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Adrenalectomy-Produced Facilitation of Pavlovian Conditioned Cardiodecelerations in Immobilized Rats

J. ROGER WILSON, C. WAYNE SIMPSON, LEO V. DICARA, AND BERNARD J. CARROLL
University of Michigan Medical Center, Ann Arbor

ABSTRACT

Previous evidence has suggested that both hormonal and behavioral aspects of adrenal stress activation may contribute to heart rate (HR) conditioning during physical/pharmacological immobilization. Accordingly, four studies were conducted to determine if bilateral adrenalectomy facilitates stimulus-control over Pavlovian conditioned cardiodecelerations in rats immobilized either through physical restraint or neuromuscular paralysis. Plasma corticosterone assays were used as an index of the effectiveness of adrenal removal. The results showed that adrenalectomy facilitated both simple and discriminated Pavlovian conditioned cardiodecelerations in rats paralyzed with d-tubocurarine chloride (dTC) without significantly altering the characteristics of EMG recovery from paralysis. Similarly, adrenalectomy facilitated simple Pavlovian HR conditioning in physically restrained rats. The results suggest that adrenal activation may disrupt the parasympathetically-mediated Pavlovian conditioned cardiodeceleration in the physically- and dTC-immobilized rat. However, the specific nature of neuroendocrine mechanisms underlying cardiovascular conditioning during immobilization remains problematical.

DESCRIPTORS: Heart rate, Conditioning, Adrenal stress, Rats.

Several reports indicate that conditioned cardiodecelerations are predominantly vagally-mediated and linked centrally to reductions in skeletal motor activity (Black & de Toledo, 1972; Obrist, Howard, Lawler, Galosy, Meyers, & Gaebelein, 1974). However, parasympathetic discharge seldom occurs in isolation but is influenced by the prevailing level of adrenergic tone. In fact, changes in adrenal secretion, as well as a constellation of behavioral defense reactions, have been a hallmark of a generalized stress response (Selye, 1950). If similar mechanisms constitute the organization of both Pavlovian conditioned cardiodecelerations and response suppression in the immobilized rat, then both the neurohormonal state and/or the characteristics of stress-elicited defense reactions may establish certain limiting conditions for strengthening the heart rate conditioned response (HR CR).

In the rat the largest portion of the adrenal medullary effluent is epinephrine and its action on cardiac β-receptors is generally stronger than norepinephrine (Brewster, Isaacs, & Wainø-Anderson, 1953; von Euler, 1967). The magnitude of the adrenal effect on cardiovascular activity also depends on the nature and intensity of the stress-eliciting stimulus as well as the preexisting state of excitability of the organism (Mellander, 1960). In this connection Obrist, Lawler, Howard, Smithson, Martin, and Manning (1974) have observed in humans that vagally-induced conditioned cardiodecelerations often mask a weak β-adrenergic cardioaccelerator influence on the heart. Accordingly, they suggest that sympathetic influences are normally held in abeyance *except* under extremely stressful circumstances.

Characteristic patterns of skeletal motor activity also tend to emerge in acutely stressful situations. For instance, crouching or "freezing" in the rat is characterized by a diminution of activity and is

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The second author is now at the Department of Psychological Sciences, Purdue University, West Lafayette, Indiana.

Address requests for reprints to: J. Roger Wilson who is now at the Department of Psychology, Neurochemical Unit, Temple University, Philadelphia, Pennsylvania 19122.

readily conditioned to stimuli associated with shock (Bolles, 1970). As might be anticipated from the "cardiac-somatic linkage" notion (e.g., Obrist et al., 1974a), increasing the shock intensity in a Pavlovian fear conditioning situation enhances CS control over both this crouching tendency, as inferred from the animal's inability to suppress the immobility response (Bolles, 1970; de Toledo & Black, 1967), and the amplitude of the conditioned cardiodeceleration (de Toledo & Black, 1966; Fitzgerald & Teyler, 1970). Moreover, unsignalled, inescapable preshock facilitates subsequent development of the fear-elicited freezing reaction (Anisman & Waller, 1973) as well as conditioned cardiodecelerations in both physically restrained and curarized rats (Wilson, Wilson, & DiCara, 1975). Therefore, procedures that strengthen response suppression in the presence of a fear-eliciting stimulus are likely to enhance its control over HR decelerations. In this regard, corticosteroid suppression of adrenocorticotrophic hormone (ACTH) may be involved in stress-elicited changes in cardiovascular conditioning. This notion is predicated on the fact that adrenalectomy-produced elevations in ACTH (Gemzell, vanDyke, Tobias, & Evans, 1951) enhance the probability of occurrence of those skeletal motor defense reactions (e.g., passive avoidance) most compatible with the development of Pavlovian conditioned cardiodecelerations (Guth, Seward, & Levine, 1971; Koranyi, Endoczi, Lissak, & Szepes, 1967; Levine & Jones, 1965; Weiss, McEwen, Silva, & Kalkut, 1970).

In view of these studies, it is interesting that the role of stress in autonomic conditioning is seldom considered with respect to the physically restrained or curarized rat. In fact, it is becoming apparent that immobilization itself can disrupt Pavlovian conditioned cardiodecelerations. Holdstock and Schwartzbaum (1965) observed HR conditioning in rats adapted to the hammock-like restrainer used during training. However, other studies (Ray, 1969, 1972; Wilson, Note 1) subsequently found that pretraining adaptation was essential for conditioning to occur. Similarly, Wilson and DiCara (1975b) reported that much of the difficulty in obtaining conditioned cardiodecelerations in curare-immobilized rats was minimized by adaptation to curare paralysis prior to conditioning. Together these studies suggest that physical/pharmacological immobilization-induced stress establishes certain hormonal and behavioral tendencies incompatible with autonomic conditioning. The following experiments were predicated on the assumption that minimizing some of these conflicting tendencies might strengthen the CS control over cardiovascular activity. Specifically, since bilateral adrenalectomy would eliminate an adrenalmedullary source of sympathetic competition and elevate ACTH levels, it might facilitate Pavlovian conditioned cardiodecelerations in curare-paralyzed or physically restrained rats.

EXPERIMENT I

Method

Subjects

The subjects were 40 naive adult male Sprague-Dawley rats. All animals weighed approximately 375–450 g at the time of testing, had no previous drug experience, and were adapted to the standard laboratory regimen of free access to food and water for at least 1 week before the initiation of the experiment. They were individually housed in wire-mesh cages in a room cycled on a 12-hr light-dark schedule.

Apparatus and Procedure

Forty rats (20/group) were randomly assigned to either adrenalectomized or nonadrenalectomized treatment groups. Ten days before Pavlovian HR conditioning those animals assigned to the adrenalectomy (ADX) group were anesthetized with Equi-thesin (.3 cc/100 g BW, Jansel Sal Products) and underwent the first phase of a two-stage surgical procedure. Adrenals were removed unilaterally under aseptic conditions. Animals in the Non-adrenalectomy (N-ADX) group were anesthetized, shaved, incised unilaterally in the paralumbar region; then closed and sutured. Three days later the contralateral adrenals were removed in the ADX animals and another sham operation was performed in the N-ADX rats. All animals were then permitted a 1 week recovery period. During this time they had free access to isotonic saline, to compensate for aldosterone depletion, and to their regular food and water.

On the first day of conditioning each animal was lightly etherized to minimize struggling and given a single 2.5 mg/kg BW intraperitoneal injection of d-tubocurarine chloride (dTc) in a 3.0 mg/cc solution (Squibb). When the rat showed signs of respiratory distress it was fitted into a face mask modified from a rubber balloon. The balloon was positioned by placing the lower lip behind the upper incisors and by pulling the upper lip tightly over the rat's snout. The balloon was attached to a rubber stopper which held a Y-tube connected by polyethylene tubing to the inspiration and expiration ports on a small animal respirator (Harvard Model 680). The rats were respirated at a 1:1 I/E ratio at 70±2 cpm with 6 cc positive pressure vol/stroke. Because of certain slow mechanical variability in the respiratory cycle there was some small drift in the cycle speed such that each animal's respiratory parameters ranged between 68–72 cpm. Since the running sequence for each of the four groups was counterbalanced and there was no detectable temporal trend exhibited for this mechanical artifact, it was felt to be of negligible importance, vis-a-vis, the nature of our results. During paralysis the rat lay on folded surgical padding and was respirated until it regained sufficient motor control to sustain respiration without aid (Wilson & DiCara, 1975a, 1975b; Wilson et al., 1975).

Simple Pavlovian conditioning. Half (N=20) the ani-

mals in both the ADX and N-ADX groups were randomly selected to receive paired CS-US presentations during the Pavlovian conditioning sessions. These were referred to as the Paired-Adrenalectomized (P-ADX) and the Paired-Nonadrenalectomized (P-N-ADX) groups, respectively. After a 30 min curare stabilization these rats received 35 paired presentations of a conditioned stimulus (CS) and unconditioned stimulus (US). The remaining half of the animals in the ADX and N-ADX groups served as explicitly unpaired sensitization control animals. These were referred to as the Unpaired-Adrenalectomized (UP-ADX) and the Unpaired Nonadrenalectomized (UP-N-ADX) groups, respectively. Within each session control groups received 35 independent presentations of the CS and the US, such that the interval between CS termination and US onset varied randomly from 20 to 70 sec. The time required for each conditioning session never exceeded the duration of complete neuromuscular paralysis. The CS consisted of a 5.5 sec presentation of a 1K Hz 80 dB (SPL) tone and 5 W light positioned in front of the animal. The stimulus display (a 3 in. speaker adjacent to a faceted indicator lamp) was mounted 10 cm in front of the rat, 8 cm above the chamber floor. The CS was presented against a background noise (65 dB) generated by the housing ventilation fan and coterminated with a 500 msec US of 1.2 mA RMS tailshock. The tailshock was of a series resistance regulated variety that offered a relatively constant 60 Hz AC current. The US was delivered through tailshock electrodes described by Weiss (1967), which were liberally coated with electrode paste (Redux) to maintain optimal electrical contact.

All trials were presented with a BRS film tape programmer in which the stimulus events occurring within a session were programmed according to an intertrial interval (ITI) averaging 100 sec with a range of 40–160 sec. Conditioning sessions occurred in a 180×60×38 cm BRS-Foringer sound attenuating chamber that was electrically insulated with copper mesh. Following the first conditioning session the animals were permitted to recover from paralysis and returned to their home cages. This procedure was then repeated on 2 consecutive days. At the end of the last conditioning session the rats were sacrificed by decapitation and blood samples were collected by exsanguination in heparinized Vacutainer tubes for subsequent plasma corticosterone assays.

Data Analysis. A Grass polygraph model 7B was used to record the EKG. Heart signals were recorded from two subdermal 28-ga ⅜-in. stainless steel hypodermic needles inserted laterally on the midthoracic area. The signals were led into a Grass preamplifier (model 7P5 A) and driver amplifier. Heart rate measurements were determined by manually counting the number of heart beats that occurred during the 5 sec periods preceding CS onset, during CS presentation, and following US termination. The conditioned response (CR) was defined as the CS-PreCS difference score. This was computed by subtracting the PreCS rate from the CS rate, thereby allowing HR decelerations to be plotted as negative functions. All HR values were converted to bpm for statistical analyses of variance (ANOVA). Neuman-Keuls tests comparing differences between group means followed all significant F ratios.

In order to avoid the risk of superimposing the conditioned trials on a period of EMG recovery from paralysis, the decision was made to omit the presentation of CS-only habituation trials prior to conditioning. Due to this omission, cursory examination of the data revealed an initial, unconditioned, cardiac orienting deceleration that generally habituated within 5 trials. Accordingly, the first block of 5 trials in each conditioning session was discarded from data analysis.

Plasma Corticosterone Determination. Corticosterone assays were used as an index of the effectiveness of the two-stage adrenalectomy. Corticosterone was measured in rat plasma by the competitive-protein-binding (CPB) method of Murphy (1967) using tritiated corticosterone as the isotopic tracer and florisil to separate free from bound steroid. In each assay, microscale corticosterone standards (range 0–30 ng) were run in triplicate. Duplicate aliquots of the ethanol extracts of plasma were assayed and the average value recorded. The coefficient of variation between assays was 11% and within assays was 8.5%. No statistical analyses were conducted on the corticosterone values since all the adrenalectomized animals used in these experiments assayed lower than 5 μg/100 ml while the non-adrenalectomized animals were consistently greater than 25 μg/100 ml.

Results

PreCS basal HR

A 2×2×3×6 (Treatment × Contingency × Session × Blocks) ANOVA with two repeated measures was conducted on both basal HRs and HR CRs. Although the mean basal HR of ADX rats appeared higher than shams, the elevation was not statistically significant (Treatment: $F<1$). Similarly, the absence of a contingency effect ($F<1$) indicated that the CS-US relationship had a negligible influence on basal HR. Basal HRs increased across both trial blocks ($F(5/180)=53.75$, $MS_e=70.09$)[1] and daily conditioning sessions ($F(2/72)=32.76$, $MS_e=566.81$). Closer examination of the trial blocks analyses revealed that this baseline shift ranged from $\bar{X}=404.92$ bpm, at the initiation of the session, while terminating at approximately $\bar{X}=419.75$ bpm. The Sessions effect revealed that the mean basal HR on Session 1 ($\bar{X}=404.29$ bpm) gradually increased through Sessions 2 and 3 of acquisition ($\bar{X}=415.96$ and 421.58 bpm for Sessions 2 and 3, respectively). The significant Treatment × Sessions interaction ($F(2/72)=3.99$, $MS_e=566.81$) indicated that increases in basal HR across the 3 conditioning sessions were greater for the ADX subjects ($\bar{X}=404.27$, 415.08, and 427.00 bpm for Sessions 1, 2 and 3, respectively) relative to the moderate cardioaccelerations exhibited by N-ADX groups ($\bar{X}=403.92$, 410.83, 416.71 bpm). However, group comparisons of this interaction re-

[1]The .05 rejection region was adopted in all statistical evaluations.

vealed that the ADX and N-ADX groups were statistically different for only the last conditioning session.

Pavlovian Conditioned HRs

Fig. 1 depicts the amplitude of the HR CRs across the 3 conditioning sessions as a function of 5 trial blocks. Adrenalectomy generally augmented cardiodeceleration ($F(1/36)=25.67$, $MS_e=695.56$). In addition, animals receiving paired CS-US presentations demonstrated larger HR responses to the CS than UP groups ($F(1/36)=17.64$, $MS_e=695.56$). Furthermore, the significant Treatment × Contingency interaction ($F(1/36)=9.07$, $MS_e=695.56$) indicated that adrenalectomy selectively facilitated HR decelerations for rats receiving paired CS-US presentations. Subsequent Neuman-Keuls tests revealed that the mean conditioned cardiodeceleration for the P-ADX group ($\bar{X}=-14.4$ bpm) was consistently greater across the 3 conditioning sessions than for the UP-ADX group ($\bar{X}=3.8$ bpm). These results generally indicate that the CS elicited greater cardiodecelerations in those ADX, paralyzed rats given paired CS-US presentations. Although basal HR estimates for the ADX animals gradually increased across conditioning sessions, it seems unlikely that the HR CR observed for the P-ADX group was an artifact of differences in Pre-CS basal HR: The CR magnitude for this group was most evident on the first 2 sessions, whereas the difference in basal HR that developed between the treatment groups was significant only on the third conditioning session.

EXPERIMENT II

The sensitization control used in the previous experiment is a simple procedure for estimating the relative CR strength due only to the contiguous CS-US relationship. However, one inherent problem with this control is that the variability due to spurious differences in subject population cannot be dissociated from that experimentally-induced variability attributable to the main treatment effects (i.e., CS-US contingency). Some concern for this problem is based on the tendency for the PreCS basal HR estimates of the ADX group to be faster than the N-ADX group on the last conditioning session. The possibility that this may have inflated the CR estimate for the P-ADX group on the 2 previous conditioning sessions is unlikely for the reasons mentioned above; but, when viewed in conjunction with the difficulty reported in obtaining conditioned cardiodecelerations in the nonadapted, curarized rat, it convinced the experimenters to modify the procedure. Accordingly, the discriminated Pavlovian conditioning paradigm in the following experiment enables a "within-group" evaluation of each rat's ability to discriminate between two stimuli that are differentially associated with the fear-motivating stimulus, while minimizing the bias due to uncontrolled differences in basal HR.

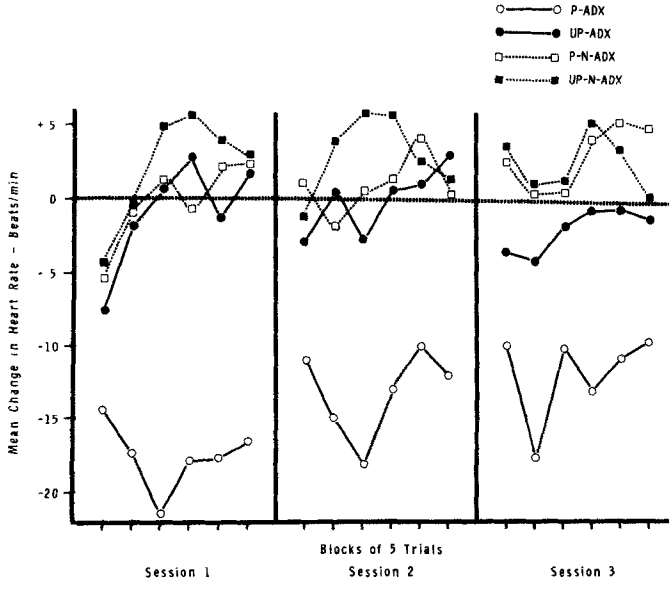

Fig. 1. Mean HR change to CS in bpm for dTC-curarized rats as a function of Adrenalectomy (ADX/N-ADX) and Stimulus Contingency (P/UP) across 3 successive 30-trial sessions of simple Pavlovian conditioning. (Abbreviations: P-ADX = Paired-Adrenalectomized; UP-ADX = Unpaired-Adrenalectomized; P-N-ADX = Paired-Nonadrenalectomized; UP-N-ADX = Unpaired-Nonadrenalectomized.)

Method

Subjects

Selection and housing of the 24 male albino rats used in this experiment were identical to that reported for Experiment I. Ten days before Pavlovian HR conditioning began, the animals were randomly assigned to either an ADX or N-ADX treatment group (N=12/group). The ADX rats were subjected to a two-stage bilateral adrenalectomy, while N-ADX animals underwent a two-stage sham operation as described in the first experiment.

Procedure

Discriminated Pavlovian Conditioning. Following a 30 min curare stabilization period in each session, the discriminated conditioning procedure was initiated. The CSs were 5.5 sec tones with frequencies of either 1K Hz or 3.1K Hz and clearly audible over the masking noise provided by the ventilation fan. The stimuli were presented such that one tone (CS^+) always coterminated with a 500 msec 1.2 mA RMS tailshock, while the other tone (CS^-) was never accompanied by tailshock. The rats were conditioned for 50 (25 CS^+ and 25 CS^-) trials for 5 consecutive daily sessions. Trials occurred, on the average, every 60 sec. The CS^+s and CS^-s occurred semi-randomly with the only restriction being that no one stimulus was presented on more than 3 consecutive trials. The frequencies of the CS^+ and CS^- were reversed for half of the rats (N=6) in both ADX and N-ADX groups to balance any differential effects that these stimuli might have had in eliciting the CR. Following the final conditioning session animals were sacrificed by decapitation and blood plasma was collected for corticosterone assays as described in Experiment I.

Data Analysis. The HR data were tabulated and converted to bpm as in Experiment I. In this case, however, PreCS basal HR estimates were determined separately for the CS^+ and CS^- trials. Accordingly, the HR CR values were calculated according to whether the CS^+ or CS^- was presented. Subsequently 2×2×5×5 [Treatment (ADX/N-ADX) × CS^+/CS^- × Session × Blocks] repeated measures ANOVAs were conducted on the resulting data.

Results

PreCS Basal HR

Basal HR was not affected by either adrenalectomy or differential conditioning (Treatment: $F<1$; CS^+/CS^-: $F<1$). The mean basal HRs averaged across treatments and sessions were slightly lower than those reported in Experiment I ($\bar{X}$=402.27 vs 413.94 bpm for Experiments II and I, respectively). However, direct comparison of basal HRs between Experiments I and II is of doubtful relevance due to inherent differences in shock density, duration of session (40 vs 50 min) and number of conditioning sessions.

Pavlovian Conditioned HRs

The mean HR CRs for the ADX and N-ADX groups are shown in Fig. 2. Bilateral adrenalectomy facilitated discriminated Pavlovian HR CRs. The Treatment effect ($F(1/12)=7.02$, $MS_e=36{,}919.93$) indicated that the mean overall cardiodeceleration to the CS, regardless of the CS^+/CS^- contingency, was

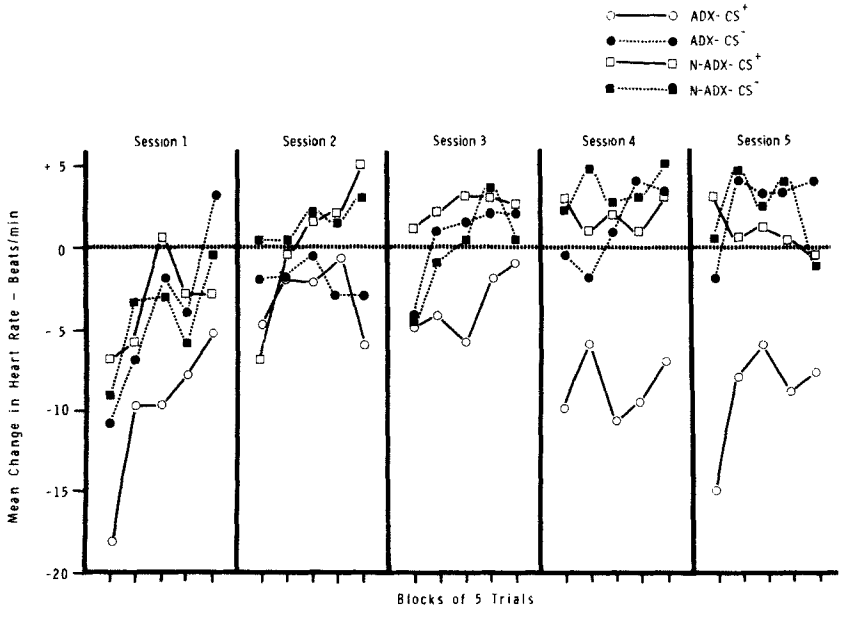

Fig. 2. Mean HR change to CS in bpm for dTC-curarized rats as a function of Adrenalectomy (ADX/N-ADX) and Stimulus Contingency (CS^+/CS^-) across 5 successive 50-trial sessions of discriminated Pavlovian conditioning. (Abbreviations: ADX-CS^+ = Adrenalectomy-CS^+; ADX-CS^- = Adrenalectomy-CS^-; N-ADX-CS^+ = Nonadrenalectomy-CS^+; N-ADX-CS^- = Nonadrenalectomy-CS^-.)

greater for the ADX rats ($\bar{X}$=3.72 vs 0.12 bpm, for ADX and N-ADX groups, respectively). The significant CS^+/CS^- Contingency effect ($F(1/12)$=10.33, MS_e=15,765.46) emphasizes the greater magnitude of cardiodecelerations to the CS^+ in both the ADX and N-ADX groups compared to the HR change elicited on the CS^- trials. The HR CR was characterized by a pronounced deceleration during the first conditioning session which then diminished over the 4 remaining sessions ($F(4/48)$=7.66, MS_e=10,090.04). A similar tendency within sessions accounts for the significant Blocks effect ($F(4/48)$=10.64, MS_e=3,521.13). The Treatment × Contingency interaction ($F(1/12)$=9.26, MS_e=15,765.46) demonstrated that cardiodecelerations to the CS^+ were greater than those to the CS^- in the ADX rats ($\bar{X}$=6.69 bpm vs $\bar{X}$=−.75 bpm). Moreover, the significant Contingency × Sessions interaction ($F(4/48)$=3.11, MS_e=7,011.46) indicated that the HR CR to the CS^+ emerged across the 5 conditioning sessions. Thus, when the Treatment (ADX/N-ADX) × Contingency interaction is viewed in conjunction with the Contingency × Sessions interaction and the robust main effects, it is evident that adrenalectomy selectively strengthened the CS^+-elicited cardiodecelerations and that this tendency developed across conditioning sessions.

EXPERIMENT III

If changes in electrolyte metabolism following adrenalectomy facilitated recovery from curare immobilization, the conditioned cardiovascular response might reflect only differences in the reinstatement of skeletal motor control. For instance, since the most stable HR CRs in the above experiments appeared in the ADX rats that had been immobilized over as many as 5 consecutive sessions, a developing drug tolerance could have facilitated the reinstatement of motor control. Accordingly, the third experiment was designed to determine if the adrenalectomy manipulation in the previous two experiments could have altered the temporal course of recovery of electromyographic (EMG) activity from dTC-induced neuromuscular paralysis.

Method

Selection and housing of the drug-naive animals used in this experiment were similar to that reported in Experiments I and II. Twelve rats were randomly assigned to one of two groups (N=6/group), an Adrenalectomized-Curare Adapted (ADX-CA) group and a Nonadrenalectomized-Curare Nonadapted (N-ADX-CNA) group. Rats in the first group received a two-stage, bilateral adrenalectomy as described above and a 1 week post-operative recovery period. They were then immobilized with a single IP injection of dTC (3.0 mg/kg BW) and artificially respirated at parameters identical to those in Experiments I and II. They were allowed to recover from paralysis and returned to their home cages. This same procedure was repeated for 4 consecutive sessions. The fifth, and last, session served as the EMG recovery testing session. Thus, the duration of curare immobilization and the adrenal status of the rats approximated those conditions that might favor drug tolerance in the previous experiments. The N-ADX-CNA rats underwent sham operations 1 week prior to the EMG testing session and were not exposed to repeated curare-immobilization. A sham operated curare-adapted group was intentionally excluded from this experiment since Wilson and DiCara (1975a), using exactly the same procedure and drug dose parameter, demonstrated that characteristics of EMG recovery following repeated curare-immobilization, when distributed over as long as 5 consecutive sessions, did not differ from those observed following a single administration.

The EMG recovery testing procedure was similar to that used by Wilson and DiCara (1975a, 1975b): On the last day of curarization for the ADX-CA group and on the first (and only) day of paralysis for the N-ADX-CNA control, dual monopolar electrodes were inserted into the large *biceps femoris* muscle of the hindlimb and the *biceps brachii* muscle of the forelimb. EMG potentials were recorded in a Grass Model 7P5-A AC preamplifier set at maximum (10 mV/cm) sensitivity. Recordings were made with the low pass filter set at 75 Hz ½ amplitude. The signals were electronically integrated, rectified, and linearly transduced into positive pen deflections to normalize the response. Continuous EMGs from baseline for each muscle group sampled were reflected by proportional adjustments in the integration reset values. In this manner, EMG integrator reset values were used as the basis for analyzing between group differences on both initial level of neuromuscular paralysis as well as the course of EMG recovery from paralysis.

Basal EMG was established 30 min after curare injection to stabilize the level of immobilization. From this point, and for the remainder of the recovery period EMGs were continuously monitored until the EMG integrator reset value attained 30% of its baseline value; the time to reach this criterion was recorded. The animals were allowed to recover sufficiently to sustain self-respiration, and were then sacrificed through decapitation. Blood samples were assayed for plasma corticosterone.

Results

Two-tailed independent t tests were conducted to obtain pairwise comparisons between N-ADX-CNA and ADX-CA groups for both the control integrator reset interval (CMS) established 30 min after curarization as well as the total time (min) needed to recover to 30% baseline paralysis. These comparisons revealed no significant difference for the initial EMG level following stabilization between the N-ADX-CNA and the ADX-CA groups ($\bar{X}$=23.13±5.80 CMS and 22.05±3.36 CMS, respectively, t=0.285). Similar comparisons for the time

to recover from paralysis also failed to show any difference. Mean recovery time of 137.2±12.4 min for the CNA group was statistically similar to mean recovery time of 138.7±8.5 min exhibited by the ADX-CA group (t=0.134). The fact that the recovery values obtained for the N-ADX-CNA group in the present study were similar to those reported by Wilson and DiCara (1975a) for a procedurally similar group ($\bar{X}_{CNA}$=24.1±6.3 min for basal paralysis and 135.2±12.4 min for recovery paralysis at 30% baseline) suggests the control values are consistent with our earlier observations. Accordingly, it is unlikely that the facilitated CS control over cardiodecelerations exhibited by the ADX animals during either the simple (Experiment I) or discriminated (Experiment II) Pavlovian conditioning paradigms can be interpreted as secondary to some nonspecific, peripheral, physiological change accompanying skeletal-motor activity.

EXPERIMENT IV

The objective of these experiments has been an assessment of adrenal effects on Pavlovian HR conditioning during immobilization-induced stress. The data supports our previous findings (Wilson & DiCara, 1975b; Wilson et al., 1975) that the pharmacological consequences of dTC-neuromuscular paralysis using conventional ventilation parameters do not preclude either simple or discriminated HR conditioning. To the extent that an adrenalectomy effect reflects the influence of nonspecific immobilization-induced stress, rather than a special interaction with neuromuscular paralysis, this surgical manipulation should be effective under other immobilization procedures. The final experiment was designed to determine if adrenalectomy also facilitates simple Pavlovian HR conditioning during conventional physical restraint.

Method

Selection, maintenance and purchase of the animals were similar to that reported in Experiments I-III. Forty rats (20/group) were randomly assigned to an adrenalectomy (Adx) group, while the remaining animals were given sham operations (N-Adx). The two-stage adrenalectomy was conducted as previously described. Following a 1 week post-operative recovery period, the animals were randomly assigned to one of two conditioning groups: 1) a Paired CS-US group (P), in which rats received 35 paired presentations of a 5.5 sec tone (1K Hz dB SPL) and light (5-W) which coterminated with a 500 msec 1.2 mA RMS tailshock, or 2) an Unpaired CS-US group (UP), in which rats received 35 independent presentations of the CS and the US. The physical properties of these stimuli, as well as their temporal distributions, were similar to those used in Experiment I. Accordingly, there were four groups (N=10/group): the paired-adrenalectomized (P-Adx); the paired-nonadrenalectomized (P-N-Adx); the unpaired-adrenalectomized (UP-Adx); and the unpaired-nonadrenalectomized (UP-N-Adx) groups.

Prior to conditioning all animals were lightly etherized to minimize struggling and then physically restrained in a U-shaped dome animal holder. Removable guillotine inserts at one end of the restrainer were positioned to hold the animals securely. The restrainer was placed in a BRS operant chamber as described in Experiment I. Two rats were conditioned concurrently, each in separate identical chambers with tailshock electrodes connected in series. Following a 15 min adaptation period, conditioning began. During training PreCS and CS heart rate measures were recorded on a Grass Model 7 polygraph for subsequent statistical analysis. The rats were then released from restraint, and returned to their home cages. The same procedure was repeated 24 hrs later. Following the second, and final day of conditioning animals were decapitated and blood samples collected for plasma corticosterone assays, as described in the first experiment. As in Experiment I, the first 5 trials of each conditioning session were omitted from the data analysis to minimize the unconditioned cardiac orienting response confounding the HR CR ANOVA.

Results

PreCS Basal HRs

The 2×2×2×6 (Treatment × Contingency × Sessions × Blocks) repeated measures ANOVA of the 5.0 sec PreCS basal HRs revealed no significant effect of surgical manipulation or CS-US contingency (F<1.00). This suggested that neither the bilateral adrenalectomy nor the paired CS-US presentations *per se* elevated basal HRs in such a manner as to invoke the Law of Initial Values (Wilder, 1957) as artificially contributing to any conditioned cardiovascular response. Nevertheless, the gradual increase in basal HR across Blocks (F(5/180)=5.72, MS_e=173.59) is consistent with similar observations in Experiments I and II, as well as those reported in previous studies (Ray, 1969, 1972; Wilson & DiCara, 1975b). However, in this instance, the basal HR shift ranged from $\bar{X}$=426.92 bpm at the initiation of each session to $\bar{X}$=437.35 bpm by session's end. Finally, comparison of group means following a significant Treatment × Blocks interaction (F(5/180)=2.32, MS_e=173.59) showed that an Adx-produced cardioacceleration was observed during each conditioning session. However, the implication of this finding for HR conditioning is unclear since similar interactions were not found in the HR CR ANOVA (cf. below).

Pavlovian Conditioned HRs

Fig. 3 shows the mean HR CRs elicited in physically restrained rats during 2 consecutive 30 trial conditioning sessions. The suggestion that adrenalectomy facilitated stimulus control over

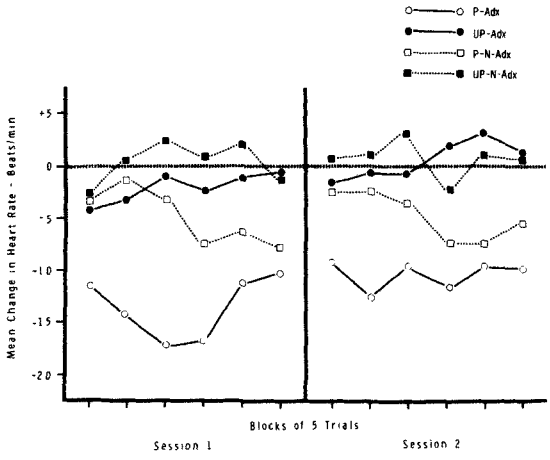

Fig. 3. Mean HR change to CS in bpm for physically restrained rats as a function of Adrenalectomy (Adx/N-Adx) and Stimulus Contingency (P-UP) across 2 successive 30-trial sessions of simple Pavlovian conditioning. (Abbreviations: P-Adx = Paired-Adrenalectomized; UP-Adx = Unpaired-Adrenalectomized; P-N-Adx = Paired-Nonadrenalectomized; UP-N-Adx = Unpaired-Nonadrenalectomized.)

conditioned cardiodecelerations has several bases of statistical support: The repeated measures ANOVA showed that cardiodecelerations were generally greater for both the Adx groups ($F(1/36)=7.83$, $MS_e=405.97$, $\bar{X}=-6.17$ vs -2.47 bpm for Adx and N-Adx, respectively) and those groups receiving paired CS-US presentations ($F(1/36)=6.33$, $MS_e=405.97$, $=-8.60$ vs 0.02 bpm for P and UP, respectively). However, the significant Treatment $\times$ Contingency interaction ($F(1/36)=7.69$, $MS_e=405.97$) and subsequent group comparisons indicated that adrenalectomy selectively enhanced HR CRs for the P animals ($\bar{X}_{P-Adx}=-11.92$ bpm) beyond the marginal responses observed for all other groups ($\bar{X}=-.45$, -5.30, and 0.35 bpm for the UP-Adx, P-N-Adx, and UP-N-Adx groups, respectively). Taken together with the earlier ANOVAs on the PreCS basal HR data, there is little indication that the Adx facilitation of the HR CR could be interpreted as secondary to some nonspecific adrenalectomy or stimulus contingency-produced shift in basal HRs. There was some tendency for the strength of HR CRs to diminish both across ($F(1/36)=6.00$, $MS_e=48.01$) and within ($F(5/180)=3.98$, $MS_e=40.23$) consecutive conditioning sessions. Specifically, the overall mean HR CR on the first conditioning session ($\bar{X}=-5.37$ bpm) exceeded that observed in the second conditioning session ($\bar{X}=-3.27$ bpm), as did the HR CRs (pooled across conditioning sessions) for the first block of 5 trials ($\bar{X}=-5.95$ bpm) compared with those observed on the sixth, and last, 5-trial block ($\bar{X}=-2.25$ bpm).

DISCUSSION

The results of this study are consistent with findings (Wilson & DiCara, 1975b; Wilson et al., 1975) that the HR CR can be disrupted by influences relatively independent of those formed during conditioning. Accordingly, both physical and pharmacological immobilization merit attention as "nonassociative" stress inducers, possibly exerting a tonic adrenal influence over Pavlovian conditioned HRs. Adrenalectomy removes this tonic control and facilitates associative control over the response. This suggests that vagally-mediated conditioned cardiodecelerations may not become dominant until the sympathetic response is suppressed, or else when circumstances are appropriate for strengthening certain defense reactions to situational stimuli associated with shock and compatible with cardiodecelerations. It also appears that the invariant HRs observed in curarized rats may perhaps be due to both inadequate ventilation or vagolytic consequences of the specific neuromuscular blocker, as well as to mechanisms common to the restraint-induced stress. The fact that demonstrating HR CRs using other paralytic agents and ventilation parameters (cf., Dworkin, 1973) has proven difficult supports this notion.

Considering the results of Experiments I, II, and III, HR CRs emerged without the development of tolerance to neuromuscular paralysis. Moreover, any suggestion that adrenalectomy increases responsiveness to shock is unfounded. Adrenalectomized rats have been found to have normal sensitivity to aversive stimulation as measured in a shock titration technique or spatial preference (Paré, 1969) or by changes in threshold to shock-induced vocalizations, flinching, and jumping (Gibbs, Sechzer, Smith, Conners, & Weiss, 1973). The failure to find stable HR CRs for the sham-operated, physically restrained rats, described in Experiment IV, is consistent with several reports (Ray, 1969; Wilson & DiCara, 1975b) but differs from the findings of Fitzgerald and his colleagues (e.g., Fitzgerald & Teyler, 1970) who reported pronounced conditioned cardiodecelerations in female rats using similar restraining procedures. However, there is increasing evidence that circulating sex hormones influence both mechanisms of cardiovascular control and emotional behavior. For instance, female rats have larger adrenals, a greater corticosteroid response to stress, and different rates of synthesis and release of ACTH (Critchlow, Liebelt, Bar-Sela, Mountcastle, & Lipscomb, 1963; Kitay, 1961; Sakiz, 1960) and these differences in pituitary-adrenal function are reflected in enhanced behavioral reactivity to fear-eliciting stimuli (Gray & Lalljee, 1974; Gray & Levine, 1964). When

these considerations are viewed in conjunction with the fact that the female rats in Fitzgerald's study were housed under continuous illumination, and therefore maintained in constant physiological estrus, it makes one reluctant to draw inferences across studies using animals of different sexes.

In our judgment the adrenalectomy-produced facilitation of the HR CR suggests that adrenomedullary catecholamines in the intact rat exert some tonic control over vagally-mediated conditioned cardiodecelerations during immobilization. Unfortunately, few studies have adequately assessed the extent to which direct sympathetic innervation and adrenomedullary catecholamines participate in basal or conditioned cardiovascular activity. What evidence does exist is consistent with our observation that adrenalectomy failed to alter PreCS basal HR during conditioning in Experiments I, II, and IV (de Champlain, 1972). Several investigators (Armour, Randall, Randall, Priola, & Stekiel, 1972; Krasney, 1967; Walker, Zileli, Reutter, Shoemaker, Friend, & Moore, 1959) have found that *both* adrenomedullary release and direct neural influences are a major source of plasma catecholamines and concomitant chronotropic effects during electrical stimulation of the stellate ganglia, prolonged hypotension, fulminating asphyxia and related physiological stresses in baboons and dogs. More recently, Obrist et al. (1974b) found that although sympathetic influences were more clearly manifested in sudomotor and vascular changes, pronounced β-adrenergic effects appear in HR in humans under conditions of acute psychological stress. What is suggested by these studies is that adrenergic influences on HR might be evoked under stressful situations through activation of either adrenomedullary release or direct cardiac innervation.

Adrenalectomy also tends to facilitate certain defense reactions, specifically response suppression (Guth et al., 1971; Levine & Jones, 1965). Thus, it might also be argued that the conditioned cardiodeceleration can be strengthened indirectly by manipulations which facilitate skeletal response suppression that counter the struggling tendencies that one might expect to predominate during immobilization-induced stress, regardless of the status of the sympathoadrenal system. Accordingly, the influence of adrenalectomy on HR conditioning is consistent with a simple activity hypothesis whose basic premise is that the direction and amplitude of the HR CR is directly related to the occurrence of response inhibitory tendencies during conditioning.

In summary, while it is clear that "associative" processes establish the limiting conditions for acquisition of the HR CR, the probability of the emergence of the cardiovascular response also seems to vary with the strength of "nonassociative" physiological and/or behavioral tendencies established during physical restraint or curare paralysis. Whether or not facilitation of the conditioned cardiodeceleration following immobilization adaptation, unsignalled, inescapable preshock, or bilateral adrenalectomy, reflects the operation of a unitary process is one of several issues to be examined. However, it seems likely that the entire sympathoadrenal system is changing during immobilization-induced stress and that aversively motivated HR CRs are probably integrated with certain neuroendocrine and skeletal motor patterns of activity.

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