Effects of infantile handling on heart rate conditioning and response suppression*

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Male and female Holtzman rat pups were either handled or left undisturbed during Days 2-5 after birth. When tested at about 55 days of age, there were no differences in heart rate during classical conditioning attributable to the handling treatment. Handling did, however, increase lick suppression during CER extinction.

Infantile stimulation has been shown to produce a variety of behavioral and physiological changes in the adult organism. Although the mechanisms by which these early treatments affect the animal remain uncertain, it is generally considered that a change in emotional reactivity is involved (Denenberg, 1964). Russell has concluded in his review of the literature on infantile stimulation in rodents that most of the data of the studies cited can be accounted for by the hypothesis that early noxious stimulation "serves to immunize the animal against excessive reaction to stress in adulthood [Russell, 1971, p. 199]." It should be noted, however, that this hypothesis often is of little help in predicting the effects of specific early treatments on various indicants of emotionality in specific test situations.

Despite the considerable effort which has been focused on this issue, few studies have employed the conditioned emotional response (CER) task to assess effects of early experience. In two studies reported by Hutchings (1963), it was found that animals that were exposed to daily severe cooling treatments during the first week resumed barpressing during CER extinction more slowly than did nontreated controls, whereas animals given a mild cooling treatment resumed barpressing faster than did the control animals. There were, however, group differences in acquisition of the barpress response which make interpretation of differences in subsequent response suppression difficult.

One purpose of the present experiment was to assess early handling effects on the extinction rate of the CER in a situation designed to preclude differences in acquisition of the response measured. Tenen (1967),

as well as Caul & Barrett (1972), has emphasized the sensitivity of measures of recovery of licking as indicants of CER strength. The second purpose was to assess possible effects of early handling on subsequent heart rate responding during classical aversive conditioning which preceded lick suppression testing. Boyles, Black, & Furchtgott (1963) have reported that manipulations of the home cage environment after weaning affected heart responsivity to stimulation during adulthood. Finally, since marked sex differences have been reported using several indicants of emotionality (e.g., Gray, 1971), possible sex effects on heart rate and lick suppression were of interest.

SUBJECTS

The Ss were the offspring of pregnant rats purchased from Holtzman Co., Madison, Wisconsin. Five male and five female pups were assigned randomly to the handling groups (HM and HF), while five animals of each sex were assigned to nonhandled groups (NM and NF).

APPARATUS

Two testing boxes were used and have been described in detail by Caul & Barrett (1972). The drinking box was a Foringer operant box modified by installing a Masonite end wall with a 2-cm-diam port centrally placed 6 cm above the grid floor. A water bottle was mounted behind the wall such that the tip of the metal drinking tube was centered in the port but did not protrude into the animal chamber. Licks were recorded via a Grason-Stadler drinkometer circuit connected to the grid floor and the drinking tube. The conditioning box had a grid floor through which the scrambled footshock US was delivered (1 mA for 0.5 sec). The conditioned stimulus (CS) was an 18.5-sec 1-kHz tone at 80 dB which interrupted the white noise background of 75 dB. The US was presented during the final 0.5 sec of the CS.

PROCEDURE

Handling procedures began when the pups were 1 day old and continued

for 4 days. The handling procedure was conducted in the colony room, which was maintained at a constant 25°C. It consisted of removing the mother from the cage, removing the home cage from the rack, and carrying it to a table. Each pup was then transferred to a tissue paper "nest" which was on the surface of a Fisher Autempt heater maintained at 30°C. Each pup was then held in the E's hand for 1 min and returned to the heated "nest." After all the pups were handled, they were replaced in their home cage, which was returned to the rack and the mother replaced. This procedure resulted in the litter being disturbed for approximately 15 min. After the fourth handling day, the handled Ss were left undisturbed until weaning at 28 days. The nonhandled animals were undisturbed during the entire preweaning period. At weaning, all Ss were rehoused in pairs of the same sex and litter in standard 18 x 18 x 25 cm wire mesh cages.

At 45 days of age, the rats were housed individually and deprived of water. Animals were given access to water for 20 min each day in their home cages for the remainder of the experiment. This procedure has been found to maintain the animals' health and body weights yet guarantee a water-deprived state at the next daily test session (Caul & Barrett, 1972). On the fourth day of this deprivation schedule, each S was placed in the drinking box with water unavailable for 10 min. After this exposure to the box, heart rate leads were implanted subcutaneously along the midline of the back under light ether anesthesia. Lick training began the following day. On each day of lick training, the rat was placed in the drinking box with the drinking tube present for 10 min or until 600 licks were recorded, whichever occurred first. Training continued daily until the criterion of 600 licks within 4 min was reached during three training sessions.

The day after a rat reached lick training criterion, it was placed in the conditioning box and given four classical conditioning trials. The first trial started 2 min after heart rate leads were attached and the animal was placed in the conditioning box. The other trials followed at intertrial intervals of 2 min. Heart rate was recorded for 18 sec prior to CS onset (pre-CS period) and during the CS.

Lick suppression testing began the day after the conditioning session. After the rat had licked 100 times, heart rate and lick responses were recorded for an 18-sec pre-CS period, as well as during the subsequent 18-sec presentation of the CS. The animal was removed from the drinking box after licking 50 times after the

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Fig. 1. Mean pre-CS heart rate and mean heart rate response for the CS over four conditioning trials.

termination of the CS or after 10 min, whichever occurred first. Each animal was given one suppression trial at the same time each day for 7 days. No footshock was ever delivered in the drinking box. The 20 min of access to water in the home cage was provided within 1 h of the lick suppression session but not immediately after it. Food was available ad lib during the entire experiment.

RESULTS

The heart rate data for the conditioning session are shown in Fig. 1. Analysis of the pre-CS heart rate shown in the left panel of the figure indicated that pre-CS heart rate decreased over the four conditioning trials ($p < .001$) and that this decrease depended on the sex of the animal ($p < .05$). From Fig. 1, it is apparent that this Trials by Sex interaction resulted from the tendency for females to show a higher pre-CS heart rate prior to the initial trial and a lower pre-CS rate by Trial 4 than was observed in the males. There was no effect of the handling treatment on pre-CS heart rates. Analysis of the heart rate responses to the CS shown on the right of Fig. 1 indicated that there was an overall response to the CS ($p < .05$) which changed in magnitude over the four trials ($p < .001$). Neither sex differences nor handling effects were apparent in this measure.

Groups acquired the licking response equivalently. All Ss, with the exception of one HF, met criterion

within 5 days. One additional training day was needed for this HF animal.

The mean lick suppression ratio (CS licks/pre-CS licks + CS licks) for each of the seven suppression trials for each group is shown in Fig. 2. As can be seen from the figure, recovery from the complete response suppression which occurred on the first trial was faster for the nonhandled groups (NM

and NF) than for the handled Ss (HM and HF) ($p < .001$). Neither the main effect of sex nor the apparent Handling by Sex by Trials interaction were reliable ($F < 1$ in each case).

Both the measures of pre-CS heart rate and heart rate response to the CS recorded during the seven suppression trials failed to show reliable sex or handling effects. While there was an overall decrease in mean pre-CS level over the seven trials from 493 bpm on Trial 1 to 481 bpm on Trial 7, there were no group differences. Similarly, while there was an overall reliable bradycardic response to the CS ($p < .001$) which decreased over trial ($p < .001$) during suppression testing, there were no differences in response to the CS attributable to sex or the handling treatment.

DISCUSSION

The finding presented in Fig. 2 that handled Ss resumed lick responding during extinction of a CER more slowly than did nonhandled controls agrees in a sense with the first study reported by Hutchings (1963), who measured extinction of the CER using a barpress response following early exposure to a severe cooling treatment. In the second experiment, however, he showed that the opposite result was obtained when the early cooling treatment was not as severe as in his original study. If, as seems reasonable, based on the data of rectal temperature changes observed with various handling procedures (Hutchings, 1963), it is assumed that our treatment was unlike his severe treatment in terms of temperature change (temperature drop of 7°-8° C),

Fig. 2. Mean suppression ratios over 7 test days of one trial per day.

then the increased response suppression observed in handled animals relative to nonhandled Ss of the present study is an unexpected finding. In the absence of data regarding rate and extent of treatment-induced temperature change in the animals used in this study, however, it is difficult to resolve the apparent inconsistency between the Hutchings results and those presented here. Such data would be relevant to the hypothesis that the effects of early handling are related to a change in body temperature which occurs during the infantile treatment (e.g., Schaefer, 1968; Hutchings, 1968; McIver, Jeffrey, Stevenson, & Neilson, 1968). Further evaluation of early treatment effects on CER extinction is needed in order to specify the components of the treatment which produce such marked changes in response suppression and to identify the mechanisms by which such effects are produced. The present results seem to support the conclusion of Russell, who, after noting data not accounted for by the cooling hypothesis, stated that "... it seems necessary to assume that at least two mechanisms are involved. One of these must be based on cooling, with the nature of the second being unclear but possibly involving some form of direct action [Russell, 1971, p. 196]."

The prolonged CER responding in the handled groups may be attributable to altered sensitivity to shock produced by the early treatment. May (1967) has reported that Holtzman pups handled once on Day 7 were more sensitive to differences in shock intensity than nonhandled controls when tested at 71 days of age. This result was obtained for groups that showed no differences in mobility scores recorded just prior to shock preference testing. In addition, it has been shown that rats handled in infancy display increased plasma corticosterone levels in adulthood at the termination of shock

(Haltmeyer, Denenberg, & Zarrow, 1967). This consideration adds to the complexity of interpreting data from behavioral test situations which involve shock. In the present study, it was hoped that any differences in shock sensitivity would be apparent in the heart rate measure taken during the four trials of CS-US pairing (e.g., Fitzgerald & Teyler, 1970). No effect of the handling treatment was observed in either pre-CS heart rate level or in heart rate response to the CS (Fig. 1).

To the extent that prolonged extinction of response suppression reflects heightened emotionality, the present results are in contradiction to the position, based largely on open-field mobility data, that infantile stimulation results in decreased emotionality. Clearly, to adequately understand the effects of handling upon measures of emotionality, research with early treatments is needed to clarify (1) the mode of action of such treatments (e.g., Russell, 1971; Thoman & Levine, 1969), (2) the conditions under which the dependent variables commonly used as indicants of emotionality covary (e.g., Candland & Nagy, 1967), and (3) the reasons for difficulty often encountered in attempts to replicate studies involving early treatment effects on subsequent adult behavior (e.g., King, 1970).

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