

Cardiac autonomic responses to intermittent social conflict in rats

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Abstract

Intermittent exposure to the same stressor can lead to a gradual decline in physiological, neuroendocrine and behavioral stress responses (habituation). We investigated possible habituation of cardiac autonomic responsiveness and susceptibility to cardiac arrhythmias in male rats exposed to either intermittent social victory (VIC) or defeat (DEF) stress (10 exposures in each case). Electrocardiograms were recorded via radiotelemetry and the sympathovagal balance at the level of the heart was evaluated via time-domain measurements of heart rate variability, namely average R–R interval (average time interval between two consecutive heart beats, RR), the standard deviation of RR (SD_{RR}) and the root-mean-square of successive R–R interval differences (r-MSSD). Values of these parameters were significantly lower in DEF as compared to VIC rats in the second part of the test period (from Minute 6 to Minute 15), suggesting a more pronounced sympathetic dominance in the former group of animals. Accordingly, the occurrence of the most frequent cardiac arrhythmias (ventricular and supraventricular premature beats) was higher in DEF rats. Habituation of cardiac autonomic responsivity was observed across repeated exposure to victory, both in terms of sympathovagal balance and susceptibility to cardiac tachyarrhythmias, whereas no habituation was found in repeatedly defeated animals. A possible explanation to this discrepancy could be the different degree of controllability characterizing the two social challenging situations. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Intermittent exposure to the same type of stressful stimulus (homotypic stressor) can lead to a gradual reduction in the physiological, neuroendocrine and behavioral response of the organism. This phenomenon is frequently called habituation and can be viewed as a process similar to habituation to a sensory stimulus [1,6,7,19,20]. Although habituation is not the rule when a homotypic stressor is repeatedly applied (meaning that also no change or increase in the responsiveness of a system can be observed) [2,18,32], it has been documented for various organismic responses and in different types of stressful situations.

In rats, for instance, repeated exposure to the same stressor over time (such as noise stress, novelty or swimming) has been shown to produce a gradual decrease in the

responsiveness of the pituitary–adrenocortical and the sympathetic–adrenomedullary systems [1,6,10,13,16]. In studies based on repeated immobilization stress, habituation developed leading to a suppression of noradrenergic response in the hypothalamic paraventricular nucleus [29].

Several variables appear to influence the patterns of neuroendocrine adaptation to a stressful situation, including predictability–controllability factors, the type, intensity and duration of the stimulus, the interval between each episode of stress and the number of presentations of the stressor [15]. For example, sympathetic–adrenomedullary responses are dampened considerably in animals exposed to a highly predictable regimen of intermittent exposure to inescapable footshock or restraint [12]. In addition, stimulus intensity appears inversely related to the magnitude of habituation. Rats exposed to repeated low-intensity shock stimuli showed habituation of plasma corticosterone response, whereas they never habituated and instead demonstrated an increased responsivity (sensitization) with higher intensity shocks [21].

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De Boer et al. [8] showed that the frequency of stressor presentation (i.e. the length of interstressor interval) also plays a role in the adaptation pattern of neuroendocrine and metabolic responses to chronic intermittent stress. Rats exposed to five trials of water immersion stress exhibited a gradual decline of plasma noradrenaline, adrenaline, corticosterone and glucose levels. The amplitude and the speed of these neuroendocrine–metabolic adaptations were inversely correlated with the interstressor interval, i.e. shorter interstressor intervals produced larger and faster habituation profiles.

Despite the wealth of studies on a number of physiological–neuroendocrine habituation processes, there is only limited information regarding cardiac autonomic adaptation to intermittent homotypic stressors, especially as far as social challenges are concerned. Tornatzky and Miczek [30] showed that autonomic (heart rate) and behavioral (ultrasonic vocalization) reactions during intermittent agonistic confrontations (social defeat) do not undergo habituation in rats.

In the present paper, we analyzed the effects of two types of intermittent social aversive experiences, namely repeated victory and defeat [3,5,17]. In the social victory test, the experimental animal was the resident in a classical resident–intruder test, which means that it frequently attacked and, finally, submitted an unfamiliar male intruding its home cage [3,17]. In the social defeat test, the experimental animal was itself the intruder in a resident–intruder test, i.e. it was introduced to the territory of an aggressive unfamiliar resident male, by which it was attacked and finally submitted [3,17].

These two series of homotypic social challenges were chosen and compared in view of their supposed difference in terms of controllability properties [26,31,34].

The aim of this study was to test whether repeated exposure to the same social stressor induces habituation of short-term cardiac autonomic responses and whether possible adaptation processes are linked to the predictability–controllability characteristics of different aversive social conditions.

2. Methods

All experimental procedures in this study were approved by the Veterinarian Animal Care and Use Committee of Parma University.

2.1. Animals and housing

We used 27 wild-type Groningen male rats (*Rattus norvegicus*, WTG), originally derived from the University of Groningen (The Netherlands) and bred in our department under conventionally clean conditions [25]. Animals were housed in unisexual groups of five individuals, from weaning until the onset of experiments (5 months of age), in clear Plexiglas cages measuring 60 × 40 × 20 cm. Addi-

tional males were used either as resident or as intruder opponents in the “resident–intruder test” (see Social challenges for details).

All the animals were kept in rooms with controlled temperature ($22 \pm 2^\circ\text{C}$) and lighting (lights on from 07:00 to 19:00 hours). The bedding of the cages consisted of wood shavings, and food and water were freely available.

2.2. Radiotelemetry system

The radiotelemetry system employed in this study consisted of flat transmitters measuring $25 \times 15 \times 8$ mm (TA11CTA-F40, Data Sciences International, St. Paul, MN, USA) and platform receivers. Two types of platform receivers were used: one for small cage recordings (RPC-1, Data Sciences International), the other (manufactured by the Electronic Department in the Biological Center of the University of Groningen, The Netherlands) for large cage recordings [24].

2.3. Surgery: transmitter implantation

The telemetry ECG transmitter was chronically implanted according to a surgical procedure that guarantees high-quality ECG recordings also during sustained physical activity [23]. 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 xiphoid process and in the anterior mediastinum close to the right atrium. Animals were anesthetized with droperidol + phentanyl citrate (Leptofen, Pharmacia, 1 ml/kg sc). Subsequently, rats were prophylactically injected with gentamicine sulfate (Aagent, Fatro, 0.2 ml/kg sc) and individually housed in clear Plexiglas cages measuring 39 × 23 × 15 cm. After 3 days of recovery, part of the animals ($n=15$, defeat group) were kept individually housed and part were paired with females ($n=12$, victory group) in larger cages (90 × 60 × 55).

2.4. Social challenges

All experimental sessions were performed in the light phase between 10:00 and 15:00 hours.

2.4.1. Intermittent defeat test

Intermittent defeat stress program was performed starting 10 days after ECG transmitter implantation. Each recording session consisted of baseline, test and recovery periods lasting 15 min each. During baseline, animals (DEF, $n=15$) were left undisturbed in their own home cages. Defeat was obtained by introducing the instrumented animal to the home cage of a highly aggressive unfamiliar male, after temporary removal of its female partner (resident–intruder test). There, it was vigorously attacked and finally submitted by the resident animal [3]. Immediately after the test, the experimental rat was returned to its own home cage

for recovery ECG recording. This procedure was repeated 10 times on alternate days, each time using a different aggressive opponent.

2.4.2. Intermittent victory test

Intermittent victory program was performed starting 10 days after ECG transmitter implantation. Each recording session consisted of baseline, test and recovery periods lasting 15 min each. During baseline, animals (VIC, $n=12$) were left undisturbed in their own home cages. Victory was obtained by introducing an unfamiliar male intruder (4 month old) to the home cage of the instrumented animal, after temporary removal of its female partner. The experimental animal frequently attacked and finally submitted the intruder [17]. Immediately after the test, the intruder was removed from the cage of the experimental animal and recovery ECG recording started. The female partner was put back to the experimental rat cage at the end of recovery phase recording. This procedure was repeated 10 times on alternate days, each time using a different intruder.

2.5. ECG data acquisition and processing

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 ECG analysis was performed by means of a software package developed in our lab (XRRECG) for quantification of time-domain indices of heart rate variability. The following parameters were quantified: (1) the mean R–R interval duration (i.e. the average time distance between two consecutive heart beats (RR, ms)); (2) the standard deviation of RR (SD_{RR} , ms); and (3) the “root-mean-square of successive R–R interval differences” (r-MSSD, ms) SD_{RR} estimates overall heart rate variability and, therefore, includes the contribution of both branches of the autonomic nervous system to heart rate variations: it measures the state of the balance between sympathetic and parasympathetic activities on the heart. The r-MSSD focuses on high-frequency, short-term variations of heart rate, which are mainly due to the activity of the parasympathetic nervous system [14,24,27,28]. Generally speaking, reductions in the values of variability indices (as compared to baseline) reflect shifts of the autonomic balance toward a sympathetic dominance, while increased values of such parameters indicate a shift of sympathovagal balance toward a parasympathetic prevalence [27]. Finally, the incidence of the most common arrhythmic events, such as ventricular (VPBs) and supraventricular premature beats (SVPBs), was quantified [4]. The identification of rhythm disturbances was based on the classical definition of arrhythmias in man and on the Lambeth Conventions for the study of experimental arrhythmias [33].

Mean R–R interval duration and heart rate variability measures were performed after removal of R–R intervals surrounding arrhythmias.

2.6. Statistical analysis

ECGs were continuously recorded during the 1st and 10th defeat and victory exposures. R–R interval parameters (mean R–R interval, SD_{RR} and r-MSSD) were quantified across each 15-min recording phase (baseline, test and recovery). Means were calculated every 3 min in order to quantify in more detail the temporal dynamics of heart rate indices. Only the last 3-min period of baseline ECG was considered as basal reference point, whereas five time points for both the test and the recovery phases were included in the data processing. Values of R–R interval parameters in the different stress situations were compared via ANOVA for repeated measures, with “type of stressor” as between-subjects factor (four levels: 1st and 10th defeat, 1st and 10th victory) and “recording period” as within-subjects factor (11 levels: one baseline, five test and five recovery periods). The incidence of arrhythmias was expressed as the number of events per each 15-min recording period. Therefore, for arrhythmias, the within-subjects factor (recording period) was characterized by three levels (one baseline, one test and one recovery). Further post hoc analyses on all electrocardiographic parameters were performed via Student’s *t* test. Values for these parameters were always expressed as mean \pm standard error of the mean (S.E.). Statistical significance was set at $P < .05$. Statistics were performed using SPSS 8.0 software package (SPSS, Chicago, IL, USA).

3. Results

3.1. Average R–R interval and R–R interval variability parameters

Fig. 1 reports the time evolution of average R–R interval and R–R interval variability parameters (SD_{RR} and r-MSSD) during the 1st and 10th exposure to victory (VIC) and defeat (DEF) experiences. Mean values were calculated for last 3-min baseline and for each 3-min period in which the test and recovery phases were divided. Detailed statistical analysis is reported in the legend of the figure

3.1.1. First victory experience vs. tenth victory experience

In the first victory experience, average R–R interval was significantly reduced all across the test duration as compared to baseline, and did not recover within the posttest recording period ($P < .05$, Fig. 1A). In addition, values of SD_{RR} were significantly lowered during the test ($P < .05$), but they recovered starting from the first minute of the posttest period. The time evolution of r-MSSD values throughout the recording session resembled that of $S.D._{RR}$, although the last two time points of the test were only tendentially different from baseline ($P = .06$ and $P = .07$, respectively).

Although the 10th victory also produced a reduction of average R–R interval, which lasted all throughout the test and recovery ($P < .05$, Fig. 1A), SD_{RR} was never signifi-

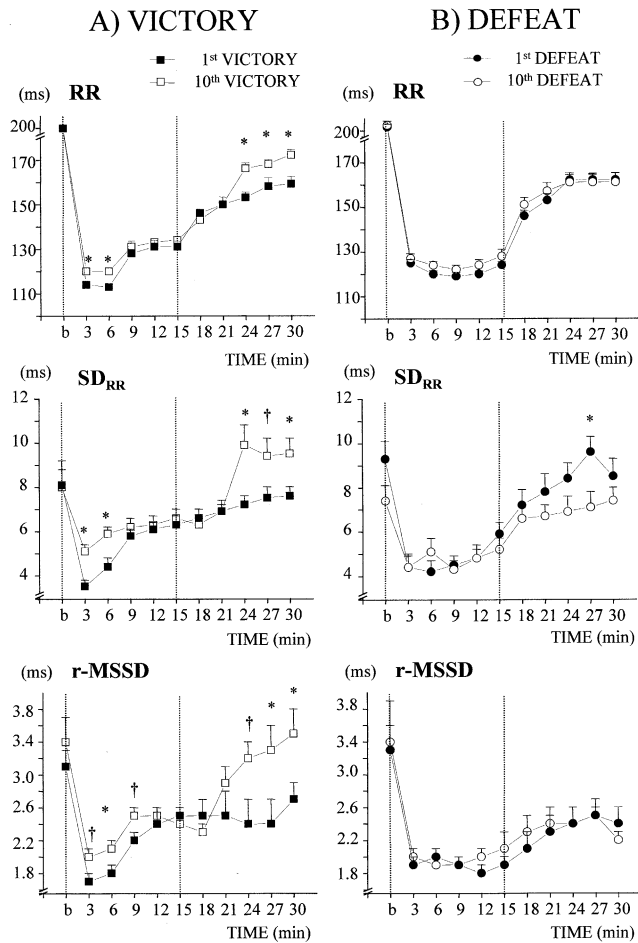


Fig. 1. Time course of heart rate parameters during and after the 1st victory (■, *n* = 12), 10th victory (□, *n* = 12), 1st defeat (●, *n* = 15) and 10th defeat (○, *n* = 15). Dotted lines indicate duration of agonistic encounter. Each time point on left dotted line indicates last 3-min baseline mean value. Values are means ± S.E. RR: mean R–R interval duration (in ms); SD_{RR}: standard deviation of average R–R interval (ms); r-MSSD: root-mean-square of successive R–R interval differences (ms). ANOVAs: Effects of “type of stressor” were significant (*P* < .05) for SD_{RR} and r-MSSD (SD_{RR}: *F* = 3.3; r-MSSD: *F* = 4.7). Effects of “recording period” (RR: *F* = 442.9; SD_{RR}: *F* = 10.9; r-MSSD: *F* = 7.5) and of stressor–period interaction (RR: *F* = 3.1; SD_{RR}: *F* = 2.1; r-MSSD: *F* = 5.7) were significant for all parameters. *Post hoc*s: Comparison between 1st and 10th stress exposure: * *P* < .05, † .05 ≤ *P* ≤ .07. Significant differences between baseline vs. test and recovery values: (1st victory) RR: vs. all. SD_{RR}: vs. 3, 6, 9, 12, 15 min (*P* = .08 vs. 18 min). r-MSSD: vs. 3, 6, 9 min (*P* = .07 vs. 12 and 15 min). (10th victory) RR: vs. all. SD_{RR}: vs. 3 min. r-MSSD: vs. 3, 6, 9, 12, 15, 18 min. (1st defeat) RR: vs. all. SD_{RR}: vs. 3, 6, 9, 12, 15, 18. r-MSSD: vs. 3, 6, 9, 12, 15, 18, 21, 24, 30 (*P* = .06 vs. 27 min). (10th defeat) RR: vs. all. SD_{RR}: vs. 3, 6, 9, 12, 15. r-MSSD: vs. 3, 6, 9, 12, 15, 18, 30 (*P* = .07 vs. 21, 24, 27 min).

cantly different from baseline (apart from the first time point of the test period). On the contrary, changes in r-MSSD resembled those observed in the 1st victory, i.e. significant reductions as to baseline were found in the test period and at the first recovery time point (*P* < .05).

The comparison between the 1st and 10th victory responses (Fig. 1A) revealed that reductions in R–R interval

parameters (RR, SD_{RR} and r-MSSD) were larger in the 1st victory experience at the beginning of the test and the end of the recovery phase (RR: *P* < .05 at Minutes 0–3, 3–6, 21–24, 24–27, 27–30; SD_{RR}: *P* < .05 at Minutes 0–3, 3–6, 21–24, 27–30, *P* = .07 at Minutes 24–27; r-MSSD: *P* < .05 at Minutes 0–3, 3–6, 24–27, 27–30, *P* = .07 at 6–9 and *P* = .053 at 21–24). Therefore, a habituation effect was observed across repeated exposure to social victory experience, clearly visible in the first minutes of the test and the last phases of the recovery period.

3.1.2. First defeat experience vs. tenth defeat experience

Average R–R interval was significantly lowered in both the 1st and 10th defeat exposures as compared to baseline (*P* < .05, all across the test and recovery periods; Fig. 1B). Values of SD_{RR} were significantly reduced for all the test duration in both the 1st and 10th defeat episodes, and were back to baseline during the recovery phase in both cases. Apart from few time points, values of r-MSSD were significantly reduced as compared to baseline for all the recording time in both the 1st and 10th defeat episodes (Fig. 1B). As illustrated in Fig. 1B, the comparison between the 1st and 10th defeat exposures revealed no statistical difference throughout the test and posttest periods, for neither average R–R interval nor heart rate variability parameters (except for one time point of SD_{RR} during the recovery phase). This suggests that no habituation effect took place when animals experienced intermittent defeat challenge.

3.1.3. First victory experience vs. tenth defeat experience

In the first 6 min of test recording and all throughout the recovery phase, values of average R–R interval (RR), SD_{RR} and r-MSSD were similar in animals exposed for the first time to victory (VIC) and defeat (DEF) (Fig. 1A,B). On the contrary, such parameters were significantly lower in DEF rats during the second part of the test period, namely at Minutes 6–9, 9–12 and 12–15 for RR, at Minutes 6–9 and 9–12 for SD_{RR} and at Minutes 6–9, 9–12 and 12–15 for r-MSSD (*P* < .05).

3.2. Arrhythmias

Fig. 2 summarizes the occurrence of the two most common cardiac arrhythmic events recorded before, during and after social stress exposures, i.e. ventricular premature beats (VPBs) and supraventricular premature beats (SVPBs). They occurred either as isolated events or grouped in doublets or triplets, the isolated form being far more frequent. The cumulative occurrence of both VPB and SVPB events was obtained by just summing up all arrhythmic beats, regardless of whether they appeared isolated or grouped.

In animals experiencing victory (Fig. 2A), SVPBs were hardly ever observed, both in the test and recovery periods. Therefore, no habituation effect in the incidence of these arrhythmic events could be seen when the 1st and 10th victory episodes were compared. On the contrary, VPBs,

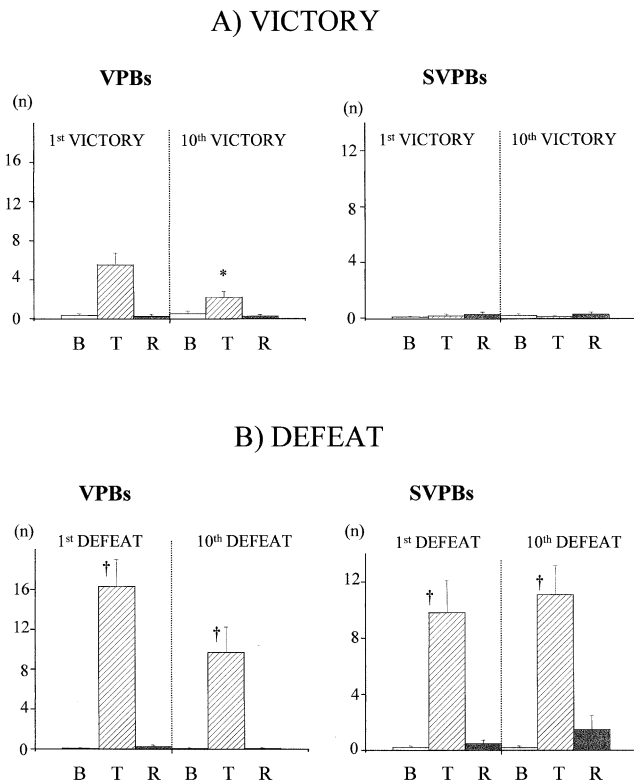


Fig. 2. Incidence (expressed as number of events, n) of cardiac tachyarrhythmias, namely ventricular premature beats (VPBs) and supra-ventricular premature beats (SVPBs) before (baseline, B), during (test, T) and after (recovery, R) the 1st victory, 10th victory, 1st defeat and 10th defeat. * Significantly different from the 1st victory corresponding value; †Significantly different from the 1st or 10th victory corresponding value.

which occurred more frequently (specifically during the test period), exhibited a clear habituation, i.e. they were significantly less frequent in the 10th as compared to the 1st victory test (2.2 ± 0.6 vs. 5.5 ± 1.2 , $P < .05$, Fig. 2A). Both kinds of cardiac arrhythmias were far more frequent in DEF rats as compared to VIC rats, regardless of whether the 1st or 10th exposures were considered (see figure legend for detailed statistics). However, no significant difference in the incidence of SVPBs and VPBs was found between the 1st and 10th defeat, i.e. no adaptation was observed for cardiac arrhythmias across repeated episodes of social defeat (Fig. 2B), though a tendency toward habituation of VPB occurrence was observed also in DEF rats ($P = .08$).

4. Discussion

The present data indicate that both defeat and victory experiences are associated with significant acute changes in cardiac sympathovagal balance. Decreased values (as compared to baseline) of average R–R interval and heart rate variability parameters (i.e. SD_{RR} and $r\text{-MSSD}$, indices of sympathovagal balance) point to a shift of autonomic balance toward a sympathetic dominance in both challeng-

ing conditions, which was particularly evident in the period of actual agonistic confrontation (test period) [24,25]. Overall, heart rate parameter reductions were similar when the animals were exposed for the first time to the two different challenging situations. However, a discrepancy in heart rate and heart rate variability responsivity between the two stressors was found in a specific phase of the test period, namely between the 6th and 15th min of recording. During this period, rats facing defeat had significantly lower values of such parameters as compared to animals experiencing victory, i.e. they were likely characterized by a larger sympathetic dominance. This finding is consistent with published data indicating a much higher catecholaminergic activation during defeat than during victory. Plasma noradrenaline and adrenaline levels, determined just at the end of the conflict, have been shown to be significantly larger in rats losing a combat as compared to rats winning a fight [11].

In the present study, also, the occurrence of cardiac tachyarrhythmias (both at the ventricular and atrial level) was significantly different in the two social aversive contexts. In fact, ventricular and supra-ventricular premature beats were far more frequent during the test in rats experiencing defeat, regardless of whether the first or tenth stress exposures were considered. The difference in arrhythmia susceptibility in the two challenging situations (both at the 1st and 10th exposure) appears far larger than expected according to the observed difference in heart rate responsivity. This suggests that, despite the fact that during defeat a higher activation of the sympathetic–adrenomedullary system (higher plasma catecholamine levels) and a larger sympathetic dominance at the level of the heart (lower values of heart rate variability) took place, the heart rate response was likely near to its physiological maximum already in the victory test.

A major difference between defeat and victory became evident when the pattern of adaptation to intermittent presentation of the same stressor was examined.

A habituation effect was observed across repeated exposure to social victory experience, clearly visible in the first minutes of the test and the last phases of the recovery period. Specifically, the decrease in average R–R interval and heart rate variability parameters during winning experience was dampened in the 10th as compared to the 1st victory exposure. Accordingly, the incidence of ventricular extrasystoles was also significantly reduced at the end of the intermittent victory program. Both experimental evidences suggest that the sympathetic prevalence taking place during victory tests was gradually reduced across the 10 homotypic stress repetitions. Interestingly, when the overall autonomic responses to the 1st and 10th victory were preliminarily compared (i.e. mean values of R–R interval and R–R interval variability were calculated for the full 15-min test and recovery period), no differences could be found. This evidence suggests that a detailed analysis of the time course of acute autonomic responsivity to a stressor is

advisable when different social (and also nonsocial) challenges are compared.

In contrast, repeated exposure to defeat did not produce any habituation of cardiac autonomic reactivity. Heart rate and heart rate variability responses were similar at the beginning and the end of the intermittent defeat protocol, and so did the occurrence of arrhythmic events.

One may argue that the habituation of heart rate response observed in animals undergoing repeated victory challenge is due to a gradual reduction in somatomotor activity across repetitions. Actually, the amount of global physical activity, measured as cumulated time spent in exploration, grooming and fighting behaviors, was not different between the 1st and 10th episodes (personal observations).

A possible explanation of the different autonomic responsivity observed in the two social challenging situations used in this study takes into account psychological factors such as controllability and predictability. A large number of experiments have been described in the literature, which show that biochemical, physiological and/or pathological changes in an organism do not seem to be caused strictly by the noxious nature of the stressor rather by the ability or inability of the individual to control or deal with it [9,31]. In animal stress research, the influence of this specific psychological factor has been studied predominantly as to behavioral and (patho)physiological reactivity by use of a classical research paradigm, which compares the consequences of controllable vs. uncontrollable yoked electric foot/tail shock [34]. In particular, it has been shown that control vs. lack of control over a stressor has a dramatic impact on subsequent changes in behavior and physiology.

In the victory paradigm used in the present paper, the experimental animal was the aggressor and was dominant in the fight: it frequently attacked and systematically submitted male conspecifics intruding its territory. On the contrary, each defeat experience took place in an unfamiliar territory, where the experimental animal was repeatedly attacked and subordinated by an aggressive resident male.

Although our measurements were limited to the short-term outcome of each stress exposure, it can be hypothesized that the larger shift to sympathetic dominance observed during social defeat and the lack of habituation across repeated exposures represent the physiological reaction to a less controllable stimulus. It is likely that an animal develops a certain degree of social control when repeatedly facing, in its own territory, an intruder that rapidly exhibits submissive behavior. On the contrary, poor control over the social stressor is reasonably the case when the experimental animal is repeatedly exposed to an extremely aggressive opponent in a totally unfamiliar territory.

Other factors, however, such as the intensity of the stressor [21] and the housing conditions of the experimental animals [22] could account for the different autonomic response/adaptation to intermittent defeat or victory challenge. As a matter of fact, neuroendocrine and cardiac autonomic data suggest that a social defeat episode has a

stronger impact than a victory experience. We cannot exclude, therefore, that this intensity difference between the two conditions is responsible for the different habituation profiles. Animals would habituate more easily to a lower intensity stressor such as victory experience, whereas they would not adapt to a higher intensity challenge such as social defeat.

In addition, recent experimental evidences underline the role of housing conditions in modulating the long-term autonomic consequences of a stressor. For instance, housing familiar male wild-type rats together sharply reduces the long-term adverse behavioral and physiological effects of a social defeat episode [22]. In the present experiment, rats experiencing victory were permanently housed with a female partner in order to induce and maintain their territorial aggression. Females were temporarily removed before each of the 10 victory episodes, but they were immediately returned at the end of the recording session. On the contrary, rats experiencing defeat were individually housed, starting from transmitter implantation and all across the experimental procedure. Therefore, it could also be hypothesized that the former group of animals benefited from the “social support” provided by the presence of a female, which might have favored habituation of autonomic stress responsivity to intermittent social challenge.

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