Effects of chronic psychosocial stress on cardiac autonomic responsiveness and myocardial structure in mice

Tania Costoli, Alessandro Bartolomucci, Gallia Graiani, Donatella Stilli, Giovanni Laviola, and Andrea Sgoifo. Effects of chronic psychosocial stress on cardiac autonomic responsiveness and myocardial structure in mice. *Am J Physiol Heart Circ Physiol* 286: H2133–H2140, 2004. First published February 12, 2004; 10.1152/ajpheart.00869.2003.—Repeated single exposures to social stressors induce robust shifts of cardiac sympathovagal balance toward sympathetic dominance both during and after each agonistic interaction. However, little evidence is available regarding possible persistent pathophysiological changes due to chronic social challenge. In this study, male CD-1 mice (*n* = 14) were implanted with a radiotelemetry system for electrocardiographic recordings. We assessed the effects of chronic psychosocial stress (15-day sensory contact with a dominant animal and daily 5-min defeat episodes) on 1) sympathovagal responsiveness to each defeat episode, as measured via time-domain indexes of heart rate variability (R-R interval, standard deviation of R-R interval, and root mean square of successive R-R interval differences), 2) circadian rhythm of heart rate across the chronic challenge (night phase, day phase, and rhythm amplitude values), and 3) amount of myocardial structural damage (volume fraction, density, and extent of fibrosis). This study indicated that there was habituation of acute cardiac autonomic responsiveness, i.e., the shift of sympathovagal balance toward sympathetic dominance was significantly reduced across repeated defeat episodes. Moreover, animals exhibited significant changes in heart rate rhythmicity, i.e., increments in day and night values and reductions in the rhythm amplitude, but these were limited to the first 5 days of chronic psychosocial stress. The volume fraction of fibrosis was sixfold larger than in control animals, although mice appeared to adapt to chronic psychosocial stress in terms of acute cardiovascular responsiveness and heart rate rhythmicity, structural alterations occurred at the myocardial level.

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STRESS RESPONSE is a set of functional and behavioral activations adopted by an organism to cope adequately with environmental challenges of varying nature and intensity. In situations in which an animal exerts limited control over environmental stimuli, such physiological and behavioral changes may ultimately produce increased susceptibility to psychosomatic disorders such as cardiovascular disturbances (11, 14). In particular, social stressors have been shown to induce robust short-term activations of the sympathetic-adrenomedullary system and the pituitary-adrenocortical axis (15). As far as cardiovascular responses to social defeat and subordinate are concerned, significant increments in blood pressure and plasma catecholamine levels have been documented in rats, persisting as long as the stimulus was present or shortly thereafter (12, 23, 31). In addition, such an experimental stress context produces a considerable increase in heart rate (HR) and a significant shift of the sympathovagal balance toward sympathetic dominance, associated with the occurrence of ventricular and supraventricular tachyarrhythmias (33).

Long-term effects of social challenges on a number of physiological and behavioral parameters have also been reported, mainly involving the daily rhythms of HR and body temperature, food intake, and exploratory and social activity (17, 24). For example, a single episode of social conflict induces persistent cardiovascular alterations (lasting up to 2 wk), mainly consisting of reductions of the amplitude of the circadian rhythm of HR (26, 35).

Scientific knowledge is contradictory about the cumulative effects of stressors. Many animal studies indicate that there is a gradual decline in the response of the organism when a homotypic stressor is repeatedly applied (habituation) for various types of physiological/neuroendocrine responses and in different stress contexts (22). However, it was shown recently that rats intermittently exposed to an uncontrollable social stressor (defeat) fail to habituate in terms of acute cardiac autonomic responsiveness (34). In other words, these animals do not seem to adapt completely, although the stressor is substantially unchanged over time.

There is a general tendency to consider the acute effects observed during and shortly after either a single or a repeated stressor as persistent changes when the stressor is applied chronically (17). To mimic the effects of challenges faced by mammalian species in real life, a new model of chronic psychosocial stress was developed recently in mice, in which a constant adverse stimulus (sensory contact with an aggressive conspecific animal) combined with daily defeat episodes was shown to affect immunologic function (3).

There are some controversies regarding the effects of high catecholaminergic activations due to real-life stressors on the structure of the heart. Sanchez et al. (29) stated that intermale aggressive confrontations should not significantly affect heart structure, as shown by the lack of increase in left ventricular and myocardial structure in mice.

Additional information related to this article can be found on the *American Journal of Physiology—Heart and Circulatory Physiology* website.
the large concomitant increase in epidermal growth factor plasma concentration might be involved in heart protection (29). On the other hand, Andrews et al. (1) reported that isolation followed by territorial stress (homing in an unstable social environment) induced myocardial fibrosis, coronary collagen deposition, increase in coronary wall-to-lumen ratio, and coronary collagen-to-vessel ratio.

Most available experimental studies focus on cardiac remodeling after the injection of adrenergic agonists. Rona (28) reported that injection of epinephrine induced cardiac alterations in rats consisting of myocardial hypertrophy and necrosis. Moreover, a single subcutaneous injection of isoproterenol in rats induced dose-dependent myocardial necrosis, ranging from patchy subendocardial cellular death to transmural infarction, finally resulting in reparative myocardial fibrosis (40, 42).

The present study was aimed at analyzing whether acute and long-term sympathovagal responsiveness to intermittent defeat episodes are affected by a chronic psychosocial challenge consisting of 15-day continuous sensory contact with a dominant animal. In addition, we tested whether such an adverse social condition can induce permanent alterations in cardiac structure, in terms of amount, geometric properties, and regional distribution of myocardial fibrosis.

METHODS

All experimental procedures in this study were approved by the Veterinarian Animal Care and Use Committee of Parma University and carried out in accordance with the European Community Council Directives of 24 November 1996 (86/609/EEC).

Animals and housing. We used twenty-four 3-month-old male Swiss CD-1 mice from an outbred stock originally obtained from Charles River Italia (Calcio, Lecco, Italy). From weaning until the onset of the experiment, the mice were housed in unisex groups of four to six individuals in Plexiglas cages measuring 39 × 23 × 15 cm.

Furthermore, ten 3-month-old sterilized females were used as partners of control mice and an additional fourteen 3-month-old males were used as residents in the “resident-intruder test” (see Surgery: transmitter implantation and Chronic psychosocial stress for details).

Before and during the experimental treatment, all animals were kept in rooms with controlled temperature (22 ± 2°C) and lighting (lights on from 0700 to 1900 h). The bedding of the cages consisted of wood shavings, and food and water were freely available.

Radiotelemetry system. The radiotelemetry system used in this study enabled ECG recordings in freely moving animals. It consisted of flat transmitters measuring 20 × 10 × 8 mm (TA10ET-A-F20; Data Sciences International, St. Paul, MN) and platform receivers measuring 32 × 22 × 3 cm (RPC-1; Data Sciences International).

Surgery: transmitter implantation. The transmitter was chronically implanted in 14 animals (SS group) according to a surgical procedure that guarantees high-quality ECG recordings during sustained physical activity (36). Briefly, the body of the transmitter was placed in the abdominal cavity and the two electrodes (wire loops) were fixed, respectively, to the dorsal surface of the xiphoid process and in the anterior mediastinum close to the right atrium. The mice were anesthetized with droperidol (2.5 mg/ml of solution) and fentanyl citrate (0.05 mg/ml of solution) (Leptofen, 3 ml/kg im; Pharmacia). Subsequently, the animals were prophylactically injected for 2 days with gentamicin sulfate (50 mg/ml of solution; Augent, 1 ml/kg sc; Fatro) and individually housed in clear Plexiglas cages measuring 39 × 23 × 15 cm. After the transmitter was implanted and before any measurement was started, the mice were allowed 15 days for recovery of body weight and circadian rhythmicity of HR.

Sham-operated animals served as experimental controls (Ctr group; n = 10). These animals underwent the same surgical procedure (although no transmitter was implanted) and the same postsurgery treatment.

Chronic psychosocial stress. On the first day of chronic psychosocial stress, each instrumented mouse was introduced into the cage of a resident animal having equal or greater body weight. In such an experimental paradigm (resident-intruder test), intruders are generally attacked and subordinated by resident animals (8). After this first 5-min social agonistic interaction (time counted starting from the first attack by the resident mouse), the two animals were divided by means of a partition (measuring 230 × 150 × 5 mm; made of a polystyrene framework and a central part of wire mesh, each square opening measuring 6 × 6 mm) that allowed continuous visual, olfactory, and acoustic (but not physical) interaction (3). The partition divided the cage into two parts of the same size and was removed daily for 5 min. This procedure was performed for 15 consecutive days at an unpredictable time between 1100 and 1500 h. Meanwhile, control animals were paired with a sterilized female for 15 days.

Behavioral analysis. Interactions were video-recorded and later scored by a trained observer by means of a series of chronometers. During the interaction the total attacking times of both the resident and the intruder were quantified and further analyzed as dependent measures.

ECG data acquisition and processing. Continuous ECG recordings were performed during the 1st, 4th, and 15th social agonistic interactions in three recording periods, each lasting 5 min: baseline (before interaction), test (during social defeat), and posttest (with animals again separated by the partition). ECG signals were fed to a PC containing ART-Silver 1.10 data acquisition system (Data Sciences International) for monitoring and acquisition of ECG waves. Offline analysis was performed by means of a software package developed in our lab (XRRTEC) for quantification of time-domain indexes of HR variability.

The following ECG parameters were quantified: 1) mean of R-R interval duration (RR, ms), 2) standard deviation of RR (SDRR, ms), and 3) root mean square of successive R-R interval differences (r-MSSD, ms). SDRR estimates overall HR variability and therefore includes the contribution of both branches of the autonomic nervous system to HR variations; it measures the state of the balance between the activities of the sympathetic component (low-frequency variations) and the parasympathetic branch (high-frequency variations). The r-MSSD focuses on high-frequency, short-term variations in HR, which are due to the activity of the parasympathetic nervous system on the heart (20, 32, 38, 41). Generally speaking, reductions in the value of variability indexes (compared with baseline) reflect shifts of the autonomic balance toward sympathetic dominance whereas increased values of such parameters indicate a shift of sympathovagal balance toward parasympathetic prevalence (38).

Finally, the incidence of ventricular and supraventricular arrhythmic events was quantified. The identification of rhythm disturbances was based on the classic definitions of arrhythmias in humans and on the Lambeth Conventions for the study of experimental arrhythmias (10, 44). Mean R-R interval duration and HR variability measurements were performed after removal of R-R intervals surrounding arrhythmias.

Around-the-clock HR and physical activity sampling and processing. HR (expressed as beats/min) and physical activity (expressed as counts) were sampled around the clock for 60 s every 60 min in the following three phases: 1) prestress (4 days): starting 15 days after transmitter implantation and with the animal in its own home cage; 2) stress (15 days): between the 1st and the 15th day, with the animal in the resident’s cage; and 3) poststress (4 days): starting 24 h after the last agonistic interaction, with the animal back in its home cage. For each individual mouse the daily amplitudes of the rhythm of HR and physical activity were calculated as the difference between average 12-h dark and 12-h light values, i.e., values for circadian activity and resting phases, respectively (26).
Cardiac morphological study. At the end of the poststress chronobiological data recording, the mice were anesthetized with Leptofen (as described in Surgery: transmitter implantation) and their hearts were arrested in diastole with an injection of 1 ml of cadmium chloride solution (100 mM iv), rapidly removed, and weighed. The ventricles were then separated and fixed in paraformaldehyde (4%). Ten 1-mm-thick slices were transversely cut from the left ventricle and embedded in paraffin. A 5-µm-thick section obtained from one of the two intermediate rings was stained with hematoxylin and eosin and used for morphometric analysis. The section was analyzed with optical microscopy (magnification ×250) to evaluate the total amount of interstitial and reparative fibrosis in the left ventricular myocardium. Reparative fibrosis describes discrete areas (foci) of myocardial scarring resulting from focal myocyte cell loss, whereas interstitial fibrosis corresponds to the widening of the interstitial space due to collagen accumulation in the absence of apparent focal cell death (4). According to a procedure previously described (9), for each section a quantitative evaluation of the fibrotic tissue was performed in 60 randomly selected fields from subendocardium, midmyocardium, and subepicardium, with the aid of a grid defining a tissue area of 0.160 mm² and containing 42 sampling points, each covering an area of 0.0038 mm². To define the volume fraction of fibrosis in the three layers of the ventricular wall, the number of points overlaying myocardi
cut and subendocardial) and experimental treatment as the between-subject factor (2 levels: Ctr and SS). Comparisons between SS and Ctr mice for heart weight normalized to body weight (HW/BW; BW = animal weight − telemetry transmitter weight) and thickness of the left ventricular free wall and post hoc analyses were performed by Student’s t-test.

RESULTS

Behavioral data. Intruder animals displayed upright posture, flight behavior, and squeaking vocalization and therefore were categorized as subordinates, whereas resident mice exhibited chasing and biting behaviors and therefore were categorized as dominants (7). Visual inspection did not reveal severe wounds on intruder animals after aggressive encounters.

During the first interaction, resident and intruder animals exhibited the same amount of total attacking time (residents 12.0 ± 2.7 s, intruders 12.7 ± 2.4 s). In contrast, in the 4th and 15th exposures, only the residents showed aggressive behavior (4th exposure: residents 25.3 ± 6.9 s, intruders 0 ± 0 s; 15th exposure: residents 8.5 ± 3.4 s, intruders 0 ± 0 s; P < 0.05).

Acute HR responses. Figure 1 depicts minute-by-minute time trend of average R-R interval and R-R interval variability indexes before, during, and after social defeat (B, T, and PT periods) at the 1st, 4th, and 15th acute stress episodes. At each defeat episode, the 5-min average R-R interval values were significantly reduced during the test compared with the baseline period and did not recover in the posttest period (1st episode: B = 113.3 ± 4.1, T = 75.7 ± 0.7, PT = 74.9 ± 0.8 ms; 4th episode: B = 105.9 ± 2.8, T = 79.3 ± 1.5, PT = 81.1 ± 1.7 ms; 15th episode: B = 106.5 ± 4.4, T = 85.2 ± 1.7, PT = 85.4 ± 1.9 ms; B vs. T and PT, P < 0.05). As far as the 5-min r-MSSD values are concerned, similar results were observed at the first and fourth defeat episodes (1st episode: B = 2.8 ± 0.4, T = 1.4 ± 0.1, PT = 1.4 ± 0.1 ms; 4th episode: B = 1.8 ± 0.2, T = 1.3 ± 0.1, PT = 1.3 ± 0.1 ms; 15th episode: B = 2.2 ± 0.4, T = 1.9 ± 0.2, PT = 1.8 ± 0.2 ms; 1st episode: B vs. T and PT, P < 0.05; 4th episode: B vs. T and PT, P = 0.054 and P < 0.05, respectively; 15th episode: no significant differences). SDAR was significantly reduced during test and posttest periods only at the first defeat episode (1st episode: B = 6.0 ± 0.6, T = 2.1 ± 0.2, PT = 1.6 ± 0.1 ms; 4th episode: B = 4.1 ± 0.7, T = 3.0 ± 0.4, PT = 2.9 ± 0.4 ms; 15th episode: B = 4.6 ± 0.4, T = 4.8 ± 0.7, PT = 3.8 ± 0.5 ms; P < 0.05). In other words, a significant HR activation was persistently observed across all repeated defeat episodes.

An overall comparison between the effects of the three defeat episodes was obtained by means of AUC (area between the response time curve and the baseline). As reported in Table 1, AUC was significantly higher for RR, SDAR, and r-MSSD at the 1st episode than at the 4th and 15th stress episodes [P < 0.05, except for r-MSSD (1st vs. 4th, P = 0.052; 1st vs. 15th, P = 0.126)]. These comparisons suggest that there was a clear habituation-like effect of HR responsiveness, which was already visible starting from the fourth defeat episode.

In the 1st, 4th, and 15th defeat episodes, the incidence of rhythm disturbances during the test period (number of events) was negligible and no habituation-like effect was observed (supraventricular premature beats: 1st, 0.50 ± 0.36; 4th, 0.57 ± 0.37; 15th, 1.19 ± 0.86; ventricular premature beats: 1st, 1.21 ± 0.42; 4th, 1.93 ± 1.85; 15th, 1.07 ± 0.92).

Long-term effects on HR and physical activity. The average night and day 12-h values of HR changed significantly during the first 5 days of psychosocial stress (except for the average
night value of the 4th day, \( P = 0.08 \) compared with prestress values. In other words, the first 5 days of chronic social challenge were characterized by increased values of HR during both phases of the light-dark cycle. Also, the amplitude of HR rhythm was significantly affected, that is, it was reduced at days 1, 2, and 4 (\( P < 0.05 \); Fig. 2). The average night and day 12-h values of physical activity changed significantly from the second until the last day of psychosocial stress compared with

Table 1. Area between the response time curve and the baseline for RR, SDRR, and r-MSSD in 1st, 4th, and 15th stress interactions

<table>
<thead>
<tr>
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<th>1st Interaction</th>
<th>4th Interaction</th>
<th>15th Interaction</th>
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<tbody>
<tr>
<td>AUC RR</td>
<td>( 362.8 \pm 37 )</td>
<td>( 242.3 \pm 31^* )</td>
<td>( 204.3 \pm 48^* )</td>
</tr>
<tr>
<td>AUC SDRR</td>
<td>( 29.0 \pm 4.4 )</td>
<td>( 10.2 \pm 5.3^* )</td>
<td>( 3.2 \pm 5.3^* )</td>
</tr>
<tr>
<td>AUC r-MSSD</td>
<td>( 13.3 \pm 3.8 )</td>
<td>( 4.5 \pm 2.0^† )</td>
<td>( 4.3 \pm 4.4 )</td>
</tr>
</tbody>
</table>

Values (in ms min) are mean \( \pm \) SE areas between the response time curve and the baseline (AUC). RR, average R-R interval; SDRR, standard deviation of the mean R-R interval; r-MSSD, root mean square of successive R-R interval differences. ANOVA significant effect of stress interaction: AUC RR: \( F = 4.3, P = 0.02 \); AUC SDRR: \( F = 7.0, P = 0.002 \); AUC r-MSSD: \( F = 2.15, P = 0.13 \). Post hoc: *\( P < 0.05 \), significant difference compared with 1st interaction values; †\( P = 0.052 \) borderline difference compared with 1st interaction values.

Fig. 1. Time course of the heart rate (HR) parameters before (baseline) during (test), and after (posttest) 1st, 4th, and 15th social agonistic interactions. Each time point on the graphs represents the value of a 1-min period. The duration of the test period is bounded by dotted lines. Time points on left dotted lines indicate the mean of the five 1-min baseline values. A: mean R-R interval duration (RR); B: standard deviation of RR (SDRR); C: root mean square of successive R-R interval differences (r-MSSD). ANOVA for 5-min period: significant effects of stress episode (SDRR: \( F = 4.9, P = 0.009 \); r-MSSD: \( F = 14.9, P < 0.0001 \); r-MSSD: \( F = 10.6, P < 0.0001 \)), and stress episode-recording period interaction (RR: \( F = 4.1, P = 0.04 \); SDRR: \( F = 5.9, P < 0.0001 \)).
prestress values (except for the average day value of the 3rd and the 15th day, $P > 0.09$). In other words, chronic social challenge produced a significant decrease in the values of activity during both phases of the light-dark cycle. Nevertheless, the amplitude of the motor activity rhythm was reduced only at days 1, 2, and 15 ($P < 0.05$; Fig. 2).

**Morphometric analysis of myocardium.** The volume fraction of interstitial and reparative fibrosis in the entire left ventricular myocardium was negligible in Ctr mice ($<0.2$%; Table 2). Reparative fibrosis was characterized by a small number of microscopic scarrings homogeneously distributed in the three layers of the ventricular wall (Fig. 3A). In SS animals, the average amount of structural damage, although still limited, was about sixfold larger than in the Ctr group, mainly as a consequence of a fourfold increase in the numerical density of fibrotic foci (Fig. 3B; volume fraction $0.98 \pm 0.18\%$ vs. $0.15 \pm 0.04\%$, numerical density of the foci $1.83 \pm 0.34$ vs. $0.43 \pm 0.10$ foci/mm$^2$; $P < 0.05$). Conversely, the cross-sectional area of the foci was only slightly affected ($5,497 \pm 483$ vs. $4,412 \pm 469$ $\mu$m$^2$; $P = 0.15$). As shown in Table 2, larger myocardial damage was found in all three layers of the ventricular wall, although at the midmyocardium the statistical significance of the differences between SS and Ctr animals was borderline ($P = 0.053$).

HW/BW was significantly lower in SS mice than in Ctr mice ($3.7 \pm 0.3$ vs. $4.9 \pm 0.1$ mg/g; $P < 0.01$), whereas the thickness of the left ventricular free wall was similar ($1.67 \pm 0.6$ vs. $1.56 \pm 0.07$ mm; $P = 0.23$). These data indicate that chronic psychosocial stress induces a cardiac structural alteration involving the extracellular matrix of the left ventricular myocardium.

**DISCUSSION**

This study analyzed the short- and long-term pathophysiological effects of chronic psychosocial stress in male mice, consisting of 15-day continuous sensory contact and daily intermittent agonistic interaction with a dominant animal (3). We evaluated the acute changes in HR and sympathovagal balance during the 1st, 4th, and 15th agonistic episodes. The assessment of long-term effects took into account the changes in the circadian rhythms of HR and the consequences on myocardial structure.

Each social defeat episode produced significant reductions in average R-R interval values compared with the baseline, indicating that a certain level of acute sympathetic-adrenomedul- lary responsiveness was present throughout the whole chronic stress protocol. However, we found a habituation-like effect for all R-R interval parameters, i.e., a gradual reduction in acute cardiac autonomic responsiveness across repeated homotypic challenge episodes (34). In other words, the shift of sympathovagal balance toward a sympathetic dominance was gradually reduced from the first to the last acute defeat experience. Indeed, during the first defeat episode the level of vagal activity (as indicated by the values of r-MSSD) was significantly reduced compared with the baseline during both the test and posttest periods. On the contrary, such a reduction was not observed at the end of the stress protocol (i.e., acute response to the last acute defeat).

**Table 2. Myocardial fibrosis in the three layers of the left ventricular myocardium in SS and Ctr groups**

<table>
<thead>
<tr>
<th></th>
<th>Subepicardium</th>
<th>Midmyocardium</th>
<th>Subendocardium</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SS</td>
<td>Ctr</td>
<td>SS</td>
</tr>
<tr>
<td>Volume fraction, %</td>
<td>0.79±0.16†</td>
<td>0.13±0.05</td>
<td>0.63±0.24†</td>
</tr>
<tr>
<td>Area, $\mu$m$^2$</td>
<td>6.210±976</td>
<td>3.818±1</td>
<td>5.993±867</td>
</tr>
<tr>
<td>Density, foci/mm$^2$</td>
<td>1.73±0.45</td>
<td>0.58±0.13</td>
<td>1.50±0.45</td>
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</table>

Values are means ± SE. SS, stressed mice ($n = 13$); Ctr: control mice ($n = 10$); area, mean cross-sectional area of the fibrotic foci; density, numerical density of the fibrotic foci. ANOVA: significant effects of myocardial layer (volume fraction: $F = 4.32$, $P = 0.017$) and of experimental treatment (volume fraction: $F = 30.22$, $P = 0.0001$; density: $F = 8.61$, $P = 0.005$). Post hoc: *significant differences between SS and Ctr groups ($P < 0.05$); †borderline differences between the 2 groups ($P = 0.053$).
to 15th agonistic interaction), suggesting that the level of sympathetic dominance occurring during each defeat episode might depend on the degree of vagal withdrawal.

On the other hand, previous studies showed that repetitive treatment with the β-adrenergic agonist isoproterenol results in a downregulation of adrenergic receptors with attenuation of the effect of subsequent dosing (5). In the present study a large repeated release of epinephrine and norepinephrine, probably occurring during the 15 days of stress stimulation, may have determined a similar downregulation of myocardial adrenergic receptors at the level of the sinus node. This could represent an alternative explanation for such a reduction in sympathetic dominance across repeated defeat challenges (habituation to a homotypic stressor).

One may argue that the present study does not provide any direct measurement of plasma catecholamines. As a matter of fact, we are not aware of any study documenting plasma catecholaminergic activation in mice across repeated social stress episodes. However, we believe we can reasonably assume that a large repeated release of catecholamines took place across repeated defeat episodes on the basis of the following considerations. Studies on rats showed that social defeat produces a robust activation of the sympathetic-adrenomedullary system, as revealed by particularly higher plasma concentra-
tions of epinephrine and norepinephrine (15). Also, previous papers from Sgoifo et al. (30, 32) showed that plasma levels of catecholamines and values of R-R interval variability indexes change coherently due to an acute challenge, i.e., eleva-
tions of plasma catecholamines are accompanied by shifts of autonomic balance toward sympathetic dominance, during both defeat and restraint; 2) higher epinephrine and norepi-
nephrine levels observed during defeat compared with restraint were associated with lower values of R-R interval variability indexes; 3) strain differences in baseline and (social) stress levels of norepinephrine are associated with coherent differences in the values of R-R interval variability indexes.

The use of time-domain measurements of HR variability as a tool to evaluate the autonomic input to the heart is not entirely free from limitations, especially when applied to short-
term recordings. The main limitation of these statistical methods is that they provide more qualitative than quantitative information. In contrast, power spectral analysis of HR variability may provide more detailed information regarding the relative contribution of the two branches of the autonomic nervous system (41). However, time- and frequency-domain measurements are closely related, i.e., for every frequency-domain measurement there is a time-domain measurement that strongly correlates with it (38). In addition, as pointed out by Lombardi and colleagues (18), despite the impressive growth of research in the field of frequency-domain measurements, rewarding results can still be obtained by utilizing time-domain indexes.

Shifts of autonomic balance toward sympathetic dominance, which are indicated by decreased indexes of HR variability, are commonly associated with an increased susceptibility to ven-
tricular arrhythmias (19, 43). In the present study, the inci-
dence of cardiac arrhythmias across the stress episodes did not show any significant reduction. The absence of a clear habit-
uation effect might be due to the limited occurrence of rhythm disturbances during acute exposures to defeat, which in turn could be ascribed to the young age of the animals and the lack of evident cardiovascular pathologies.

These experimental results are substantially different from previous data from our research group, where rats experiencing intermittent social defeat did not show any habituation of sympathetic dominance and tachyarrhythmia susceptibility (34). This inconsistency among data can be explained by taking into account species and stress protocol differences. In the previous study, rats were intermittently exposed to the same acute stressor, i.e., 10 defeat episodes lasting 15 min, each followed by return to the home cage with no additional sensory stimulation from aggressive opponents. In contrast, in the present study the mice cohabited throughout the whole 15-day stress phase with an aggressive conspecific animal.

As for long-term consequences on chronobiological parameters, average night and day values of HR rose significantly in the first days of chronic stress and were associated with a reduction in the amplitude of day-night oscillation. In addition, physical activity was significantly depressed in both circadian phases and throughout the stress treatment. This condition represents an imbalance between normally precisely orchestrated physiological and behavioral processes (a marker of maladaptation?) and may constitute a risk factor for the development of disease (27). In certain circumstances, when adaptive mechanisms fail, the increased mortality observed among subordinate individuals in rat colonies suggests that subordi-
nation may carry serious negative implications for health (6).

In addition, the consequences of social defeat in rodents seem to represent a valuable animal model of depression (16). Disturbances in circadian rhythms, although by themselves neither sufficient nor necessary to induce pathological changes in behavior and mood, may well contribute to the imbalance between neurochemical and neuroendocrine systems and thus sensitize an individual to pathological mood changes (25).

H2138 CHRONIC STRESS, CARDIAC FUNCTION, AND STRUCTURE

Morphometric measurements indicated that exposure to chronic psychosocial stress induced cardiac structural alterations involving the extracellular component of the left ven-
tricular myocardium. Compared with control animals, stressed mice exhibited a higher percentage of fibrosis, mainly consisting of a higher number of scattered, small scarrings homoge-
neously distributed throughout the ventricular wall. This kind of cardiac remodeling may be considered as a consequence of focal myocyte loss (2) due to repetitive, transient increases in circulating catecholamines after stress-induced, acute sympa-
thetic activations (5). In fact, it is well recognized that the sympathetic nervous system has a major role in the cardiovas-
cular response to stress and there is a relationship between adrenergic stimulation and ventricular remodeling (28, 40).

It has long been postulated that exposure to high levels of catecholamines might have toxic effects mediated by β-adre-
nergic receptors of cardiomyocytes (21, 37). Furthermore, our data are in accordance with previous studies showing that injection of isoproterenol results in myocyte death, fibroblast proliferation, and connective tissue accumulation (5). The small amount of damage observed in our study may be due to the fact that the acute increase in circulating catecholamines occurring during each defeat episode was probably much lower compared with β-adrenergic agonist concentrations deriving from isoproterenol injection.

Henry et al. (13) reported that psychosocial stress, obtained by changing group composition twice a week for 6 mo, can
induce chronic hypertension and increased heart weight in normotensive strains of rats. In our stress model, where the duration of forced cohabitation was limited to 15 days, the thickness of the left ventricular wall did not change but the heart weight of SS mice was lower than that of Ctr mice. It can be hypothesized that in normal hearts, in the absence of a pronounced compensatory hypertrophic response, myocyte death and the associated development of replacement fibrosis lead to a decrease in heart weight. Indeed, Stilli and colleagues (39) showed that there is a significant negative correlation between the volume of fibrosis and the weight of the left ventricle in normal rat hearts.

In summary, during chronic psychosocial stress, animals exhibited HR rhythmicity disturbance that was substantially over after a few days of chronic adverse cohabitation. This evidence was in line with the observation of a clear habituation-like effect in terms of acute cardiac autonomic responsiveness. This link between habituation of acute cardiovascular responses and relatively rapid (yet gradual) normalization of HR rhythmicity suggests that mice adapt to adverse social conditions. Despite this coping capacity, we provided evidence that chronic psychosocial stress induces permanent cardiac structural changes with the appearance of numerous scattered microscopic foci of fibrosis. This allows us to hypothesize that a psychosocial challenge of longer duration might be able to produce a more severe structural damage, which in turn represents a substrate predisposing to more susceptibility to arrhythmias (40).

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