

Chronic psychosocial stress persistently alters autonomic function and physical activity in mice

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Abstract

We investigated heart rate (HR), temperature (T), and physical activity (Act) (by means of radiotelemetry) in male mice subjected to chronic psychosocial stress. Resident/intruder dyads lived in sensory contact for 15 days with the possibility to physically interact daily during the light phase for a maximum of 15 min. Intruders becoming dominants (InD) or subordinates (InS) were investigated here. The aims were to investigate; if a daily aggressive interaction would result in adaptation of autonomic responses; the effects of the social stress on daily rhythmicity and the way these effects change over time; whether acute and long-term autonomic changes do correlate; to compare dominants and subordinates. InD and InS showed a strong autonomic activation during the interactions, with moderate (InS) or no (InD) habituation over time. On the long term, InD showed tachycardia and marked hyperthermia but normal physical activity, while InS showed tachycardia, slight hyperthermia, and depressed physical activity. No correlation emerged between the acute and the long-term autonomic responses. These results highlight the existence of a sustained autonomic activation under chronic stress, which was also affected by mice social status. © 2003 Elsevier Inc. All rights reserved.

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1. Introduction

Acute and long-lasting changes in autonomic function are the hallmarks of the canonical stress response [1,2]. Acutely, when an individual is exposed to a challenge, heart rate and temperature rise as described in the fight–flight response [2]. With a different temporal dynamic, all systems will return to their pre-challenged baseline level [3]. Such an allostatic response raises two relevant questions: one regards the response in a subsequent acute challenge exposure; the second regards the long-term effects of a challenge on baseline autonomic function. Adaptation of a response to a given challenge has been indicated by McEwen [4] as one of

the key features of the normal ability of an organism to cope with the external world. If such an adaptation is lacking, a state known as allostatic load may develop with possible health consequences. Adaptation is commonly observed at several levels, i.e., behavioral responses to a stimulus, pattern of *c-fos* expression in the brains, immune functions, corticosteroid release, and so on [5–8]. Autonomic functions are less likely to adapt to both social and psychological stress repeated over time [9–11]. However, the absence of adaptation of a physiological response does not imply the existence of a sustained chronic imbalance of the system. A few studies demonstrated a sustained activation of the autonomic function in rats even 3 weeks after a single social defeat [3,12]. However, this observation has not been confirmed in studies on mice [13]. In addition, many of the defeat-induced behavioral and physiological alterations are buffered by a positive social context [14–16].

In the present study, we investigated autonomic functions and activity in male mice in a model of chronic psychosocial

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stress, adapted from those developed with tree shrews and mice [17,18], in which two previously unfamiliar male mice are housed in sensory contact with the possibility to physically interact once a day [19]. Intruders becoming dominants (InD) and subordinates (InS) were here investigated. To assess autonomic function (heart rate and temperature), mice were chronically implanted with a radio telemetric device allowing them to freely move and freely behave [1]. The aims of this study were (i) to verify whether daily social interaction, in the context of a chronic sensory contact between two subjects, would result in adaptation of acute autonomic responses; (ii) to investigate the effects of social stress on daily rhythmicity and the way these effects change over time; (iii) to investigate whether acute and long-term autonomic changes do correlate; (iv) to compare dominants and subordinates.

2. Methods

2.1. Animals

The subjects were 3-month-old male Swiss CD-1 mice from an outbred stock originally obtained from Charles River Italia (Calco, Lecco). The mice were born and reared in a colony room at the University of Parma at 22 ± 2 °C in a 12-h light–dark cycle (lights on at 0700 and off at 1900). After weaning (25–28 days of age), they were housed in same-sex-sibling groups (4–7 per group) in Plexiglas cages ($45 \times 25 \times 20$ cm) furnished with wood shaving bedding. Food (Mil Topi e Ratti, Morini, Reggio Emilia) and water were available ad libitum.

2.2. Procedure

Forty-two adult males were individually housed in Plexiglas cages ($45 \times 25 \times 20$ cm). The morning after, 21 mice were randomly assigned to surgery, further serving as intruders. The animals that did not undergo surgery served as residents. The whole procedure consists of 23 days: (i) pre-stress, 4 days in order to determine pre-stress circadian rhythmicity in heart rate (HR), temperature (T), and activity (Act); (ii) stress (see below), 15 days; (iii) recovery, 4 days—starting the morning of the 16th day, the experimental animals were rehoused in isolation in Plexiglas cages ($45 \times 25 \times 20$ cm).

2.3. Surgery: transmitter implantation

Animals were anesthetized with droperidol ($2.5 \text{ mg} \times \text{ml}$ of solution) + phentanyl citrate ($0.05 \text{ mg} \times \text{ml}$ of solution) (Leptofen, Pharmacia, $0.03 \text{ ml} \times 10 \text{ g}$ body weight, sc). The radiotelemetry electrocardiogram (ECG)/T/Act transmitter was chronically implanted according to a surgical procedure that guarantees high quality ECG recordings even during sustained physical activity [20]. Briefly, the

body of the transmitter was placed into the abdominal cavity and the two electrodes (wire loops) fixed respectively to the dorsal surface of the xyphoid process and in the anterior mediastinum close to the right atrium of the heart. Subsequently, mice were prophylactically injected for 2 days with gentamicine sulfate ($50 \text{ mg} \times 1 \text{ ml}$ of solution) (Aagent, Fatro, $0.01 \text{ ml} \times 10 \text{ g}$ body weight, sc). After implantation of the transmitter, mice were allowed 10 days for recovery of body weight and circadian rhythmicity of HR, T, and Act [21].

2.4. Chronic psychosocial stress

The procedure is a modified version of our standard procedure [19]. An experimental animal played the role of the intruder and was placed into the cage of a resident male having equal or higher body weight. After the interaction, the two animals were divided by means of a perforated polystyrene–metal partition allowing a continuous visual–olfactive–acoustic but not aggressive interaction. The partition bisected the cages in two symmetrical rectangular compartments. Both animals had food and water ad libitum.

The perforated partition was removed daily (for a total of 15 days) at an unpredictable time between 0900 and 1200 h (therefore, all interactions took place during the light phase). The interaction lasted 5 min after the first attack. If no attack occurred, the maximum interaction time allowed was 15 min. The chasing and biting animal was defined as dominant, while the mouse displaying upright posture, flight behavior, and squeaking vocalization was categorized as subordinate. According to the outcome of the agonistic encounters, four groups of animals were categorized: residents becoming dominants (RD), intruders becoming dominants (InD), residents becoming subordinates (RS), and intruders becoming subordinates (InS). The autonomic function of intruder mice only (both InD, $n=5$, and InS, $n=16$) was investigated in the present study.

2.5. Behavioral analysis

Interactions were video recorded and later scored by a trained observer by means of a series of chronometers. During the interaction, the latency to the first attack and total attacking time [22] of both the resident and the intruder was quantified and further analyzed as dependent measures. To compare the amount of aggression between RD–InS vs. RS–InD, the sum of the total attacking time of the two animals of each dyad (RD + InS and RS + InD) was computed.

2.6. Radiotelemetry system

The radiotelemetry system employed in this study enabled the recording of ECG, T, and Act from freely moving,

freely behaving animals. It consisted of flat transmitters measuring $20 \times 10 \times 8$ mm (TA10ETA-F20, Data Sciences Int., St. Paul, MN, USA) and platform receivers (RPC-1, Data Sciences Int., $32 \times 22 \times 3$ cm). ECG, T, and Act data were acquired following the procedures described below in the Sections 2.7 and 2.8.

2.7. Acquisition and analysis of heart rate, body temperature, and physical activity data during interaction sessions

Recording of HR, T, and Act were performed during the 1st (I1), 4th (I4), and 15th (I15) interaction episodes in three recording periods each lasting 5 min: pre-interaction (animals undisturbed in their home cages before partition removal), interaction (after partition removal), and post-interaction (animals separated again with the partition). HR, T, and Act were acquired with ART-Silver 1.10 data acquisition system (Data Sciences Int.). Data were stored as mean values of each recording minute (15 time points overall) and further computed as mean values for each 5-min recording period. In order to verify the effects of the agonistic encounter (I1, I4, and I15), values of each interaction and post-interaction were compared to the corresponding pre-interaction value. In addition, the effects measured in the pre-interaction, interaction, and post-interaction of the three different sessions (I1, I4, and I15) were compared among each other. An overall estimation of HR, T, and Act response to acute interactions was obtained by computing the area comprised between the response time curve and the pre-interaction (AUC; [10]).

2.8. Chronological data acquisition and analysis

Heart rate (bpm), body temperature ($^{\circ}\text{C}$), and physical activity (counts) were recorded in the three phases described above: (i) pre-stress, 4 days; (ii) stress, 15 days; (iii) recovery, 4 days. HR, T, and Act were sampled around-the-clock for 60 s every 30 min. The dark phase was sampled through the entire 12-h period. The light phase was sampled starting at 1400. This is due to the fact that during the stress phase, there is a time window comprised between 900 and 1230, where interactions and continuous acquisition of heart rate, body temperature, and physical activity occurred (due to system constraint, it is impossible to acquire in the same time acute and chronobiological data). The pre-stress values were averaged to obtain a reference value for the dark and light phases. For each individual mice, the daily amplitude of the rhythms of HR, T, and Act was calculated as the difference between average dark and light values, i.e., values for circadian activity and resting phases, respectively. An overall estimation of HR, T, and Act was obtained by computing the area comprised between the pre-stress values and the daily dark and light phases separately, recorded during the stress phase (AUC).

2.9. Statistical analysis

Data were analyzed by means of Statistical 5.0 (Stat-Soft, Tulsa, OK, USA). All parameters were expressed as means \pm standard error (S.E.) of the mean.

2.9.1. Behavioral data

Total attacking time was analyzed by means of two-way ANOVA with “resident–intruder” and “interaction session” as within subjects factors. Latency of the first attack (either by the resident or the intruder mice) was analyzed by means of one-way ANOVA with “interaction session” as within subject factor. Comparisons between RD–InS and InD–RS dyads in total-attacking time and latency were performed via two-way ANOVA with “dyad outcome” as between subject factor and “interaction session” as within subject factor.

2.9.2. Acute physiological responses

HR, T, and Act were analyzed with two-way ANOVA with “phase” and “interaction session” as within subject factors, while AUC was analyzed by means of one-way ANOVA with “interaction session” as within subject factor. Comparisons between InS and InD were performed using the AUC and analyzed by means of a two-way ANOVA with “social status” as between subject factor and “interaction session” as within subject factor.

2.9.3. Long-term physiological responses

Mean dark and light phase HR, T, and Act were analyzed by means of two-way ANOVA with “light–dark” and “interaction session” as within subject factors. Daily changes in HR, T, and Act were analyzed by means of two-way ANOVA with “light–dark” and “day” as within subject factors. Daily amplitude of HR, T, and Act were analyzed by means of one-way ANOVA with “day” as within subject factor. Comparison between InS and InD were performed using the AUC for the dark and the light phase and analyzed by means of a two-way ANOVA with “social status” as between factor and “light–dark” as within subject factors. Post hoc analyses were always performed by means of Tukey’s HSD test. Correlations were conducted between acute and long-term AUC values, and between the behavior and the long-term parameters, by means of Spearman Rank correlations.

3. Results

Acute automatic responses to daily agonistic interaction, as well as long-term stress-induced consequences, were first described separately for InD and InS. Afterwards, InD and InS were directly compared by means of the AUC values applied to the parameters recorded. Finally, correlations between acute and long-term autonomic functions were analyzed.

3.1. Intruder-dominant responses to chronic stress

3.1.1. Behavior

As evident in Fig. 1 (upper panel), after the first interaction, only the intruder-dominant animals showed attack behavior. The latency of the first attack did not change across session (data not shown). Intruder dominants showed no changes across interactions in their total attacking time.

3.1.2. Acute responses

Heart rate was significantly increased as compared to the pre-interaction phase during the interaction and post-interaction phases in all testing sessions (I1, I4, I15; $P < .001$, Fig. 2, upper left). A habituation effect was found only in the post-interaction of the 15th session as compared to the post-interaction of the 1st ($P < .05$). Similarly, no habituation emerged when comparing the area under the HR response curve of the three experimental sessions recorded (Table 1).

Body temperature significantly increased between pre-interaction phase and interaction and post-interaction phases in all testing sessions ($P < .001$, Fig. 2, mid left). No habituation in any interaction and post-interaction phases, as well as in the area under the temperature response curve of the 4th and 15th session, emerged when compared with the 1st session (Table 1).

Activity significantly increased between pre-interaction phase and interaction and post-interaction phases of the 1st session ($P < .01$, Fig. 2, lower left), while only between the pre-interaction and the interaction phases of the 4th ($P < .01$) and the 15th ($P < .05$) sessions with the post-interaction value going back to pre-interaction levels. There were no differences in any interaction and post-interaction phases as well as in the area under the activity response curve of the 4th and the 15th sessions when compared with the 1st (Table 1).

3.1.3. Long-term responses

In Fig. 3, telemetry data from one representative intruder dominant are provided. Heart rate for both the light and dark periods was increased as soon as the stress phase started, the tachycardic effect lasted a few days (see Fig. 4, upper), HR values for the light period increased as compared to pre-stress values from days 1 to 4 ($P < .001$), and HR values for the dark period increased from days 1 to 5 of the stress phase ($P < .01$). The daily amplitude of the HR rhythm (dark–light value) was reduced with respect to pre-stress only on day 1 ($P < .01$) of the stress phase (data not shown).

Temperature was dramatically affected in dominant animals with the light and especially the dark phases being increased with respect to pre-stress. As soon as the recovery phase started, temperature returned to pre-stress values. Temperature (Fig. 4, mid) values during the dark period were increased as compared to pre-stress value from day 1 until the end of the stress phase, i.e., day 15 ($P < .001$). Furthermore, temperature values during the light phase were increased in days 1 ($P < .001$), 2 ($P < .01$), and 4 ($P < .05$) of the stress phase as compared to pre-stress. The daily amplitude of body temperature was reduced as to pre-stress on day 1 ($P < .01$) of the stress phase, while from day 3 onward, a significant increase was noticed ($P < .01$), with values going back to normal in the recovery phase (data not shown).

Activity was slightly increased during the stress phase although not significantly. On a daily basis (Fig. 4, low), the only significant increase emerged in the light period of the first few days of the stress phase ($P < .001$). In the recovery, instead, an increase in activity was noticed for days 1 and 2 ($P < .001$). Daily amplitude of the activity was reduced with respect to pre-stress in the first day of the stress phase ($P < .01$), increased with respect to pre-stress in days 4 ($P < .01$), 5 ($P < .05$), and 6 ($P = .06$), as well as during the first 2 days of the recovery phase ($P < .01$, data not shown).

3.2. Intruder-subordinate responses to chronic stress

3.2.1. Behavior

As evident in Fig. 1 (lower panel) after the first interaction, only the residents showed aggressive behaviors while the intruder subordinates showed no further sign of attack. The resident dominants increased their total attacking time in the 4th interaction ($P < .01$) with respect to the 1st and the 15th, while after the first interaction, the subordinates

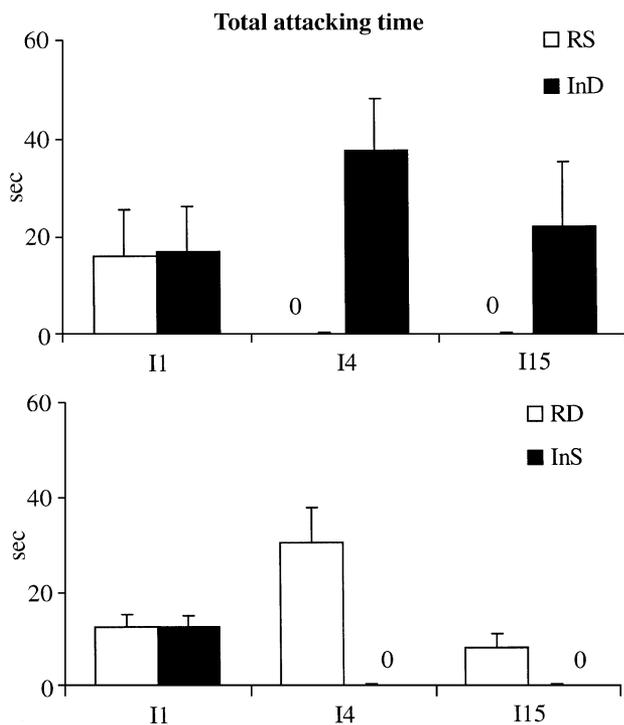


Fig. 1. Total attacking time displayed by mice during interactions I1, I4, and I15. Upper panel: resident subordinates (RS) and intruder dominants (InD). Lower panel: resident dominants (RD) and intruder subordinates (InS).

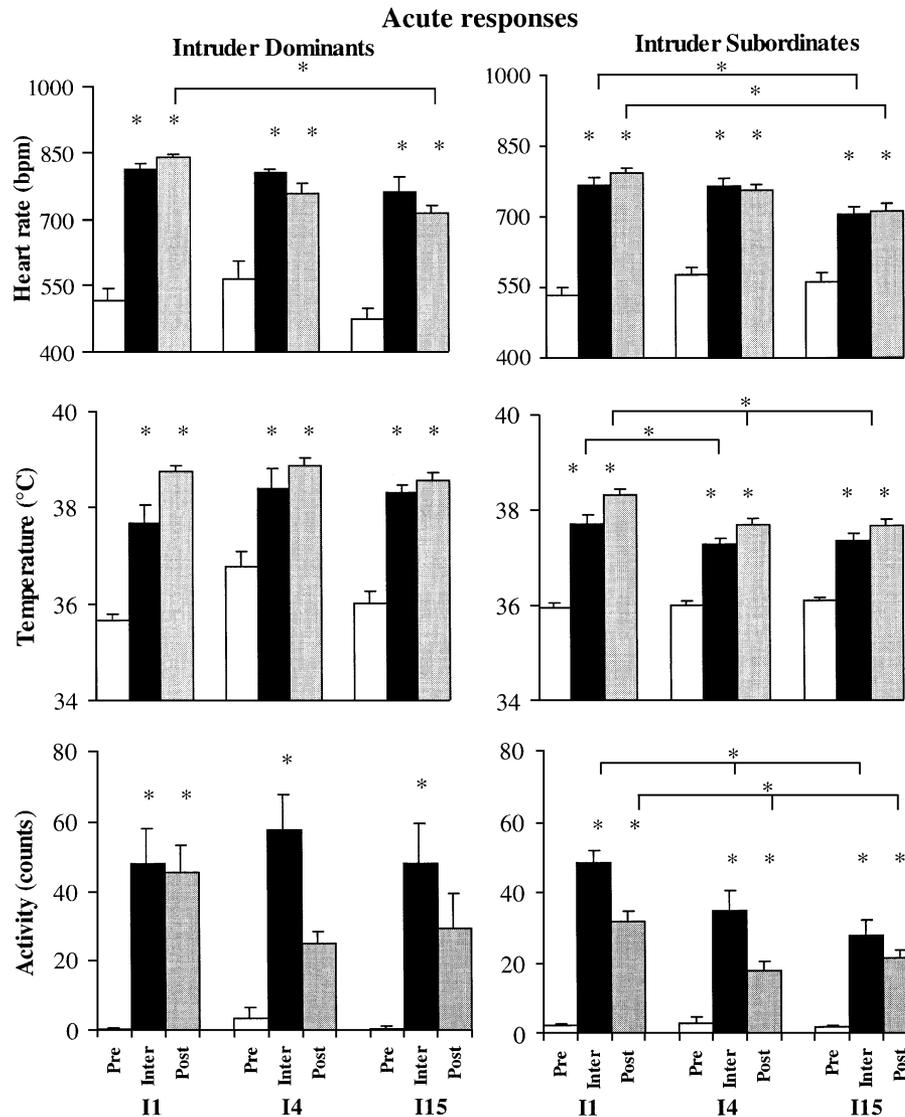


Fig. 2. Acute HR, T, and Act responses during interactions I1, I4, and I15. Values (mean of 5-min interval recordings) are presented for pre-interaction, interaction, and post-interaction phases. Left graphs: responses measured in intruder-dominant mice. Right graphs: responses measured in intruder subordinates. * $P < .05$ vs. pre-interaction or respective I1 value.

performed no further attack. The latency of the first attack increased in the 15th interaction when compared to the 1st ($P < .05$, data not shown).

Table 1
Area under the curve for acute responses to daily aggressive act

		InD	InS
Heart rate (bpm \times min)	I1	2915.4 (176.6)	2342.8 (231.7)
	I4	2063.4 (330.7)	1810.5 (241.7)
	I15	2493.4 (407.7)	1494.0 (251.4)***
Temperature (°C \times min)	I1	24.2 (2.3)	19.5 (1.5)
	I4	17.7 (3.5)	15.9 (2.0)*
	I15	23.1 (2.0)	13.2 (2.2)***
Activity (counts \times min)	I1	447.1 (53.6)	362.4 (28.3)
	I4	366.3 (65.0)	242.5 (34.9)*
	I15	368.0 (62.1)	226.2 (36.3)***

* $P < .05$ vs. I1.

** $P < .05$ vs. corresponding phase InD.

3.2.2. Acute responses

Heart rate significantly increased between pre-interaction phase and interaction and post-interaction phases in all testing sessions ($P < .001$, Fig. 2, upper right). A habituation effect was found in the 5th session, with the values being reduced when compared to the 1st and the 4th session ($P < .05$), while the 15th post-interaction showed reduced values compared to the post-interaction of the 1st ($P < .05$). To a similar extent, the area under the HR response curve was reduced in the 15th session when compared with the 1st one ($P < .01$, Table 1).

Body temperature significantly increased during interaction and post-interaction phases as compared to pre-interaction in all sessions ($P < .001$, Fig. 2, mid right). However, a habituation effect was found in the post-interaction phase of both the 4th and the 15th sessions when compared with the post-interaction of the 1st ($P < .05$). The temperature rise

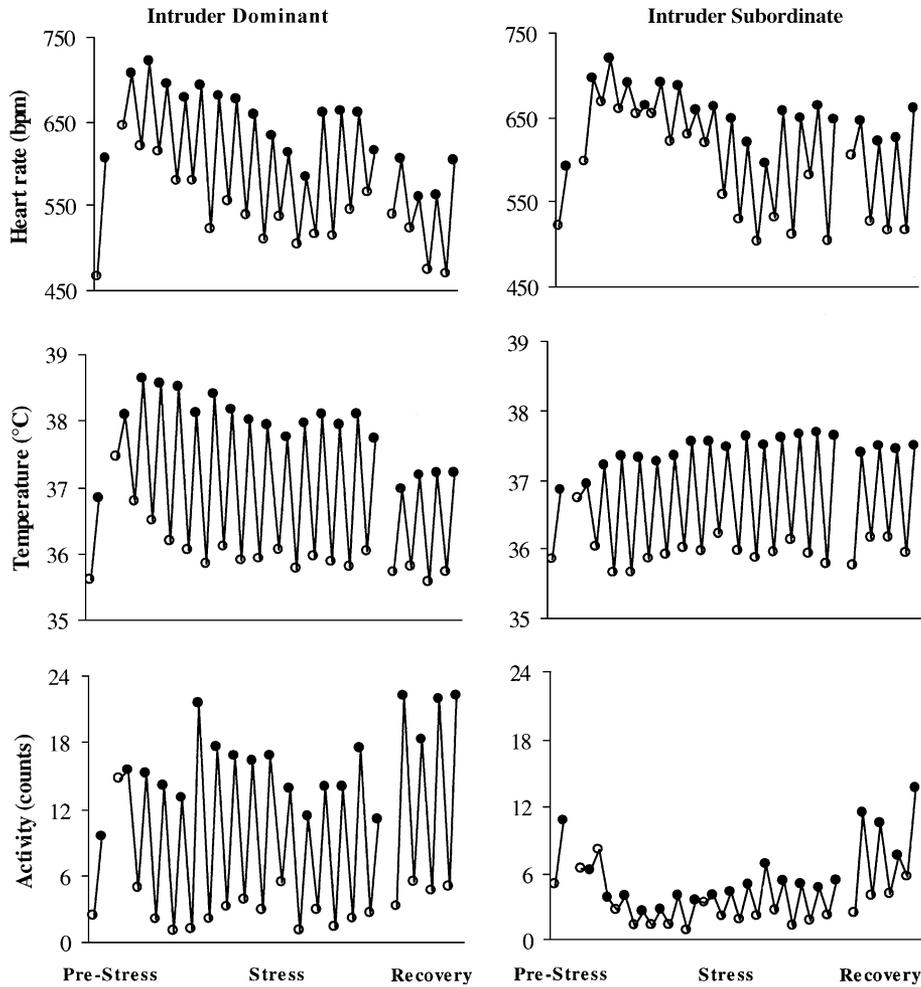


Fig. 3. HR, T, and Act data from one representative intruder dominant (left side) and one intruder subordinate (right side). Values are presented as mean daily dark (filled circles) and light (empty circles) phase values for pre-stress (mean of four days), stress, and recovery.

in the 4th interaction was reduced compared to the 1st interaction ($P < .05$). The area under the temperature response curve was reduced in both the 4th and the 15th sessions when compared with the 1st one ($P < .05$, Table 1).

Activity significantly increased between pre-interaction phase and interaction and post-interaction phases in all interactions ($P < .001$, Fig. 2, lower right). Both values for the interaction and the post-interaction phases of the 4th ($P < .01$) and the 15th ($P < .01$) sessions were reduced compared with corresponding values of the 1st. To a similar extent, the area under the activity response curve was reduced in both the 4th and the 15th sessions when compared with the 1st one ($P < .05$, Table 1).

3.2.3. Long-term responses

In Fig. 3, telemetry data from one representative intruder subordinate are provided. Heart rate was strongly increased during the stress phase with both the mean light and dark values significantly higher as compared to pre-stress. The HR (Fig. 5, upper) for the light period increased as compared to pre-stress light value from days 1 to 6 ($P < .001$)

and in days 7 and 8 ($P < .05$) of the stress phase. The HR for the dark period, instead, increased as compared to pre-stress corresponding value in days 1, 2, 3, and 5 of the stress phase ($P < .01$). Finally, the amplitude of HR rhythm was only transiently reduced as compared to pre-stress reaching a significant reduction in days 1 ($P < .01$) and 2 ($P < .05$) of the stress phase (data not shown).

Temperature was only affected in the dark period during the stress phase. In details, it is evident that hyperthermia develops from day 8 afterwards (Fig. 5, mid). In fact, the temperature for the dark period increased with respect to the pre-stress value in day 1 ($P < .001$) and from days 8 to 15 of the stress phase ($P < .01$, except day 9). Furthermore, the temperature for the light phase was increased only in day 1 of the stress phase compared to pre-stress. As soon as the recovery phase started, temperature returned to pre-stress values. The amplitude of the daily rhythm of temperature was reduced with respect to pre-stress on day 1 ($P < .01$) of the stress phase, while from day 9 onward, a significant increase was noticed ($P < .01$, data not shown).

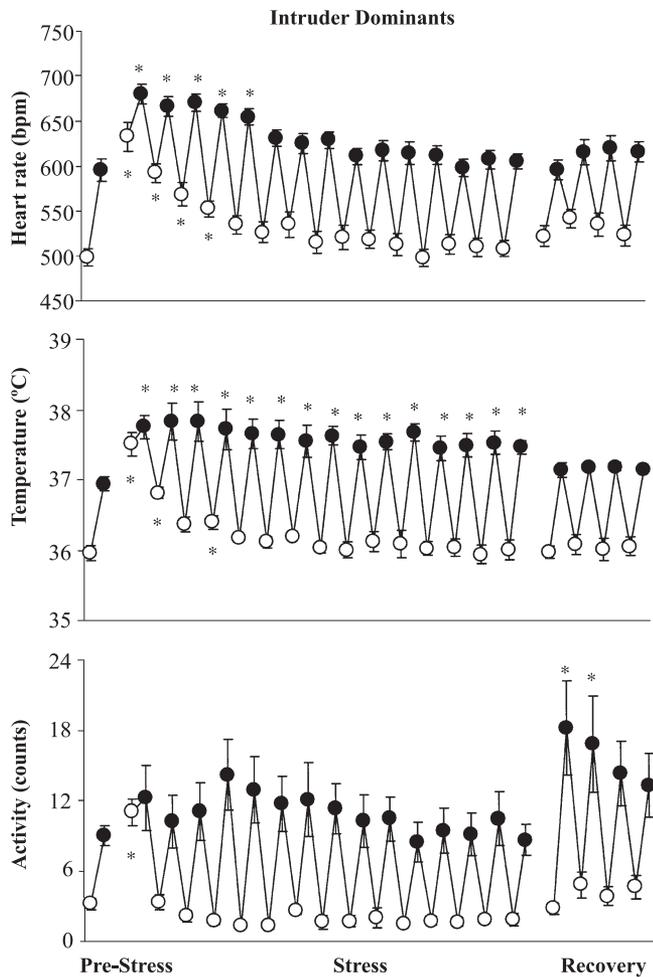


Fig. 4. HR, T, and Act values measured during the dark (filled circles) and the light (empty circles) phases of the daily cycle in intruder dominants. Values are presented for pre-stress, stress, and recovery. * $P < .05$ compared to pre-stress corresponding value.

Activity was strongly depressed mainly during the dark phase. During recovery, instead, an increase in dark phase activity emerged. On a daily basis (Fig. 5, lower), the activity in the dark period was reduced compared to the pre-stress level from day 2 onward ($P < .001$). On the contrary, in the recovery phase, an increase in activity was recorded during all days ($P < .0001$). The activity in the light phase was significantly reduced during days 9, 11, and 13 of the stress phase ($P < .05$). Daily amplitude of the activity daily rhythm was reduced with respect to pre-stress across the first 2 days of the stress phase ($P < .05$) and on day 15 ($P < .05$), while during the recovery, a significant increase was noticed ($P < .01$, data not shown).

3.3. Comparing intruder dominants and intruder subordinates

3.3.1. Behavior

When comparing the latency to the first attack and the total attacking time, no difference can be found between the

two categories RD–InS and RS–InD (data not shown). This suggests that resident and intruder dominants did not differ in terms of aggression level; therefore, intruder and resident subordinates mice were subjected to a similar degree of aggression across the chronic protocol.

3.3.2. Acute responses

Comparisons were performed by means of the area under the response time curve for each parameter (Table 1). Dominants displayed an overall higher HR [$F(1,19) = 4.71$, $P < .05$], T [$F(1,19) = 7.03$, $P < .05$], and Act [$F(1,19) = 5.18$, $P < .05$] response during the daily interaction when compared to subordinates. When the comparison was performed within each agonistic session, dominants showed significantly higher HR and temperature but not activity response only at the 15th session ($P < .01$).

3.3.3. Long-term responses

No difference between intruder dominants and intruder subordinates emerged when comparing HR, T, and Act recorded during the pre-stress phase (see Figs. 4 and 5 for basal levels). Comparisons among stress phases were per-

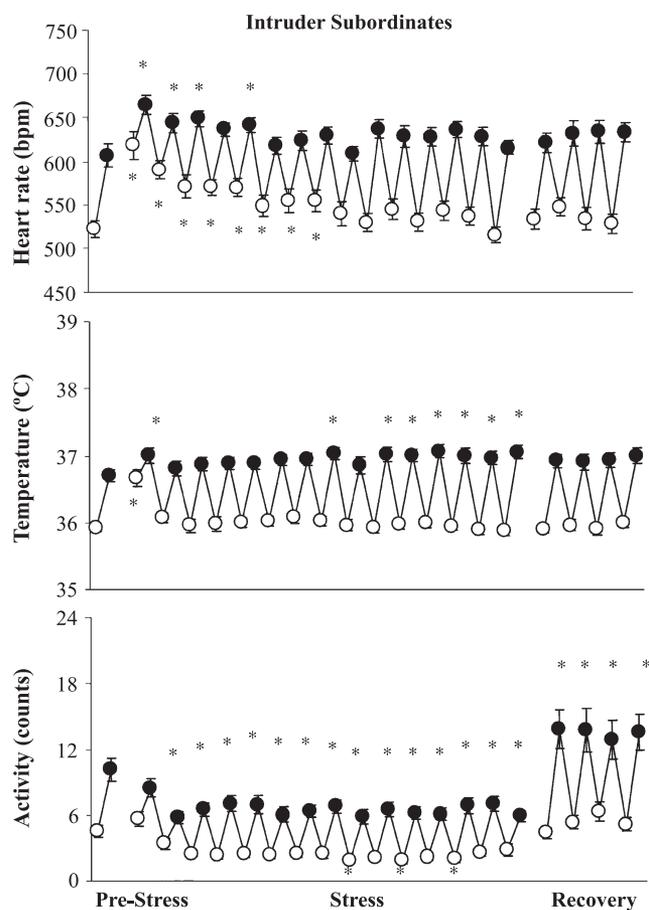


Fig. 5. HR, T, and Act values measured during the dark (filled circles) and the light (empty circles) phases of the daily cycle in intruder subordinates. Values are presented for pre-stress, stress, and recovery. * $P < .05$ compared to pre-stress corresponding value.

formed by means of the area under the curves of the dark and the light phase for each parameter. Results showed that dominants and subordinates differ in their temperature and activity responses to stress, but not in HR (Fig. 6). Effects were limited to the dark phase, with dominants showing higher AUC for the dark phase temperature than subordinates ($P < .05$). AUC for the dark phase activity was affected in opposite direction, with dominant showing a modest increase in activity while subordinates showing a reduction ($P < .05$). Light phases were never different between dominant and subordinates.

3.4. Correlations between acute and long-term parameters

No significant correlation emerged between the acute responses (calculated as AUC) measured in I1, I4, and I15 for HR, T, and Act, and the long-term responses (calculated as AUC) for the dark and the light period of the stress phase (data not shown). Similarly, no significant correlation

emerged between the behavioral parameters and the AUC for the long responses (data not shown).

4. Discussion

The purpose of this study was to investigate the acute and long-term alterations in heart rate, body temperature, and physical activity in male mice under chronic psychosocial stress. In our model, a chronic extension of the classical resident–intruder test [23], two previously unfamiliar mice, lived in a cage under continuous sensory contact, with the possibility to physically interact briefly once a day [19]. Autonomic function and activity of InD and InS were investigated in this study.

In the acute responses to aggressive interactions, InS showed a marked autonomic activation induced by the confrontation with their dominant counterparts (i.e., resident dominant) as proved by the strong increase in both heart rate and temperature over the pre-interaction level. A partial habituation was found for all the parameters measured. On the other hand, InD responded to the daily physical interaction (with resident subordinate mice) with marked tachycardia and hyperthermia without any sign of habituation over sessions. Therefore, despite subordinate partially habituate to the procedure, it is important to highlight the strong and persistent autonomic activation occurring even after a daily based aggressive interactions with the same opponent along 15 days.

The common procedure employed to study the effect of repeated social stressors in mice and rats requires a repetitive forced removal of the experimental animal from his familiar environment and the intrusion in the territory of a novel resident animal on consecutive sessions, i.e., a winner/loser context [24]. Under these conditions, mice and rats showed a mixed profile. In some studies, a partial habituation in the expression of *c-fos* in several brain regions was noticed, while other studies did not find habituation in the autonomic function when measured with radiotelemetric devices [8–11]. Sgoifo et al. [10,11] showed a substantial lack of habituation in stress-induced heart rate and temperature rise in response to both social and psychological intermittent homotopic stressor. In this view, our study provides the first investigation of the effects of intermittent (on a daily basis) aggressive encounters between mice in a situation of chronic sensory contact with an aggressive opponent. In this situation, the result remained substantially unchanged with respect to studies employing intermittent stress; both dominants and subordinates responded with a strong autonomic activation during every aggressive encounter, with some sign of (subordinate) or no (dominants) habituation over time. Furthermore, very few studies directly compared dominants/winners with subordinates/losers in their response to repetitive aggressive encounters. Interestingly, we found that InD responded to the daily fight with a greater HR and T increase with respect to pre-interaction than InS did. In

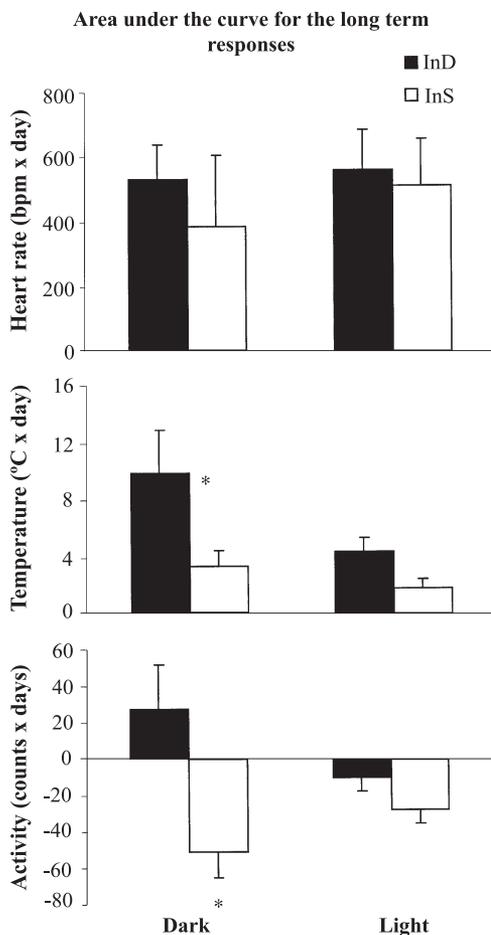


Fig. 6. Area under the curve above pre-stress for HR, T, and Act. Values are presented as mean values of the dark and the light phases of the daily cycle for intruder dominants (InD) and intruder subordinates (InS). * $P < .05$ InD vs. InS.

contrast, Sgoifo et al. [11] found that intermittent exposure of an intruder rat to a resident resulted in a partially habituating profile for the resident/winner, while no habituation was noticed for the intruder/loser. Species and procedural differences do not allow a straightforward comparison of the two data sets. However, our results strongly suggest that dominant/subordinate and winner/loser categorizations cannot be regarded as overlapping concepts.

The temporal dynamic of the stress response is one of the more intriguing aspects of the organism reaction to a challenging situation [3,25]. In the seminal studies conducted by Meerlo et al. ([26–28], Ref. [12] for review), a series of long lasting alterations have been observed in male rats after a single social defeat. However, relatively few studies investigated the long-term effect, as well as the temporal dynamic, of the stress response in animals subjected to an intermittent social defeat stressor [10,11,29,30], or with intermittent defeat and chronic stress continuing through (e.g., Ref. [13]). Due to this procedural variability, it is still a matter of debate how much the differences observed among studies depends on the procedure applied (chronic vs. single/intermittent), to the species employed (mice vs. rats), or even to the time of the day in which the interaction occur (light vs. dark). In the present study, we investigated the long-term effects on autonomic functions and physical activity in nonmanipulated and chronically stressed animals. InD and InS mice responded to the stress procedure with a strong long-term alteration in heart rate, temperature, and activity, which was also affected by social status. The temporal dynamic of events followed a multistep pattern (see Figs. 3–5). On the first day, a very strong effect was noticed for heart rate and temperature showing a blunted rhythmicity and values increasing dramatically above the pre-stress level. This applies to both dominants and subordinates. Despite no clear status definition was evident during the first interaction (see Fig. 1), the physical activity of future subordinates (InS) was not altered, whereas dominants (InD) showed a blunted circadian variation due to a very high light phase value. Following the very first day, both dominants and subordinates showed a strong tachycardia (about 50–100 bpm higher than pre-stress values) for about 6 days that was evident both during the light/inactive and the dark/active phase of the daily cycle. At that stage, differences between dominants and subordinates became more evident. Dominants maintained for the whole stress phase a strong dark phase hyperthermia, while subordinates showed a much smaller dark phase hyperthermia that appeared only some days later (8 days after stress onset). Importantly, subordinates showed a dramatic depression in activity, particularly in the dark phase, which is the active phase for rodents, while activity of the dominants was not statistically changed, or if anything, slightly increased.

The chronic stress model employed, with animals fighting on a daily basis while living in continuous sensory contact, allows investigating the impact of acute stress response on long-term changes in HR, T, and Act. Specifically, do the HR

and T rise measured during the daily aggressive interactions correlate with long-term changes in the same parameters? The clear answer of the present study is not. A related question is, do the behavior (in terms of latency of the first attack and total attacking time) during the interaction correlates with the long-term changes? Again, the clear answer from the present study is not. This result appear surprising given the literature addressing a role, for example, to counterattack frequency in determining the subsequent rhythmicity on T and HR in rats [31]. However, the current experimental setting differs from those measuring the long-term effects of single or intermittent events, first of all, because we employed mice, and second, because mice live under a continuous social challenge. In this context, it appears that a daily fight is perceived as highly arousing event (as proved by the marked and poorly habituating increase in HR and T), but this physiological activation does not correlate with the changes observed in the long term. In other words, these effects are more likely due to the psychological perception of the stressful context, i.e., to the continuous forced sensory contact, that the animal experience than to the daily fight. In agreement with this interpretation, the more dramatic stress-related alterations of homeostatic processes have been documented in animal model of chronic stress based on a continuous exposure to a social stimulus rather than to intermittent fights (e.g., [32–38]). Summarizing, our data clearly point toward a distinct mechanism for acute (likely adaptive) and long-term (likely maladaptive) changes on autonomic responses to a stressful context, i.e., they further support the validity of the distinction between the concepts of allostasis and allostatic load [4,39].

A further point to be discussed is the prolonged effects of stressful events after the cessation of the stimulus. In the pre-sent experimental context, HR and T went back to pre-stress level soon after the cessation of a chronic stress, i.e., in the recovery when the animal lived individually housed. Indeed, it has been reported a long-term alteration in rhythmicity for up to 3 weeks after a single social defeat [3] and after repeated conflicts [30]. In general, the effect observed is a dampening in the rhythm of HR, T, and Act [3,12]. The dampening in circadian rhythms, however, is not a constant finding [11,13], and the same is true for the carry-over effects of repeated social defeat [13]. In addition, in rats, the rhythm dampening after a single or double defeat may be determined by the further individual housing, since recent evidences suggest that group housing after such event can abolish physiological and behavioral effects [14–16]. Besides the already discussed procedural and species differences, the fast recovery in T and HR we observed after stress cessation does not mean that the animal would not have had long lasting effects of the chronic challenge. Indeed, these mice suffered an increased rate of fibrotic tissue development in the heart as compared to non-stressed counterparts (Costoli et al., unpublished observations).

Mechanisms involved in the chronic increase of autonomic functions observed under stress involve the hyper-

activation of the HPA axis and the sympathetic nervous system [1,40–42] with central CRH playing a main HPA-related and unrelated role [40–43]. Interestingly, *c-fos* responses to intermittent social stress were found to habituate in several brain regions, but not in the PVN, the anterior hypothalamus, and in several brain stem nuclei (see Ref. [8,9,44] for review), all brain structures related to temperature and autonomic functions control [46–48]. Chronic central CRH infusion was found to induce a long lasting (but habituating) hyperthermia and elevated corticosterone secretion in rats [41,49]. On the other hand, chronic corticosterone treatment, (3 mg day) able to mimic the circadian peak, did not induce rhythm disturbance in rats [50]. Therefore, it is possible to attribute to a sustained stimulation of the central sympathetic activity the results here observed, particularly in InD. Sickness behavior refers to a coordinated set of behavioral changes observed in individuals under infectious diseases or inflammation [51]. Sick individuals develop many physiological and behavioral changes including, for example, fever, depressed physical activity, inability to concentrate that also resembles depression-like states [51,52]. The profile of subordinate mice observed in the present study, i.e., hyperthermia that develops despite depressed home cage physical activity, as well as high corticosterone level [19] shares some similarity with a sickness behavior state.

In line with the abovementioned findings, it is tempting to suggest that the experimental procedure employed was able to modulate two different physiological responses depending on the social status of the subject. Namely, dominants seem to develop a hyper-activation of the autonomic nervous system, while subordinates develop a state resembling sickness behavior. This conclusion needs further experimental evaluations but opens an intriguing possibility, i.e., to investigate the development of different stress-related responses despite the same challenge presented to the subjects. A possible relevance of this finding is the link between individual vulnerability, psychopathologies, and unbalanced autonomic function [4,36,53–56].

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