AUTONOMIC AND BEHAVIORAL RESPONSES TO AN AVERSIVE STIMULUS

EFFECTS OF REPEATED TESTING AND EARLY HANDLING ON SKIN CONDUCTANCE, DEFECATION AND ACTIVITY IN AN AVERSIVE SITUATION

By

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SCOPE AND CONTENTS:

The purpose of the present experiments was to determine whether a change in skin conductance is a reliable component of the fear pattern in the mouse. In these experiments, the sight of E was employed as an aversive stimulus. SC and defecation increased and activity decreased when the stimulus was presented. The SC and defecation responses tended to adapt with repeated testing. Decreases in activity on Day 1 were replaced by increases on subsequent days. Early handling severely attenuated the SC, defecation and freezing responses that would be normally seen on the first day of testing.

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Table of Contents

Introduction . . . . . Page 1

Experiment 1 . . . . . Page 3

Experiment 2 . . . . . Page 14

Discussion . . . . . Page 18

References . . . . . Page 24

List of Figures and Tables

		Page
Figure 1	Effects of stimulus presentations on SC, defecation and activity for the first day of testing.	8
Figure 2	SC, defecation and activity before and during stimulus presentations as a function of trials.	9
Figure 3	SC, defecation and activity before and during stimulus presentations as a function of days of testing.	11
Figure 4	Amplitude of the activity response (levels before subtracted from levels during) as a function of trials and days of testing.	12
Figure 5	Minute by minute plot of SC, defecation and activity for the handled and nonhandled groups (Day 1 only).	17
Figure 6	SC response observed in one of the nonhandled Ss.	20
Table 1	Between-Ss correlations.	23

INTRODUCTION

When an aversive stimulus is presented to a mouse, a pattern of responses is observed that includes defecation, freezing behavior, and sometimes urination as well. The usual interpretation of this observation is that the responses of the pattern are regulated by a central neural mechanism for fear. The objective of the present research was to determine whether a change in skin conductance (SC) is a reliable component of this pattern.

The question of whether a change in SC is a component of the fear pattern may be investigated in two ways. First, if SC is regulated by the same mechanism that governs defecation and freezing behavior, there should be a reliable change in the level or probability of all these responses when an aversive stimulus is presented. Furthermore, these responses should return to their pre-stimulus levels when the stimulus is removed. Experiment 1 tested these predictions, and examined changes in the response pattern as a function of repeated testing. Second, experimental manipulations which reduce emotional defecation and freezing should also attenuate the SC response to an aversive stimulus. Experiment 2 tested these predictions by subjecting Ss to early handling, which has been shown to reduce defecation and freezing in the open field (Levine, 1962).

The present experiments employed a grid method to assess SC responses to presentations of an aversive stimulus. Although grid methods have the advantage of allowing S freedom of movement, they are

also susceptible to a variety of artifacts that can contribute to the electrodermal phenomena recorded (Roberts, 1967). The problem posed by these artifacts is that it is difficult to determine whether the SC changes observed are due to the central neural control of epidermal conductivity, or to artifacts inherent in methods of electrodermal recording. Although the present experiments were not specifically designed to evaluate the contribution of central and artifactual processes to electrodermal correlates of fearful behaviors, they do provide data relevant to this problem.

EXPERIMENT 1

Roberts (1965) has observed that freezing, defecation and a marked increase in SC accompanied the mouse's catching sight of E. Thus, the sight of E appeared to be an adequate stimulus for eliciting fearful behaviors. The present attempt to determine whether a change in SC is a reliable component of the fear pattern involved a systematic application of this procedure.

METHOD

Subjects and Apparatus

The Ss were 25 female mice, 55-93 days of age, drawn from the F_2 population of a cross between the C57BL/6J and A/J inbred lines. Ss were housed with like-sexed littermates in cages with sawdust floors from birth until testing. The experimental and colony rooms were illuminated 12 hr. daily, starting at 7:30 A.M. All testing was done between 7 and 11 P.M.

The apparatus was a shuttlebox 3½ X 11 X 6 in. Two 7½ w. 110 v. frosted lights were mounted directly above the Plexiglas lid of the shuttlebox, and the entire apparatus was securely fastened in a sound-attenuating, air-ventilated cubicle equipped with a one-way mirror. The floor of the shuttlebox was divided into two independent grids constructed of 1/8 in. stainless steel rods centered 5/16 in.

apart. Each grid was spring-suspended and equipped with a set of normally-closed electrical contacts that could be held open by a weight of 5 gm. (the lightest S weighed 18.1 gm.). The maximum vertical movement of the grids was about 1/16 in. This arrangement permitted recording of locomotor activity (crosses between grids) by a marking stylus on the oscillograph used for recording SC.

Instruments used in measuring SC included a 4.8 v. battery which served as power supply, a Davis 255 grid-shock scrambler, and a Hewlett-Packard 7701A oscillograph. The power supply was connected to the grids through the scrambler and a 280 kilohm series resistor. The voltage developed across the series resistor varied with the conductance of S and was amplified and recorded by the oscillograph. Conductance was determined by comparing each voltage record (herein called a conductance record) to a calibration curve constructed when 15 precision resistors of known value were substituted for S before testing. This recording method maximized the resolution of conductance records in the range where changes due to fear were most likely to occur. The scrambler was used to eliminate gaps in the conductance record that would otherwise have occurred when S contacted a pair of equipotential grid bars. The average current flow, taking S's resistance into account, was about 5μa.

Procedure

S was placed in the apparatus for 35 minutes. At the start of

minutes 6, 12, 18, 24 and 30, E opened the lids of the test cubicle and shuttlebox, and brought his face to within about 10 in. of S. Opening of the lids was accomplished in about 1-2 sec. After one minute, E withdrew and replaced the lids. These one minute periods will be referred to as stimulus presentations. Ss were tested for 35 minutes on each of four consecutive days. SC and crossing activity were recorded throughout, and occurrences of defecation and urination were noted by E.

Analysis of Data

Five SC readings were taken at 12 sec. intervals for each of the minutes before, during and after a stimulus presentation. Medians of these five values provided a measure of SC for each minute of the trial. SC medians were then estimated for each of the intertrial minutes by visual inspection of the conductance records. Conductance readings distorted by feces shorting out the grid were excluded from the analysis.

Response amplitudes were determined for SC, defecation and activity by subtracting levels observed before a stimulus presentation from those observed during. Analyses of variance were then performed on SC, defecation and activity response amplitudes, with Ss, trials and days as variates (lindquist, 1956, p. 237). SC measurements before and during stimulus presentations were analyzed further by averaging SC over days and then over trials. These means were then subjected to

three-way analyses of variance, in which Ss, minutes (before or during), and either trials or days served as variates (Lindquist, 1956, p. 237). The significance levels reported herein were taken from these analyses.

RESULTS

Figure 1 shows the effect of stimulus presentations on SC, defecation and activity for the first day of testing. Here, the three dependent variables are plotted for the minutes before, during and after each stimulus presentation. It is clear that presentation of the stimulus led to marked increases in SC and defecation on all five trials. On Trial 1, for example, SC increased for 23 of the 25 Ss, and defecation increased for all 25. Locomotor activity was depressed in the presence of the stimulus, indicating increased freezing behavior, but this did not persist beyond the third trial. The incidence of urination was very low. In particular, six Ss urinated once in the presence of the stimulus on the first day of testing, whereas on subsequent days a total of 11 urinations was observed during stimulus presentations.

Figure 2 shows in more detail how the response pattern changed as a function of trials. In this graph, levels before and during stimulus presentations are averaged over the four days. The amplitude of the conductance response did not change appreciably, but there was a significant decrease in pre-stimulus and during-stimulus levels of SC ($p<.01$). The amplitude of the defecation response underwent a clear decrease ($p<.01$); examination of Figure 2 reveals that this was due to a decline in the amount of defecation occurring in the presence of the stimulus. Changes in the amplitude of the activity response were more complex. The first two trials were characterized by decreases due to

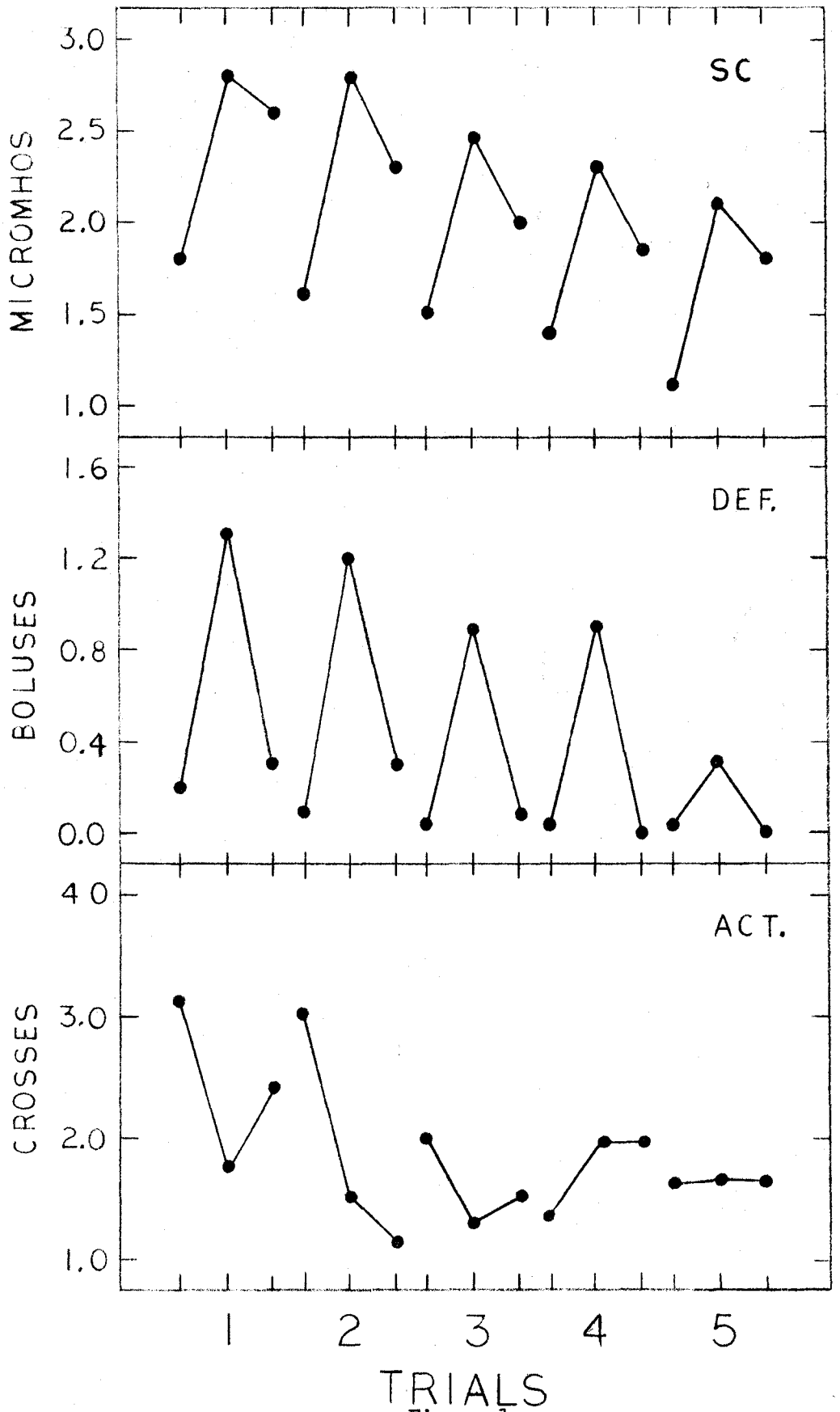

Figure 1

Effects of stimulus presentations on SC, defecation and activity for the first day of testing.

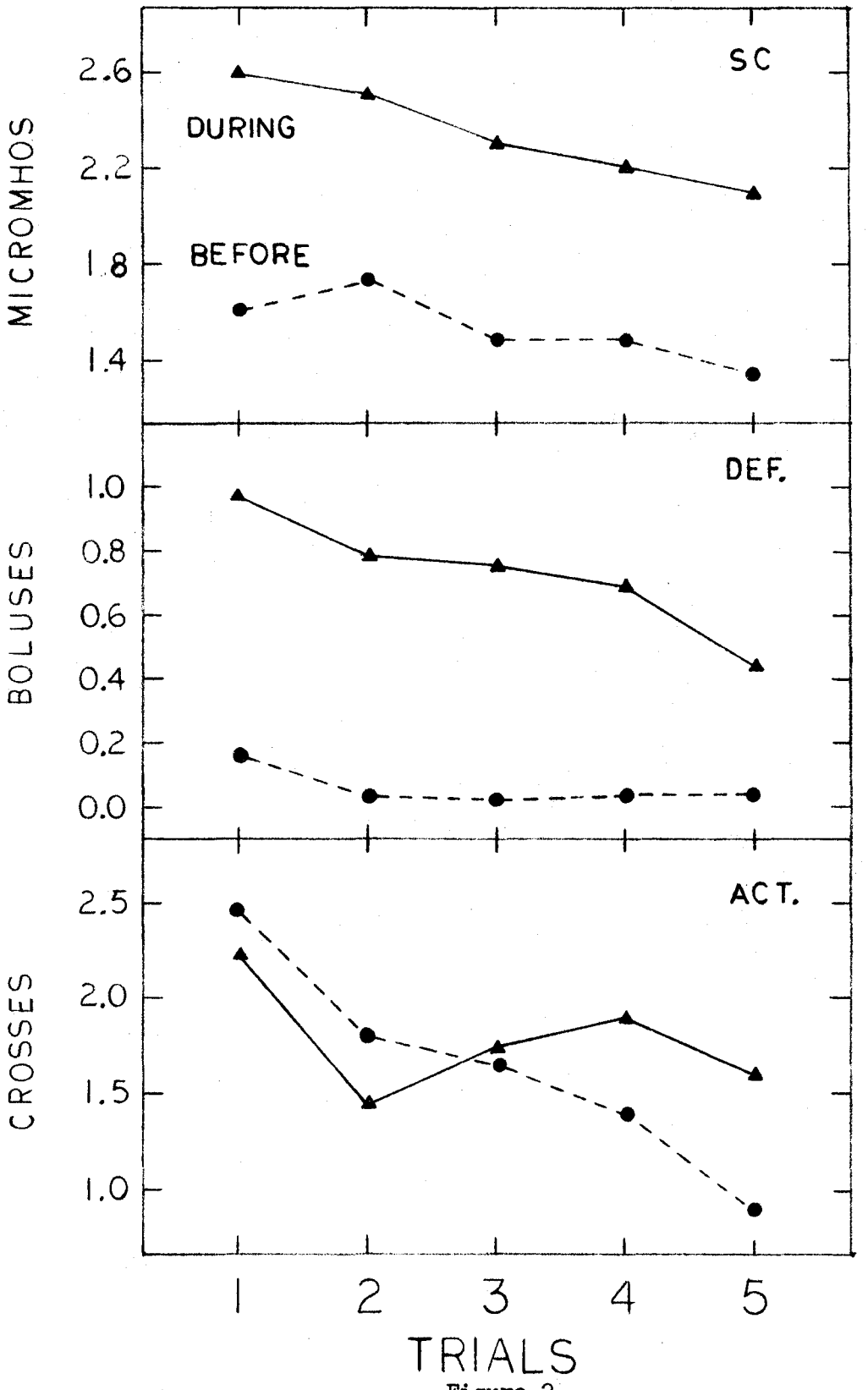

Figure 2

SC, defecation and activity before and during stimulus presentations as a function of trials.

freezing, whereas on the remaining trials an increase in activity was observed.

Figure 3 describes changes in the response pattern as a function of days, averaged over trials. The amplitudes of the SC and defecation responses both showed significant decreases here ($p<.01$), and activity again exhibited the reversal effect found for trials. Inspection of the activity data of Figures 2 and 3 reveals that the change in the direction of the activity response was attributable to a steep decline in pre-stimulus activity, rather than to major changes in activity observed during stimulus presentations.

In addition to the trials and days effects reported above, there was a significant Trials X Days interaction for activity. The nature of this interaction is examined in Figure 4, where the amplitude of the activity response (level before subtracted from level during) is plotted as a function of trials for each of the four days. It may be seen here that most of the decreases in activity occurred on the first three trials of Day 1, and that increments in activity became increasingly more prominent as testing progressed. Observation of the animals suggested that the increments in activity were due to an increase in frequency of escape attempts. Therefore, this interaction indicates that the activity response to an aversive stimulus may consist of either freezing or escape behavior, and that the degree of experience with the stimulus is one of the factors which determines which behavior will be elicited. Interactions involving SC and defecation were not significant.

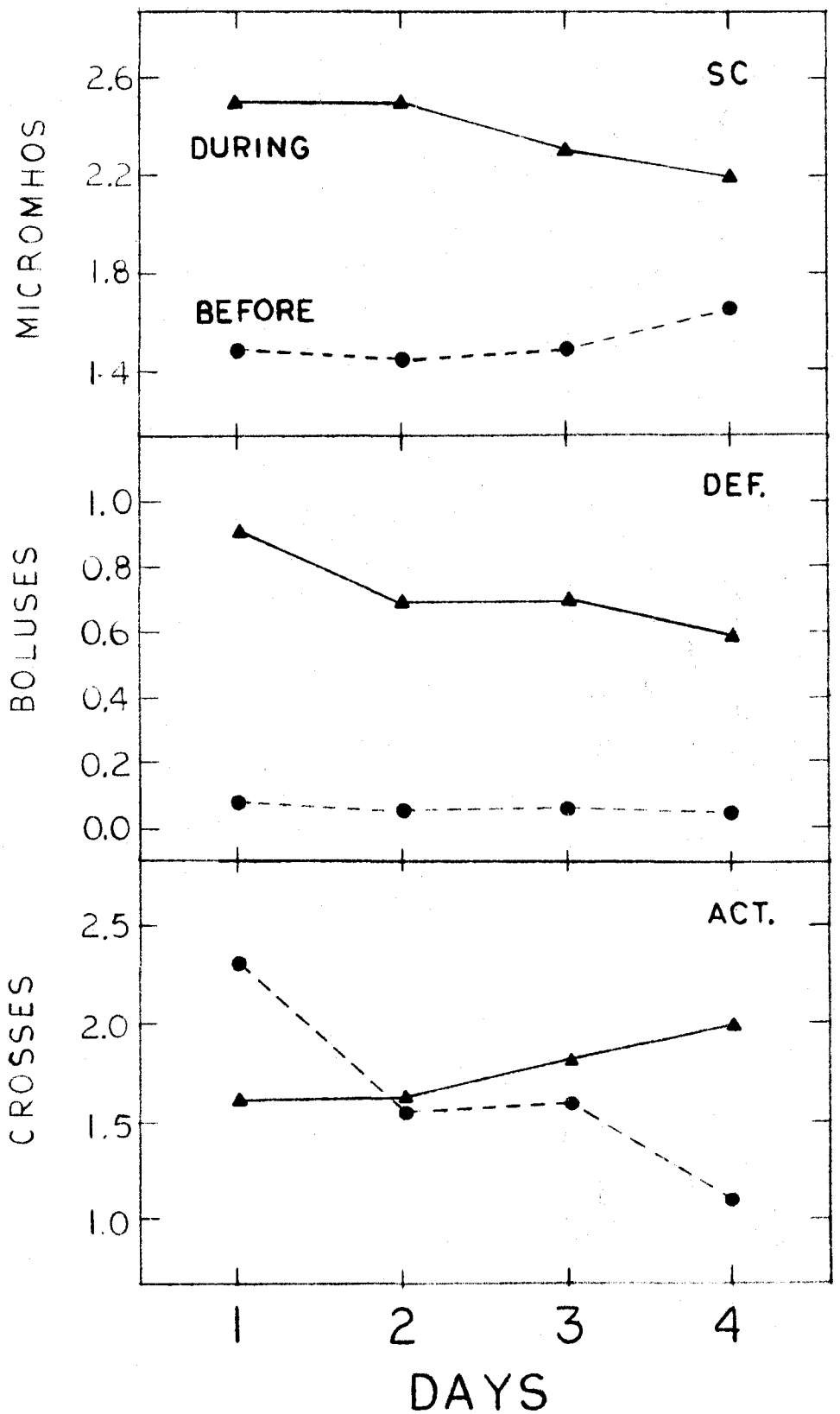

Figure 3

SC, defecation and activity before and during stimulus presentations as a function of days of testing.

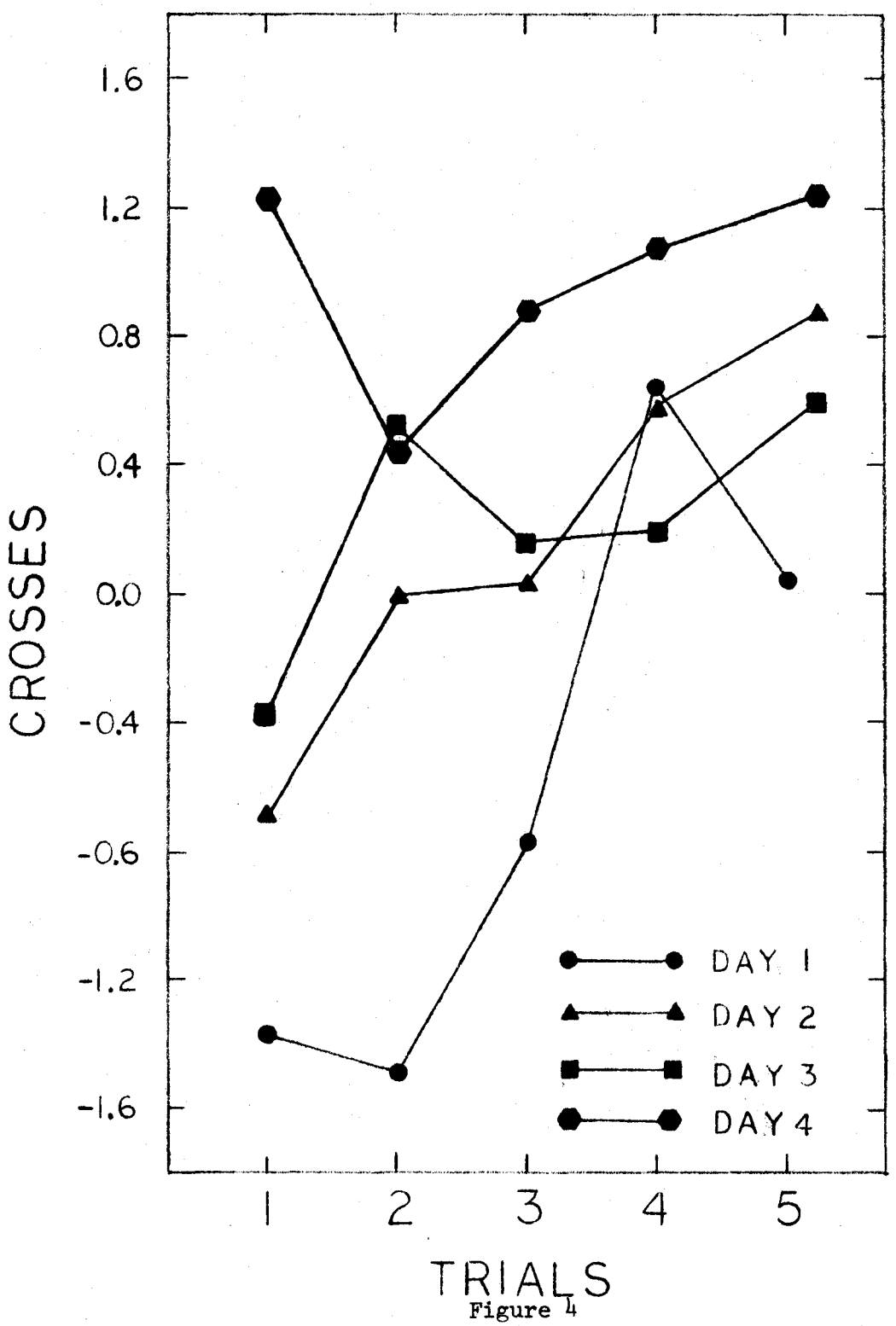

Figure 4

Amplitude of the activity response (level before subtracted from level during) as a function of trials and days of testing.

In summary, Experiment 1 has shown that increases in SC reliably accompany repeated elicitations of defecation and freezing or escape-oriented behavior. These results lend support to the contention that a change in SC is a component of the fear pattern, or, stated somewhat differently, that SC is regulated by the same mechanism that governs these fearful behaviors.

EXPERIMENT 2

If SC is regulated by the same mechanism that governs defecation and freezing or escape behavior, then an experimental manipulation which reduces the defecation and activity responses to an aversive stimulus should also attenuate the SC response to that stimulus. Experiment 2 provided a direct test of this hypothesis. The experimental manipulation was early handling, a procedure which is known to reduce emotional reactivity in the open field (Levine, 1962).

METHOD

Subjects and Apparatus

Ss were 12 female mice, drawn from two litters of the same F_2 population that was the source of Ss for Experiment 1. The apparatus was the same as in Experiment 1.

Procedure

Ss were subjected to daily handling from 3 to 28 days of age. Briefly, this consisted of removing S from the home cage, stroking its back for approximately one minute, and then depositing S on the sawdust floor of a fresh cage. After Ss had been handled in this manner, they

were returned to the home cage one-by-one. The time spent away from the home cage was approximately 15 minutes. At 28 days of age, Ss were weaned and thereafter received treatment identical to that given to Ss in Experiment 1, which consisted of routine cage cleaning every two weeks.

Ss were tested for a single 35 minute session at 80 days of age, according to the procedure outlined in Experiment 1.

RESULTS

Figure 5 compares the behavior of the handled group with the Day 1 data for Ss used in Experiment 1, which were not exposed to a specific handling procedure. Here, SC, defecation and activity are plotted for each of the 35 minutes of the test session. It is clear that each component of the response pattern was greatly reduced or completely abolished by early handling. Also, the overall level of activity was much higher in the handled group ($p<.01$), a frequent finding in early handling experiments. Furthermore, Figure 5 demonstrates that SC decreased in both groups during the first five minutes of the test session, a finding which replicates the results reported by Roberts (1967), Walters and Tullis (1966), and other investigators.

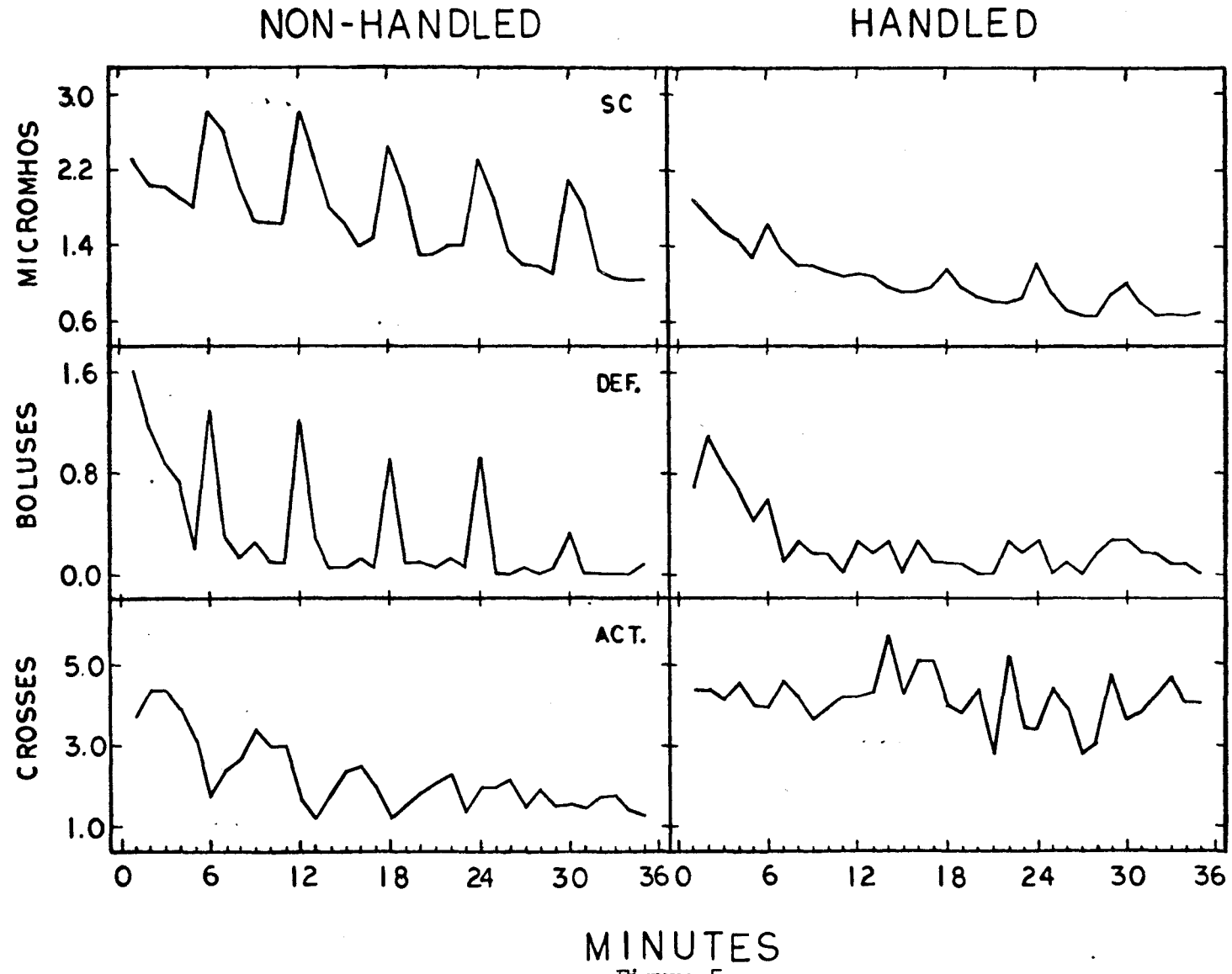

Figure 5
Minute by minute plot of SC, defecation and activity for the handled and non-handled groups (Day 1 only).

DISCUSSION

The first of the present experiments has shown that reliable increases in SC accompany defecation and freezing or escape behavior in the presence of an aversive stimulus. Experiment 2 has shown that early handling severely reduces the SC, defecation and freezing responses that would normally be observed on the first day of testing. These results indicate that the SC response is controlled by the same mechanism that governs fearful behaviors.

A question of considerable importance, however, concerns the way in which the SC response is determined. Two fundamentally different processes may contribute to the electrodermal correlates of fearful behaviors. First, it is possible that epidermal conductivity is increased by a sudomotor volley originating in brain centers that control sweating (Wang, 1964). Alternatively, one could argue that the SC response is the result of artifacts that are peculiar to grid methods of electrodermal recording. For example, the urination response that sometimes appears when a mouse is made fearful can result in an increase in conductance simply because the grid and the mouse's footpads are moistened. Another way in which an artifactual increase in SC could be generated is by an increase in contact area as a result of freezing. According to this view, a mouse displaying a freezing response could contact more grid bars, thus providing additional paths of current, or he might grip the grid bars with greater force, thereby increasing

contact area and perhaps contact pressure as well.

Although the present experiments were not specifically designed to evaluate the contribution of each of these artifacts to the conductance responses, they do provide data relevant to this problem. Urination artifacts could not have played an important role in these experiments because urination occurred on less than 4% of all stimulus presentations. The contribution of contact area artifacts to the SC response is more difficult to evaluate. Although it is likely that increases in the number of grid bars contacted contributed to some of the conductance responses, there were many instances in which this artifact could not have been involved. Consider, for example, the response shown in Figure 6. The smooth texture of the recording shown here is due to the action of the grid-scrambler in the recording system, and indicates periods when the mouse's position on the grid remained constant. Thus, the response of Figure 6 cannot be attributed to an increase in the number of contact sites because S's position on the grid did not change when the stimulus was presented. Increments in SC in the absence of apparent movement were elicited by a total of 86 of the 500 stimulus presentations. It is also worthwhile noting that in order to attribute responses like those of Figure 6 to strengthening of grip attending freezing, one would have to assume that strength of grip increases, not suddenly, as seems most likely, but gradually over a period of 2 to 10 sec. Further evidence against the hypothesis that the SC response is due solely to contact

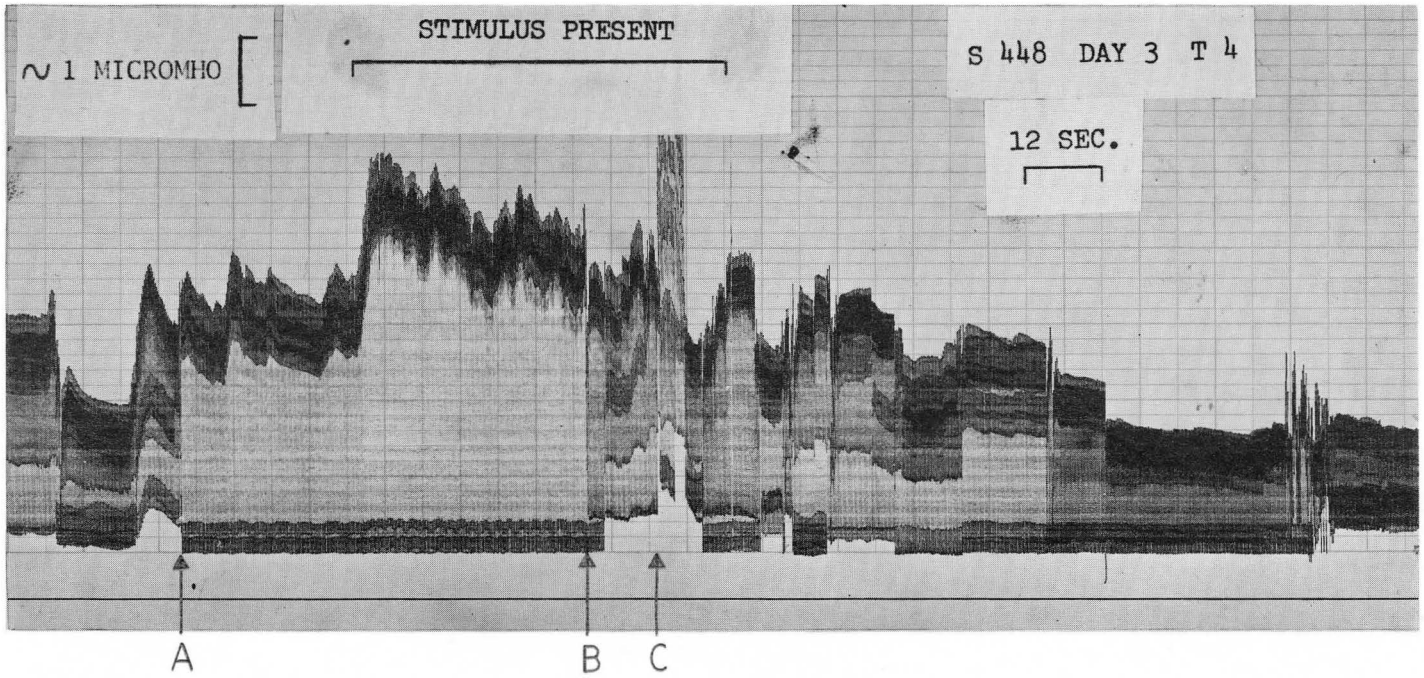

Figure 6

SC response to presentation of the stimulus observed in an S from the nonhandled group. The smooth texture of the portion of the record between arrows A and B indicates that S was motionless on the grid during that period. At the onset of the stimulus, SC increased by about 2.4 micromhos. The abrupt increase in SC at arrow C is an example of a feces short.

area artifacts attending freezing is provided by the finding that the direction of the activity response reversed as testing progressed, whereas conductance continued to change in the same direction.

Other evidence suggests that central neural processes contribute to conductance responses which accompany fearful behaviors. It is known that rats, and probably all rodents, have sweat glands in the footpads that are regulated by the sympathetic branch of the autonomic nervous system (Ring and Randall, 1947; Wagner, 1950). The defecation data of Figure 1 indicate an increase in autonomic activation that would have been expected to involve the sweat apparatus as well. The view that central neural factors contribute to the electrodermal correlates of fearful behaviors is given additional support by Roberts and Young (1968), who attached electrodes to the rear feet of restrained rats, and recorded SC and skin potential (SP) during discriminative CER training. They found that a large increment in SC (about 1 micromho) attended the suppression of operant behavior, and that the SC response was accompanied by increased negativity of SP. These electrodermal responses could not be attributed to contact area artifacts, because the recording electrodes were attached directly to the feet, and because SP, which is determined at least partly by the effector for SC, is independent of contact area, at least in the human (Lykken, Miller and Strahan, 1968). These results suggest that, while artifacts may have contributed to the conductance responses observed in the present experiments, there are reasons to believe

that there was an important central contribution as well.

The present research has shown that a change in SC as measured by grid methods is a reliable component of the fear pattern. This suggests that grid assessments of SC may provide a reasonably good measure of changes in the level of fear within an individual animal. A second question concerns the sensitivity of SC as an indicator of individual differences in fearfulness. In an attempt to answer this question, between-Ss correlations were computed among various indices of SC, defecation and activity, and are presented in Table 1. The most salient feature of these correlations is that they are very low. In fact, only 4 out of the 36 correlations reach statistical significance. These results indicate that SC, as presently measured, does not appear to be a sensitive indicator of individual differences in fearfulness. This is not particularly surprising, since there are probably several factors, such as thickness of the skin, density of sweat glands and other characteristics of the electrodermal effector, which are not related to individual differences in the level of fear, but are nevertheless involved in the generation of differences in SC.

TABLE 1

Between-Ss Correlations

	SC-Defecation			SC-Activity			Defecation-Activity		
	Before	During	Amplitude	Before	During	Amplitude	Before	During	Amplitude
Day 1	.199	-.052	.147	-.476	-.269	-.071	-.260	.388	.159
Day 2	.403	-.328	-.078	-.269	-.439	-.215	-.152	.215	-.309
Day 3	.012	-.126	-.020	-.191	-.509	-.177	.040	.222	-.024
Day 4	.078	.201	.133	-.283	.101	-.163	-.140	.324	-.070

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