC accompanying a decrease in BP or vice-versa, a minus (-) was assigned. No change in one or both of the two variables meant that trial was assigned a zero, but these cases were few. The data, thus transformed, were then treated according to the formula: $d = \frac{P-M}{N}$, a modification of the Strahan formula, where P = the number of pluses, and M = the number of minuses, and N = the number of CS+ trials. It can be seen that if SC and BP changed in the same direction on all trials, the correlation would be +1.00, while if the two responses changed in opposite directions, the correlation would be -1.00.

The results of the directional correlation test are given in Table 6. These results show a high negative correlation for both the experimental Ss (r = -.75) and for the control Ss (r = -.67). These correlations show that, for both groups, an increase in SC was reliably accompanied by a decrease in bar-pressing rate, as is also evident from Figures 2 and 3. However, it is interesting to note that the directional correlations for both these groups are of similar magnitude, as are the Product-Moment Correlations, while the rates of acquisition of the SC response and suppression, as evaluated by t-tests, appeared to be disparate for control Ss but not for experimental Ss. Thus, the t-tests suggest that the SC response and suppression were conditioned at the same rate in experimental Ss but at different rates in controls, whereas the correlational analyses suggest equal rates of acquisition in both groups. The simplest explanation of this discrepancy is that the responses were conditioned at equal rates in both groups, an inspection of figures 2 and 3 suggest, but that the small number of control Ss tested prevented the SC response from achieving significance until response amplitude was large.

Background Level of SC and Bar-Pressing

A systematic relationship between the background levels of SC and bar-pressing is revealed by inspection of the pre-CS values shown in Figures 2 and 3. The tonic level of SC for experimental Ss was lowest on the pre-test day and C-1, increased on C-2 and C-3 to their highest levels, and declined until C-8, where a slight reversal occurred. This pattern was approximately the same for the background rate of bar-pressing. Rate of background was quite fast on the pre-test day and C-1, and decreased on C-2 and C-3 with a tendency toward recovery appearing from C-4 onward. This pattern was roughly the same in the control Ss, except that there was an unaccountable increase in SC level on C-8 through C-10. The relationship between the tonic level of SC and bar-pressing may be seen more clearly in Figure 4, where the mean pre-CS values of SC and bar-pressing, averaged across positive and negative trials for each session, have been plotted for control and experimental Ss. While the relationship is discernable in both groups, it is much stronger for experimental than for control Ss, the Product-Moment Correlations being -.59 and -.37, respectively.

It is of interest to compare the tonic SC levels of the experimental and control groups. Since in the subdermal reference electrode system current need flow only through the resistance of a single footpad, while in the traditional grid system it must flow through two resistors (footpads) in series, one would expect the tonic conductance levels of control Ss to be approximately half that of experimental Ss. The mean pre-CS level of SC for the experimentals was 3.54 micromhos, while the corresponding value for the controls was 1.56 micromhos. This result is in approximate accord with what one would expect.

Contact-Area Artifacts

The occurrence of freezing behavior during Conditional Suppression (Roberts, 1967) might lead S to touch additional grid bars, or grip them more firmly. Both of these effects would increase contact area and cause an artifactual increase in the recorded level of SC.

The question of whether Ss contacted additional grid bars during suppression was evaluated for experimental Ss by recording signal frequency during positive trials. Since for these Ss, signal frequency was proportional to the number of grid bars contacted, dividing frequency by the speed at which the commutator revolved gave a direct measure of the number of contact sites. The mean numbers of contact-sites before and during the CS+ are given in Table 7 for each rat on the days frequency analyses were performed. The average number of contact-sites during the pre-CS+ period was 2.34, while the average number during the CS- period was 2.83. Of the ten rats tested, seven showed a greater number of contact-sites during the CS+ period. Of the three rats who showed a decrease, two rats had negligible SC responses on the day of the decrease. One rat, rat No. 31, showed a large SC response while having fewer contact-sites on the grid than before. Although these data are not extensive enough to permit a firm conclusion, they suggest that additional grid bars are touched during conditional suppression, and that SC as measured by traditional grid systems is increased accordingly. However, this factor did not contribute to the SC responses displayed by experimental Ss in the present study, since the improved grid technique used with these Ss was immune to this type of contact-area artifact.

An attempt was made to test the gripping hypothesis by measuring SP, using the subdermal electrode system on the eleventh day of conditioning for rat No. 59. However, large and rapidly fluctuating potential changes were observed which did not follow any evident pattern; these were caused by the rat's movement about the grid and were large enough to mask any smaller SP changes due to electrodermal response. The fluctuations observed ranged from approximately 10 mv. to 125 mv. SP responses measured from one footpad of the hind leg of restrained rats have been recorded during conditioned suppression and found to be on the order of 10 mv. (Roberts and Young, 1968). Since the amplitude of movement artifacts greatly exceeded the size of the true signal, it was not possible to evaluate the gripping hypothesis as planned.

DISCUSSION

Measurement Artifacts

The main finding of this study was that large increments in SC reliably accompanied conditioned suppression of bar-pressing in the experimental rats. A secondary result was that the frequency analysis demonstrated a slight tendency for Ss to show an increased number of contact-sites during the CS+ period. Because the nature of the measuring system did not allow additional contact-sites to contribute to SC amplitudes, the observed SC increases cannot be accounted for by this artifact. An individual case which demonstrates this fact is that of Rat 31, whose frequency analysis suggested no increase in contact-sites, while his SC responses were greater than the mean. These results indicate that SC responses can exist in the absence of contact-site artifacts, and, moreover, constitute evidence for the existence of neural control of epidermal conductivity in rodents.

However, it is possible that the SC increases found here could be accounted for by one of two other artifacts: the gripping artifact already discussed in the introduction, and a urination artifact, since urination on the grid would provide extra ions for current conduction or enlarge contact area, both of which would increase the recorded level of SC. The most compelling evidence against such hypotheses is given in a study by Roberts and Young (1968), who measured SC in restrained rats with a technique that did not permit the occurrence of either type of artifact. Roberts and Young's (1968) system measured SC and SP simultaneously, one from each of the hind feet of the rat, while the rat followed a CER schedule similar to the one used in this study. SC and SP responses of approximately 1 micromho and -12 mv., respectively, were consistently elicited in conjunction with Conditioned Suppression. Even without this evidence, however, it would seem unlikely that urination artifacts could produce results of the nature seen in the present experiment. One would have to postulate that the rat urinated each time he heard the CS+, and that the effects of the urination lasted exactly the duration of the CS+. Also, there is reason to believe that urination is not a reliable component of the fear response. Deutsch (1967) observed consistent increases in defecation and conductance to a fear-producing stimulus (sight of E), but observed urination on less than 4% of 500 stimulus presentations. Hall (1934) tested defecation and urination as measures of individual differences in emotionality, and concluded that defecation is a more valid measure of emotionality than urination, and recommended its use alone as a measure. McClearn and Meredith (1964) in a factor analytic study also concluded that urination is not associated with the same factors as defecation. Therefore, these experiments suggest that urination may not be a reliable component of the pattern of responses observed when an animal is frightened.

There are several implications of the findings of this study for other grid studies of electrodermal responses. First, the present results, complemented by those of two previous experiments in which SC was recorded from electrodes attached directly to the rat's footpads (Roberts and Young 1968; Holdstock and Schwartzbaum, 1965), provide evidence for the existence of electrodermal responses as true physiological phenomena distinct from measurement artifacts. The existance of true electrodermal responses is also indicated by certain studies using grid methods which produce effects difficult to attribute to measurement artifacts. Deutsch (1968) found that the pattern of locomotor activity displayed by

_S_ over repeated exposures to aversive stimulation was initially one of freezing, followed on later trials by escape. Throughout the experiment, however, conductance changed in the same direction, that of increasing SC. Roberts (1967) studying mice in CER training observed a decline in the amount of time spent in freezing behavior as testing progressed, while SC increases continued in the same amount. Another source of evidence is derived from studies which demonstrate the existence of sweat-glands in the footpad of the rat both by means of stimulation of the sciatic nerve and by histological examination (Ring and Randall, 1948 , Wagner, 1950). The present study, taken together with these other studies lends validity to the existence of true electrodermal responses, and therefore to the hypothesis that other grid studies were examining, at least in part, real electrodermal phenomena.

Secondly, however, the results of this study indicate that contact area artifacts exist, and may complicate interpretations of electrodermal data obtained by grid techniques. The data from the frequency analysis, while limited in quantity, suggest that an increase in contact-sites does take place during conditioned suppression, where _S_ is typically freezing (Roberts, 1967). Thus, the problem may be particularly severe if the test situation encourages freezing behavior, because contact-site increases appear to be a consequence of freezing. A detailed analysis of freezing behavior during Conditioned Suppression, reported by Roberts (1967), indicates that total time spent freezing becomes maximal on about the 5th day of CER training, and then declines thereafter. Although their analyses are not as extensive, the experiments by Anderson et al. (1967) and Deutsch (1968) also suggest that freezing behavior declines over repeated presentations of an aversive stimulus. It should, therefore, be noted that the frequency analysis was done on the last days of conditioning, when contact-site increases due to freezing were most likely to have been minimal. The contact-site increases recorded in Table 8 therefore, may be underestimated. With this consideration taken into account, the results of the frequency analysis seem to indicate that a bias can occur in studies using a grid technique of electrodermal measurement. An increase in contact-sites may have contributed in some degree to electrodermal responses reported by Roberts (1967), Deutsch (1968), Anderson et al. (1967), Kaplan (1963), Kaplan and Kaplan (1963), and Walters and Tullis (1966).

The Conditioning of SC and Suppression

Some researchers believe that electrodermal responses cannot be conditioned, but that all studies of electrodermal conditioning can be considered as examples of sensitization or pseudo-conditioning (Stewart, Stern, Winokur and Fredman, 1961). Sensitization has been defined as:

> "The increase in the strength of a reflex originally evoked by a conditioned stimulus through its conjunction with an unconditioned stimulus and response. (It) differs from conventional conditioning in that the response which is strengthened is appropriate to the conditioned stimulus, not to the unconditioned stimulus." (Kimble, 1961)

The problem for SC conditioning studies in particular is that almost every CS initially causes some electrodermal response, however slight. So it remains a very difficult problem to prove that any particular case of SC conditioning is true conditioning and not pseudo-conditioning or sensitization in which an undetectable Unconditioned Response (UCR) has been augumented.

Kimmel (1964) has suggested a way out of this dilemma. His solution is based upon the view that true conditioning consists of"any S-R alterations directly attributable to the paired presentation of the CS and the UCS". He suggests that the experimental design include a sensitization control group which undergoes all of the procedures of conditioning that the experimental group undergoes except for pairing of the CS and US. A second method for differentiating between conditioning and sensitization employs two differentially reinforced CSs. Either procedure is an appropriate control for sensitization.

The present experiment used the second design, a discrimination procedure. Since complete discrimination between the CS+ and the CS- was achieved in this experiment, it is clear that the SC changes observed were due to a specific CS-US pairing and not to a lowering of the threshold of the SC response system to any stimulus or to an increase in the general arousal level of the rats. Therefore, employing Kimmel's definition of conditioned response as a behavioral change directly attributable to the CS-US pairing, the changes in SC observed here were conditioned SC changes.

The fact that conditioned increments in SC attended suppression of bar-pressing suggests that both responses provide measures of emotional arousal or fear. Baseline rates of bar-pressing and SC also co-vary in a way which suggests that they, too, are sensitive to fear. Suppression of the base-line rate of bar-pressing in a CER situation has been reported by other researchers (e.g. Brimer and Kamin, 1963), and it is not surprising to find that the SC baseline shows the same effect once conditioning has begun. The correspondence of the two measures both in response amplitudes during the CS+ and in the baseline levels during the pre-CS periods is taken to mean that the measures are each part of a pattern of behavior indicating emotional arousal.

The close relationship found for the experimental rats between conditioned SC increases, which are regulated by the Autonomic Nervous System (ANS), and the suppression of the bar-pressing response, which is a function of the voluntary motor centers, raises the question as to whether autonomic and behavioral variables are equally good indices of conditioned fear. The present findings for the experimental subjects as well as two other studies (Roberts, 1967; Roberts and Young, 1968) suggest that SC and suppression are equally sensitive, but studies on heart rate raise the possibility that not all autonomic variables are equally sensitive measures of emotionality. de Toledo and Black (1966) have correlated changes in Heart Rate (HR) and suppression of bar-pressing during conditioning, discrimination and extinction in a CER arrangement. They found that HR conditioned more slowly and was extinguished more quickly than suppression. These findings were replicated by Parrish (1967), using roughly the same CS and UCS intensities. Goldberg and Schuster (1967) also correlated changes in HR with suppression of bar-pressing in morphine addicted monkeys, using as the UCS, nalorphine, a drug antagonistic to morphine which causes bradycardia as well as other ANS effects. They found HR conditioned slightly after suppression and that it was less resistant to extinction that suppression. Overmier (1967), measuring HR and instrumental responding, also confirms this finding of the lower sensitivity of HR in comparison with motor variables.

The differences in the conditioning rates of SC and HR with respect to suppression can be explained in two ways, not mutually exclusive. First, variations in experimental procedures could produce different rates of conditioning, discrimination or extinction. de Toledo and Black (1968), in an extension of their work, found that rats in a CER situation given a CS+ consisting of white noise interrupted three times per second acquired HR and suppression responses at approximately the same rate, while rats given a CS+ consisting of white noise interrupted fifteen times per second showed suppression well in advance of HR responses. The relative conditioning rates were also affected by variations in intensity of the UCS. The two responses conditioned most nearly together at the lowest shock intensity (0.8 ma.) and showed the greatest disparity in rate of acquisition at the highest shock intensity.

The magnitude of different responses as well as the time of conditioning may also be affected differently by changes of UCS intensity, as shown in the work of Holdstock and Schwartzbaum (1965) comparing SR and HR responses, conditioned to a clicker. These authors found no significant difference in magnitude of the SR response between low-shock (0.5 ma.) and high-stock (1.5 ma.) groups, while they found that the low-shock group showed a much greater HR response. Therefore, it is quite clear that variations in experimental parameters have an effect on the relationship of two conditioned variables to each other in both rate of conditioning and magnitude of response and probably along other dimensions, as well.

Secondly, there is the possibility that different autonomic response systems have different characteristics. In the Holdstock and Schwartzbaum (1967) experiment, some differences between HR and SR appear in their data which seem not to be attributable to anything but differences between the two response systems. Conditioning trials took place over five days with one session per day. Each session consisted of 32 conditioning trials, 16 of which were shocked and 16 of which were unshocked. Within each day's session both HR and SR showed the same pattern of decline in magnitude of response with repeated trials through the session, but HR conditioning showed an increasing response magnitude over the five days conditioning while SR response magnitude dwindled over the same period in the low-shock condition for both. Moreover, the HR baseline (the pre-CS level) remained at a steady level throughout each session, while the SR baseline increased during each day's session.

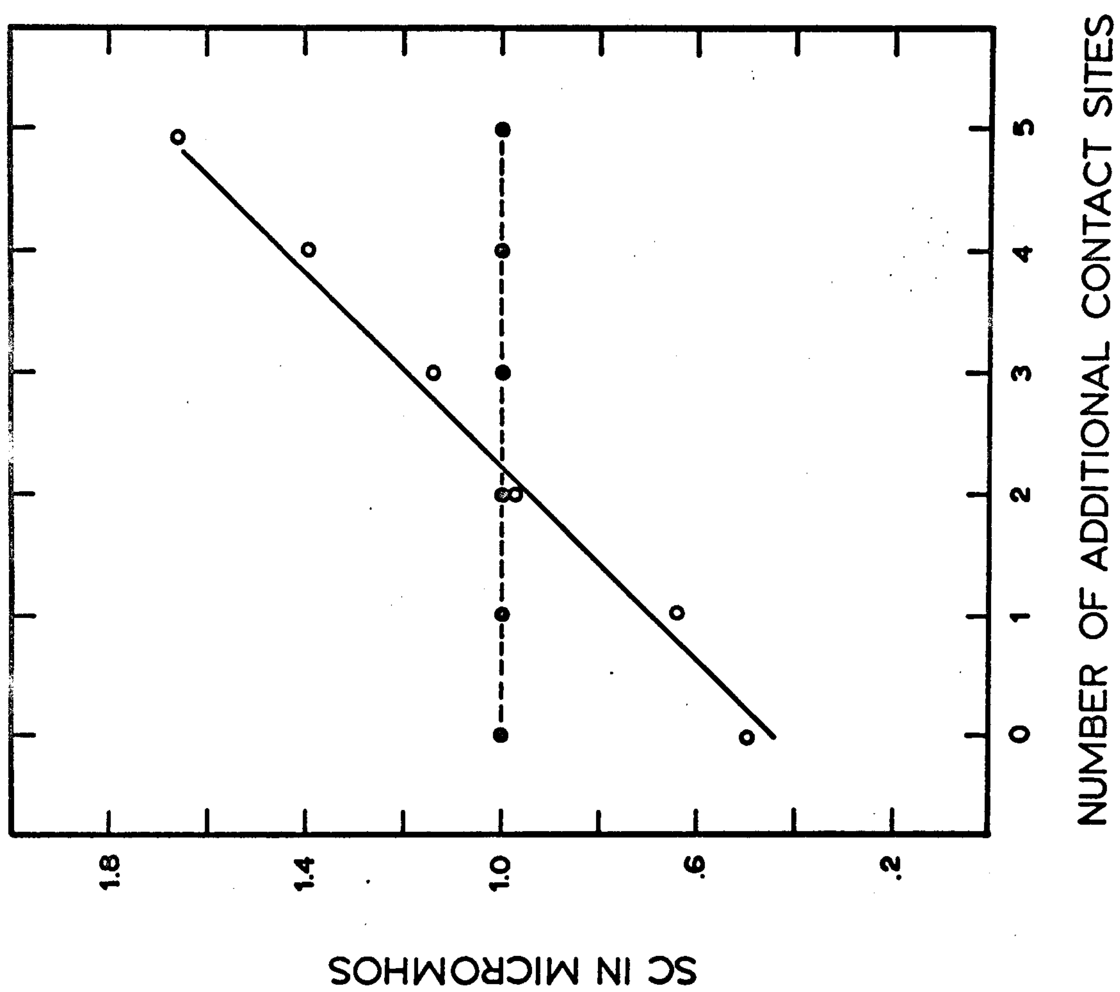
SUMMARY

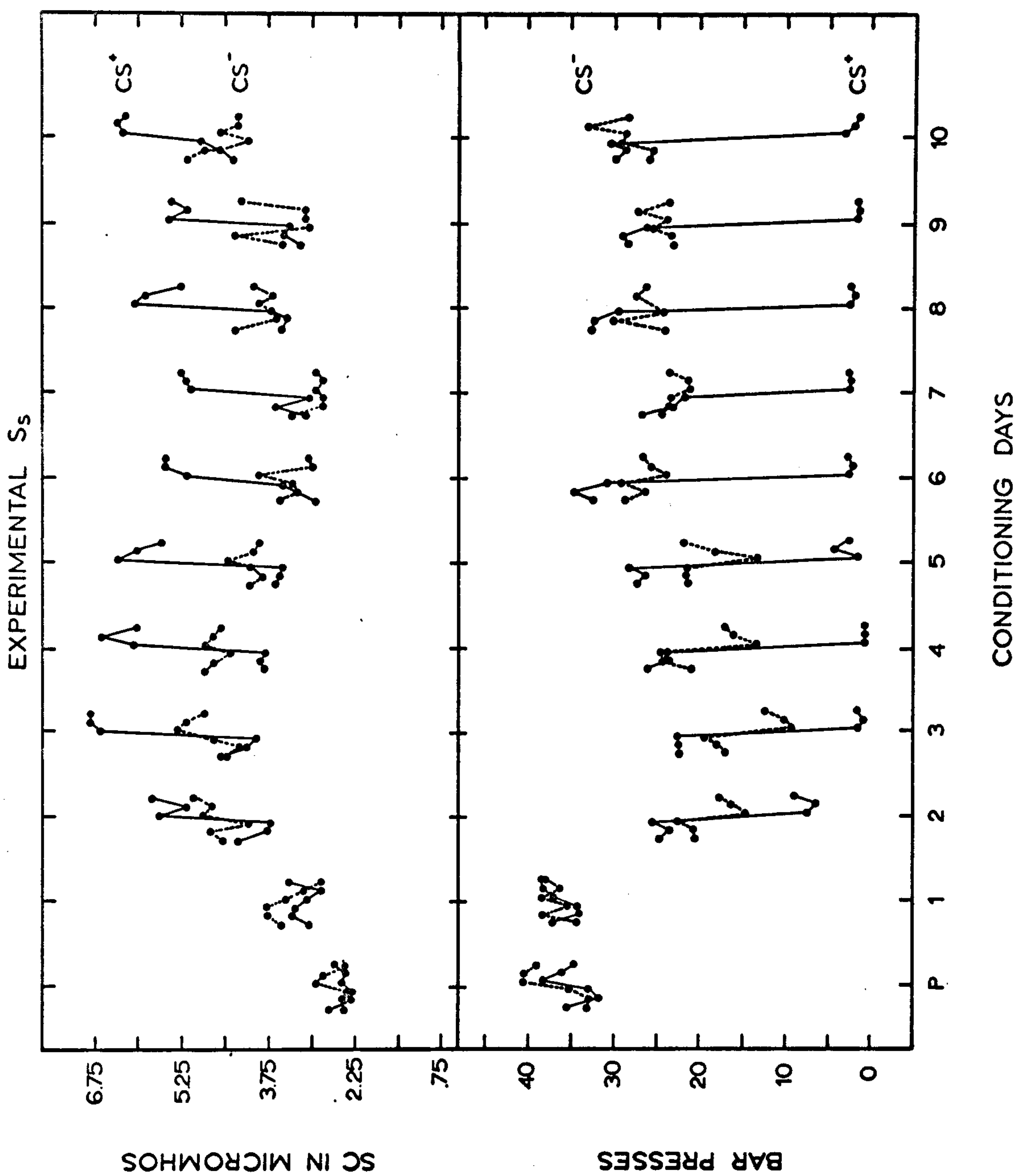
Traditional grid techniques are subject to contact-site artifacts, mainly due to the animals' contacting an increased number of grid bars when presented with an aversive stimulus. In view of the inadequacies of the standard grid methods, an improved recording technique was devised, which measured Skin Conductance (SC) from only one contact-site at a time and at the same time enabled a count of the number of recording sites in contact with the grid at any one time to be made. This technique employed a subdermal reference electrode chronically implanted under the skin of the rat's skull. A commutator connected each grid bar in turn to the subdermal reference electrode two times per second. SC was thus the conductance recorded between the rats' footpad and the reference electrode. Measurements were made in this improved way on one group of rats, the experimental group, and then also on a control group of rats whose SC was measured by the traditional grid method, but which was in all other ways treated exactly the same as experimental _S_s.

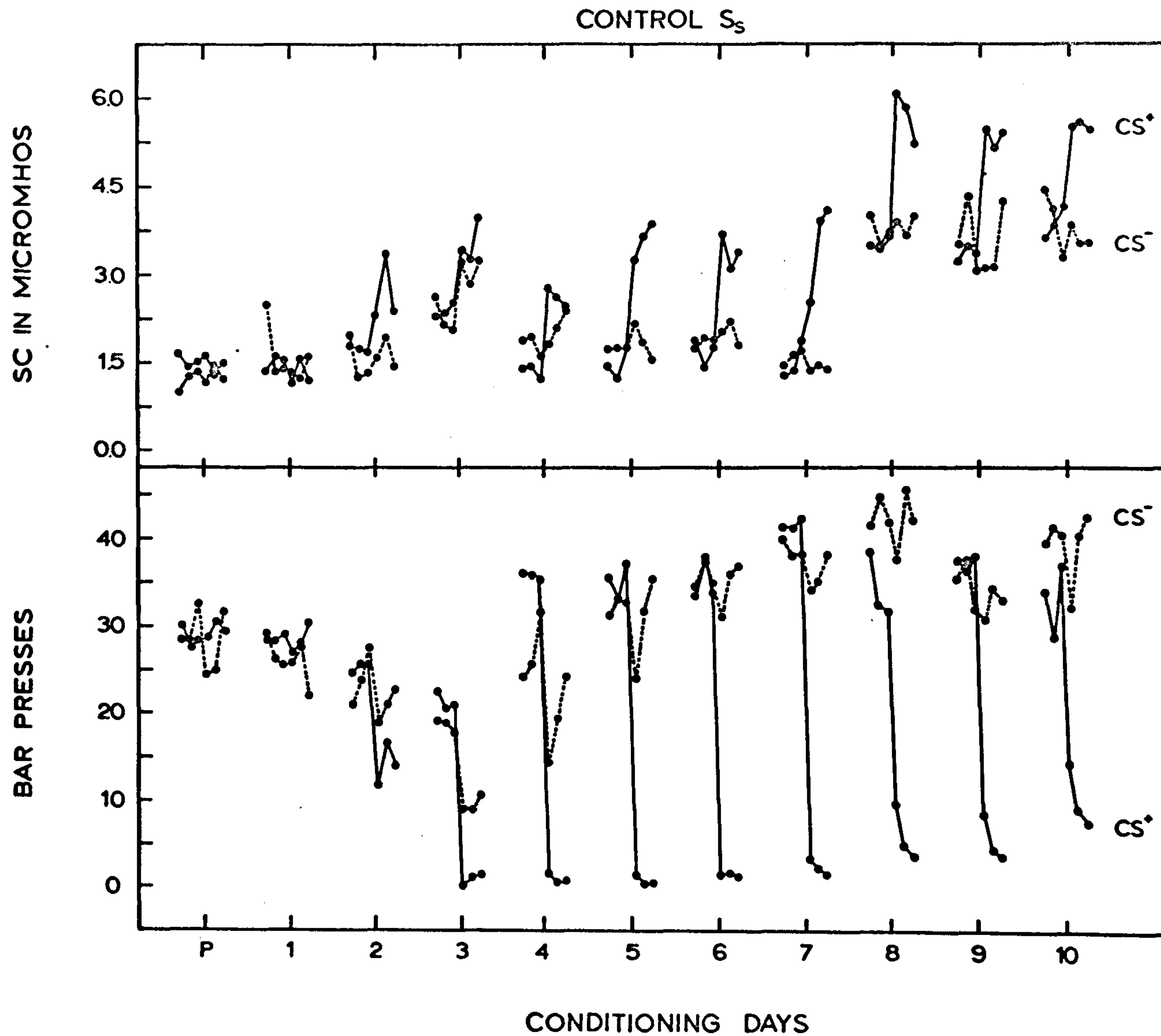
An increase in contact-sites was recorded from the experimental animals during the CS+, indicating that this contact area artifact does occur and so complicates the interpretation of results of studies where electrodermal responses are recorded by traditional grid methods. However, the second finding of this study was Conditioned suppression

of operant behavior was accompanied by an increase in SC amplitude (approximately 2.0 micromhos) in the experimental animals whose SC was measured by the improved grid technique which was free of contact area artifacts. The control group also showed reliable SC increases (approximately 1.0 micromho) attending conditioned suppression of bar-pressing. Suppression and SC responses appeared to be conditioned simultaneously in the experimental Ss. The results regarding SC conditioning were attributed to neural control of electrodermal activity rather than to measurement artifacts.

FIGURES AND TABLES


SC IN MICROMHOS
1.8
1.4
1.0
.6
.2
NUMBER OF ADDITIONAL CONTACT SITES
0
1
2
3
4
5


EXPERIMENTAL Ss
SC IN MICROMHOS
6.75
5.25
3.75
2.25
.75
CS^+
CS^-
BAR PRESSES
40
30
20
10
0
CS^-
CS^+
P
1
2
3
4
5
6
7
8
9
10
CONDITIONING DAYS


CONTROL Ss
SC IN MICROMHOS
6.0
4.5
3.0
1.5
0.0
CS+
CS-
BAR PRESSES
40
30
20
10
0
CS-
CS+
P
1
2
3
4
5
6
7
8
9
10
CONDITIONING DAYS

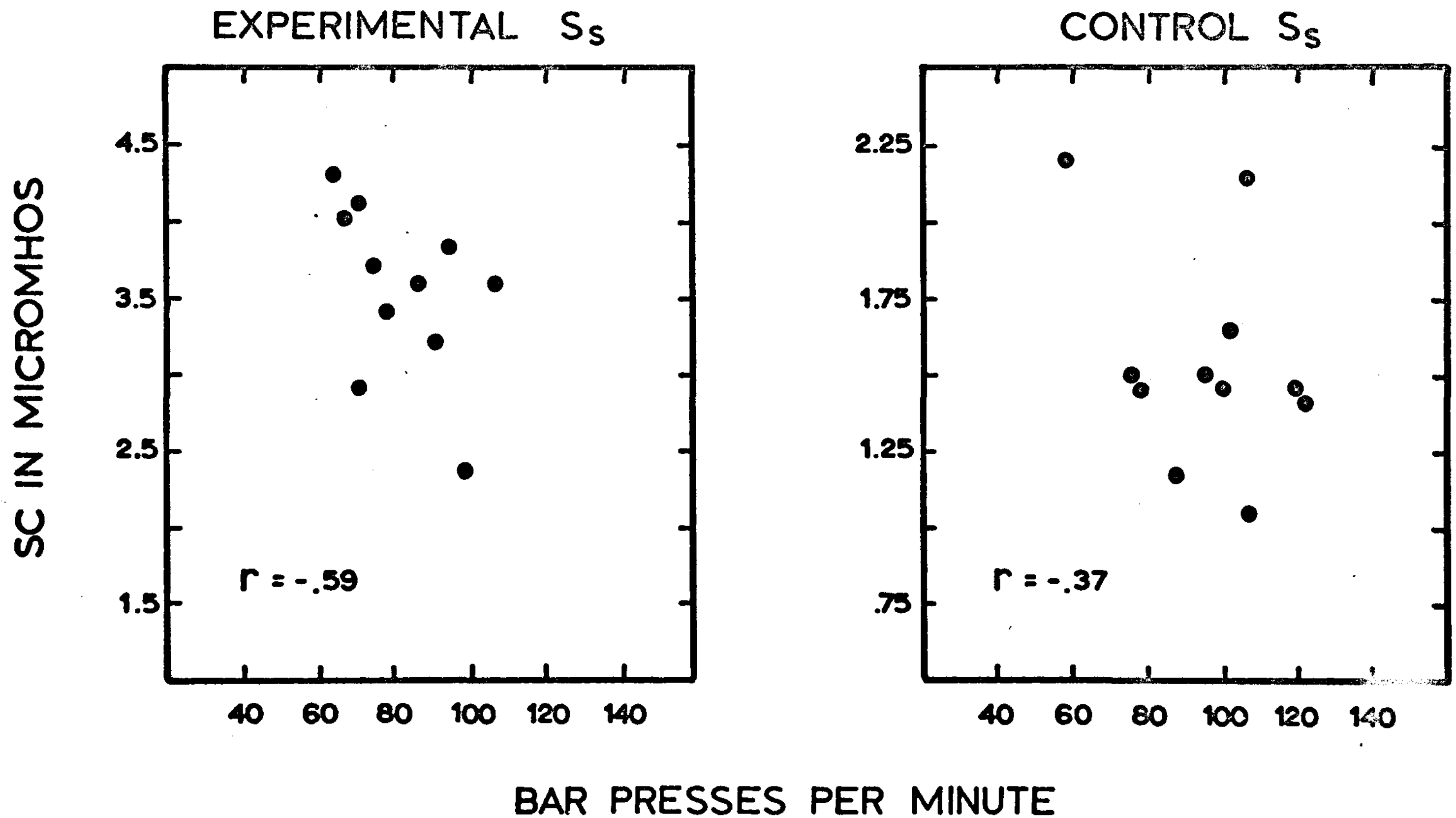

EXPERIMENTAL Ss
CONTROL Ss
SC IN MICROMHOS
4.5
3.5
2.5
1.5
2.25
1.75
1.25
.75
r = -.59
r = -.37
40 60 80 100 120 140
40 60 80 100 120 140
BAR PRESSES PER MINUTE

TABLE 1

Days on which frequency analysis was performed for each rat

Rat	Days Tested
21	C-11
31	C-10
32	C-10
43	C-10
44	C-10
54	C-10
46	C-10 & C-11
47	C-10 & C-11
48	C-10 & C-11
60	C-8 & C-9 & C-10

TABLE 2

T-tests on Response Amplitudes for Experimental Ss.

	PT			C-1		
	CS+	CS-	CS+-CS-	CS+	CS-	CS+-CS-
Δ Conductance	.301	1.07	.111	.67	-.71	.59
Δ Bar-pressing	.81	2.76*	.68	-.21	-.06	.49

	C-2			C-3		
	CS+	CS-	CS+-CS-	CS+	CS-	CS+-CS-
Δ Conductance	** 4.41	1.54	** 3.47	** 4.66	.72	** 3.64
Δ Bar-pressing	** -7.02	-1.79	** 3.75	** -23.25	-2.55	** 3.65

* $p < .05$ two-tailed test

** $p < .01$ two-tailed test

TABLE 3

T-tests on Response Amplitudes for Control Ss.

	PT CS+	PT CS-	PT CS+-CS-	C-1 CS+	C-1 CS-	C-1 CS+-CS-
Δ Conductance	-.85	.40	.61	-1.46	1.61	.50
Δ Bar-pressing	-2.50	-.096	1.18	-.71	-.31	-.52

	C-2 CS+	C-2 CS-	C-2 CS+-CS-	C-3 CS+	C-3 CS-	C-3 CS+-CS-
Δ Conductance	.94	1.92	.44	1.51	2.39	.28
Δ Bar-pressing	* -3.26	-2.50	* 3.07	** -19.62	-1.20	** 4.69

	C-4 CS+	C-4 CS-	C-4 CS+-CS-	C-5 CS+	C-5 CS-	C-5 CS+-CS-
Δ Conductance	1.81	.33	2.31	* 2.96	.16	* 2.76
Δ Bar-pressing	** -34.36	-2.36	** 9.10	** -64.98	-1.21	** 33.74

* $p < .05$ two-tailed test

** $p < .01$ two-tailed test

TABLE 4

Product-Moment Correlations from Pre-test through C-10 for all Ss.

RAT	EXPERIMENTALS	RAT	CONTROLS
21	-.52	59	-.41
31	-.31	61	-.30
32	-.48	62	-.22
43	-.38	66	-.56
44	-.51	67	-.52
46	-.28		
47	-.62		
48	-.41		
54	-.07		
60	-.41		
Mean	r = -.40		r = -.40

TABLE 5

Product-Moment Correlations for Days C-1 through C-4

EXPERIMENTAL		CONTROL	
Rat	r	Rat	r
21	-.60	59	-.53
31	-.38	61	-.17
32	-.50	62	-.11
43	-.57	66	-.65
44	-.60	67	-.62
46	-.49		
47	-.72		
48	-.43		
54	-.20		
60	-.32		
Mean	-.48	Mean	-.42

TABLE 6

Directional Correlations for all Ss.

EXPERIMENTAL						CONTROL					
Rat	Trials	+	0	-	d	Rat	Trials	+	0	-	d
21	33	3	0	30	-.82	59	35	3	3	29	-.74
31	30	3	1	26	-.77	61	30	9	3	23	-.40
32	34	1	0	33	-.94	62	35	8	1	26	-.51
43	36	4	3	29	-.69	66	32	0	0	32	-1.00
44	31	2	2	27	-.81	67	36	5	1	30	-.69
46	36	5	0	31	-.72						
47	36	2	0	34	-.89						
48	36	5	2	29	-.67						
54	36	8	1	27	-.53						
60	30	5	0	25	-.67						
Total	338	38	9	291	-7.51	Total	168	25	8	140	-3.34
Mean	33.8	3.8	0.9	29.1	-.75	Mean	33.6	5.0	1.6	28.0	-.67

TABLE 7

Mean Number of Contact-Sites on Grid
Before & During CS+

		Number of Contact-Sites	
RAT	TEST DAY	PRE CS+	CS+
21	C-11	2.57	3.34
31	C-10	2.85	2.26
32	C-10	1.94	2.45
43	C-10	2.51	2.53
44	C-10	2.48	3.54
54	C-10	2.76	2.19
46	C-10	2.54	2.50
	C-11	2.09	2.10
47	C-10	2.33	3.20
	C-11	2.30	3.15
48	C-10	2.47	2.98
	C-11	2.75	3.75
60	C-8	1.88	2.90
	C-9	1.88	2.84
	C-10	1.72	2.70
TOTAL:		35.07	42.43
MEAN:		2.34	2.83

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