DIFFERENTIAL CONDITIONAL EMOTIONAL AND CARDIOVASCULAR RESPONSES—A TRAINING TECHNIQUE FOR MONKEYS¹

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A training technique has been developed which combines classical differential conditioning and conditional emotional response (CER) procedures. After monkeys were trained to lever-press on a variable-interval schedule, two stimulus lights were presented. One light (CS) was always followed, upon termination, by electrical shock; the other light (DS) was never followed by shock. The response to the CS was a decreased rate of lever-pressing and increased heart rate and blood-flow velocity. None of these responses occurred to the DS. This technique eliminates the possibility of pseudoconditioning and provides measurement of both somatic and autonomic responses in a CER situation.

The present training technique was developed during a study of the neural control of the cardiovascular system in unanesthetized monkeys (Smith and Nathan, 1964). A reliable cardiovascular response was needed to serve as an endpoint in determining the effects of various central neural lesions. A differentiated classical conditioning paradigm with electric shock as the unconditional stimulus (US) was first chosen as the technique to gain control over the cardiovascular system. Two stimulus lights, which differed in color and position, were presented to the subject. One light, always followed by unavoidable electrical shock, was the conditional stimulus (CS), and the other, never followed by shock, was the differential stimulus (DS). Blood-flow velocity, heart rate, and general somatic activity were recorded. This procedure demonstrated differentiation of the cardiovascular response (Smith and Stebbins, 1965), but lacked a measure of operant behavior which might have aided the interpretation of the motivational significance of the cardiovascular response.

A procedure for conditioned emotional response (CER) training, originally formulated by Estes and Skinner (1941), was added. While the animal was lever-pressing on a variable-

interval schedule, CS-US pairings were presented. Lever-pressing rate decreased during the CS concurrently with increases in the magnitude of the cardiovascular variables (Stebbins and Smith, 1964).

The differentiated classical conditional response and conditioned emotional response have now been combined into a training procedure which may be described as a "differential CER".

METHOD

Apparatus

Flow sections operating on the Doppler principle (Franklin, Schlegel, and Watson, 1963) or pulsed ultrasound (Franklin, Baker, and Rushmer, 1962) were aseptically implanted on the terminal aorta in six pigtailed monkeys (*Macaca nemestrina*). Instantaneous blood-flow velocity and heart rate were recorded from these transducers. The monkeys were kept in restraining chairs for the duration of the experiment. Gross body movement was recorded by strain gauges attached directly to the chair.

All sessions were conducted in sound-shielded rooms (IAC-400) with 60 db (C-flat reference) white noise as additional protection against extraneous sounds.

Two visual stimuli were used. One light stimulus was a single, 120 mv, white indicator bulb, placed in the center of a gray box measuring 7.5 by 10 by 13 cm. The other consisted of three red indicator bulbs, arranged to form an equilateral triangle in a similar box. The boxes were placed side-by-side for some ani-

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mals, 15 cm apart for others, and about 25 cm in front of all animals. The selection of one or the other stimulus as the CS or DS was randomly determined for a given subject. Background illumination was provided by an indirect incandescent light source of 7.5 w.

Shock was administered between a collar electrode and the metal seat bars of the restraining chair. A constant-current generator, delivering 60 puses/sec monophasic square waves of about 8.4-ms duration was used.

Presses on a lever placed close to the animal's left hand delivered 90-mg banana-flavored pellet reinforcers into a food cup placed either where the animal could lick up the pellets or pick them up with its right hand.

Digital equipment was used for programming lights, shock, and reinforcement schedules. All variables were recorded in analog form on Sanborn or Offner multichannel recorders. Cumulative lever-presses were recorded with solid-state, digital-to-analog converters.

Procedures

The monkeys were brought to the sound-shielded rooms five days a week and trained for 1 hr a day on a variable-interval schedule with a mean interval of 30 sec and a range of 2 to 227 sec. After 10 to 32 daily sessions, a stable lever-pressing rate was attained. The stimulus lights were presented without shock (light-control conditions) to extinguish any "orienting response", as measured by the lever-pressing and cardiovascular variables. Each light was presented for 60 sec, four times during a session. The intertrial interval was 3, 7, or 10 min, and the two types of light stimuli were alternated. The number of light-control sessions needed to extinguish the orienting response varied from 1 to 10.

CER sessions normally consisted of seven DS lights (light not followed by shock) and one CS light. Each session lasted 1 to 1.5 hr and each stimulus was presented for 1 min. At the termination of the CS, shock was delivered for 1 to 3 sec with a current of 10 to 15 ma. The shock parameters used for each animal were of the shortest duration and least current needed to elicit a maximum cardiovascular unconditional response. This level was generally determined in the first few training sessions, although changes in these parameters were required later for some monkeys. The

lights were presented in a random sequence except that the first light of a session was always a DS. A 7-to-1 ratio of DS to CS was maintained when possible. With many animals, however, some sessions in which all eight lights were DS's were necessary to prevent stimulus generalization or elevation of cardiovascular basal levels.

RESULTS

The top record of all figures shows cumulative lever-presses. The pen reset to zero at the beginning of the 1-min basal period which preceded every stimulus period. The pen again reset at the beginning of the CS or DS period and at the beginning of the US period following CS or DS. These three 1-min intervals were used to measure all variables and constituted a trial. The decrease in lever-pressing rate (suppression) during the CS or DS period compared to the basal period was computed according to the inflection ratio formula of Hunt and Brady (1951a). A ratio of -1.00 represents complete suppression, 0.00 represents no suppression, and a positive ratio represents acceleration of lever-pressing rates over the basal level.

Peak blood-flow velocity, general somatic activity, and heart-rate responses (second, third, and fourth records in all figures) to CS, DS, or US were compared to the maximum response rate during the basal period of that same trial. Flow velocity and activity were qualitatively measured by the number of millimeters' deflection of the recording pen. Heart rate was quantitatively measured by tachometer.

Figure 1 shows somatic and autonomic responses of S783 during light-control conditions. There were no systematic variations in lever-pressing rate, activity, blood-flow velocity, or heart rate to any of the stimuli. The 18-mm increase in activity in the 1 min following the DS trial was unrelated to the stimulus.

Figure 2 shows the degree of stimulus generalization between CS and DS by the fifteenth training session. There was substantial generalization as indicated by the flow and heart-rate changes; however, no generalization was evident in the lever-pressing variable.

Generalization or "sensitization" to the experimental environment, as well as between CS and DS, is indicated by the increase in basal heart rate by CER 15. The peak heart

Fig. 1. Lever-pressing, blood-flow velocity, general activity, and heart rate responses to the stimulus lights before any CS-US pairings. Records taken from the last light control (LC) session. Blood-flow velocity and activity are shown in millimeters increase or decrease on the recording paper. In all figures, CS trial on the left is separated from DS trial on the right by the solid vertical line. Data records of CS and DS trials are divided into three 1-min periods. Periods for CS trials are, from left to right: basal period, CS period, and US period (this period contains no US for trials either during LC conditions or during any DS, and in these instances simply designates the period following a light).

Fig. 2. Generalized cardiovascular responses to DS after 24 CS-US pairings. Records taken from CER session 15. US was delivered immediately after CS termination.

rate during the basal period of the light-control session was 172 bpm. The peak heart rate during the basal period 15 training sessions later had increased to 204 bpm. Visual comparison of the basal periods of Fig. 1 and 2 show an average increase of about 20 bpm.

The criterion for lever-pressing differentiation was six CS trials in a row of -0.60 suppression with little or no suppression to DS. The suppression during the intervening DS trials never reached this level. Animals achieving this criterion can be maintained easily at this level of proficiency and are therefore stable preparations for assessing the effects of neural damage, *etc.*, upon such behavior.

The UR (Fig. 2) is seen as a further increase in peak flow velocity and heart rate after the CS terminated. The initial transient depression of blood-flow velocity immediately after CS termination is an artifact due to delivery of the electrical shock.

Complete differentiation is shown in Fig. 3 with perfect lever-pressing suppression to CS and no suppression to DS. There was also a sustained peak flow velocity CR reaching a maximum of 22 mm above basal level with an accompanying 60 bpm increase in heart rate. By comparison, there was no cardiovascular

response to DS. The basal heart rate returned to a maximum of 172 bpm, the same as that observed during the light control conditions before pairing of the CS and US.

The UR (Fig. 3) after CS termination is seen only as a transient increase in peak flow velocity and a maintenance of the already elevated heart rate.

Table 1 summarizes the mean inflection ratios and mean cardiovascular response increases, relative to basal level, of the last six CER sessions for all subjects trained by this technique. Criterion, as determined by lever-pressing suppression ratios, had been met at the conclusion of these six trials. All subjects developed excellent differentiation of the lever-pressing response rate between CS and DS. Criterion was met in 16 to 31 sessions for all monkeys with a median of 24.5 sessions. The most generalization was by S64844 whose average inflection ratio to the DS was -0.30 . This amount of suppression was still only half the criterion value of -0.60 required to CS and less than half the average inflection ratio of -0.80 actually shown by this subject in response to CS. The differential cardiovascular responses for the first four subjects are shown in Table 1. However, the last two subjects

Fig. 3. Differentiated cardiovascular and lever-pressing responses from a criterion session of S783. Downward deflections of heart rate on DS trial are due to artifact.

Table 1

Summary of mean lever-pressing and mean cardiovascular responses during the CS, DS, and, in some cases, US periods for each animal. An inflection ratio was computed on each of the six criterion sessions for the CS and DS periods of each animal. A mean inflection ratio of these six sessions was then computed and is tabulated below. A maximum CR, DR, and UR response, relative to basal level, was computed for the six criterion sessions of each subject. A mean CR, DR, and UR was then computed on these six maxima and is tabulated below.

Subject	Lever-Pressing		Blood-Flow Velocity			Heart Rate		
	CS	DS	CS	US	DS	CS	US	DS
783	-1.00	-0.13	25	29	6	61	41	15
64844	-0.80	-0.30	14	10	2	67	45	18
6441	-0.64	0.00	15	14	1	47	39	5
65228	-0.90	0.03	14	15	2	31	35	7
65230	-0.94	0.00	4	7	4	10	29	10
612	-0.83	-0.13	2	13	1	4	43	4

showed little cardiovascular CR and no differentiation. For example, S612 had 4 bpm cardiac CR which was less than that shown as a DR shown by any other subject. On the other hand, S65230 showed a cardiovascular CR but it was not differentiated from the DR.

All subjects showed cardiovascular URs, *i.e.*, all responses consistently exceeded the maximal basal level responses enough times during the six criterion sessions to yield increases in blood-flow velocity of at least 7 mm and in heart rate of at least 29 bpm for the smallest UR observed.

DISCUSSION

An important principle in the establishment of the discriminated CER is that CS trials should not be massed. Increases in basal cardiovascular levels and cardiovascular generalization may occur when the ratio of CS to DS trials is increased within a session. Figure 2 is an example of such heart rate basal level increases and cardiovascular generalization to CS and DS. This subject generally had been given at least two CS trials and six DS trials for the first 10 CER sessions. As shown in Fig. 3, a return of basal heart rate to control levels and cardiovascular differentiation was finally accomplished, but only after seven more CER sessions containing just one CS trial and seven or more DS trials per session. These CER sessions were also separated from one another

by one to two sessions consisting of nothing but DS trials.

Another procedural consideration is that supramaximal shock, *i.e.*, an intensity higher than the minimum level that elicits a maximum cardiovascular response, should be avoided. High currents may also produce generalization to the entire experimental situation and a general elevation of basal levels.

There are several advantages to concomitant measurement of differentiated lever-pressing and cardiovascular response: (1) it provides an empirical autonomic measure of the "anticipation" of shock described by Estes and Skinner (1941) in their explanation of the decreased rates of lever-pressing in CER experiments; (2) it provides simultaneous measurement of both the somatic and the autonomic aspects of conditioning.

The use of a differential training technique forces the animal to make a discrimination, thus obviating the argument of possible sensitization or pseudoconditioning (Singh, 1959). The addition of the measure of general somatic activity serves to indicate the degree of relationship between general muscular movement and cardiovascular responses. All figures illustrating a cardiovascular CR fail to show a concomitant increase in activity during the CS interval over that of the basal period. This indicates that the cardiovascular changes are not secondary to gross muscular movement.

The acquisition of consistent lever-pressing suppression took much longer in these monkeys than in rats used in other studies. In general, suppression of similar or greater magnitude occurs in rats within eight trials; the monkeys used in this study required 16 to 31 trials. The investigations of Kamin (1963) and de Toledo and Black (1966) have apparently ruled out pseudoconditioning as an explanation for this difference. The procedures used have not been identical, but it is not possible to identify a difference in method which distinguishes all experiments with rats from the present studies with monkeys. The most plausible explanation seems to be that the well-known freezing response of rats makes it easier to induce inhibition of on-going behavior and lever-pressing suppression is thus acquired more rapidly in the rat than in the monkey.

The lack of a differentiated cardiovascular response by S65230 and the absence of a cardiovascular response by S612 are enigmas.

S65230 had a cardiovascular UR somewhat less than that of the other subjects (Table 1). However, this subject did show a cardiovascular CR, but it was simply undifferentiated. On the other hand, S612 showed a UR that exceeded that of some of the other subjects, yet it failed to show a cardiovascular CR. Clearly a Pavlovian interpretation of stimulus substitution is not satisfactory. The explanation that the decrease of lever-pressing rate during the CS is a function of the interrupting effects of respondent conditioning on an operant response (Kamin, 1965) is also not satisfactory if one assumes that respondent conditioning is indicated by a cardiovascular CR. The lack of conditional cardiovascular responses concomitant with lever-pressing suppression in S612 may be a function of punishment of the lever-pressing response rather than a function of interruption of on-going operant behavior by respondents elicited by a CS. Gottwald (1967) and Azrin (1956) pointed out that apparent suppression in a CER procedure may partly be due to punishment of the lever-pressing response. S612 had a lever-pressing rate during the last light-control trial that was more than four times as great as that observed for any other animal. It is therefore highly probable that some lever-presses could have been punished. It would appear then, that in some animals lever-pressing suppression might occur not as a result of respondent conditioning, but simply because a response had been punished. This interpretation does not explain the lack of cardiovascular conditioning in S612. Hunt and Brady (1951b) demonstrated that rats punished for lever-pressing, i.e., electrical shock was delivered for each lever-press, differed from rats trained on the usual CER procedure. The punished subjects showed not only lever-pressing suppression but also a decrease in defecation and a stable lever-pressing response rate between trials as contrasted to the CER animals. If defecation can be considered an index of autonomic reactivity, then the lack of cardiovascular CRs in S612 agrees with the observations made by Hunt and Brady.

Apparent lever-pressing suppression in the CER situation may be a function not only of the interrupting effects of respondent conditioning, but could also be a function of negative reinforcement. To circumvent this alternative, subjects could be conditioned in a

situation apart from lever-pressing training, and then the CS-US pairings could be superimposed on the lever-pressing situation, as Hunt and Brady (1951a) originally did. This would be easily accomplished when cardiovascular variables are recorded because the cardiovascular CRs could indicate the most propitious time to combine the classical and operant training procedures. Lever-pressing suppression in such a situation could not possibly be a function of punishment.

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