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Published in:
Physiology & Behavior

DOI:
10.1016/S0031-9384(99)00134-1

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Document Version
Publisher’s PDF, also known as Version of record

Publication date:
1999

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Different Sympathovagal Modulation of Heart Rate During Social and Nonsocial Stress Episodes in Wild-Type Rats

ANDREA SGOIFO,*† JAAP M. KOOLHAAS,* EZIO MUSSO† AND SIETSE F. DE BOER*

*Institute for Behavioral and Cognitive Neurosciences, Department of Animal Physiology, University of Groningen, Kerklaan 30, P.O. Box 14, 9750 AA Haren, The Netherlands; and †Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Parco Area delle Scienze 11/A, 43100 Parma, Italy

Received 1 April 1999; Accepted 21 June 1999

SGOIFO, A., J. M. KOOLHAAS, E. MUSSO AND S. F. DE BOER. Different sympathovagal modulation of heart rate during social and nonsocial stress episodes in wild-type rats. PHYSIOL BEHAV 67(5) 733–738, 1999.—The acute consequences of a social aversive stimulus (defeat) on the autonomic control upon the electrical activity of the heart were measured and compared to those observed in three nonsocial stress paradigms, namely restraint, shock-probe test, and swimming. Electrocardiograms were recorded from rats via radiotelemetry, and the autonomic neural control of the heart was evaluated via measures of heart rate and heart rate variability, such as the average R-R interval (RR), the standard deviation of RR (SD), the coefficient of variance (SD/RR), and the root-mean-square of successive R-R interval differences (r-MSSD). Although all stressors induced significant reductions of average R-R interval, the effect of defeat was significantly larger ($p < 0.05$). The social stimulus also determined a significant decrease in the variability indexes ($p < 0.01$ for all), whereas in the other stress conditions they were either unchanged or increased (SD/RR during restraint, $p < 0.05$; SD and SD/RR during swimming, $p < 0.05$ and $p < 0.01$). Cardiac arrhythmias (mostly ventricular premature beats, VPBs) were far more frequent during defeat than during the other challenging situations ($p < 0.01$), with an average of $33.5 ± 6.5$ VPBs per 15-min test recording. These data suggest that during defeat autonomic control was shifted toward a sympathetic dominance, whereas in rats exposed to nonsocial stressors, although significant heart rate accelerations were also found, sympathovagal balance was substantially maintained. These differences in autonomic stress responsivity explain the different susceptibility to ventricular arrhythmias and indicate that a social challenge can be far more detrimental for cardiac electrical stability than other nonsocial aversive stimuli. © 1999 Elsevier Science Inc.

Stress Autonomic nervous system ECG Arrhythmias Heart rate Variability

CLINICAL reports and animal studies have provided extensive information about the influence of stressful stimuli on cardiovascular function, with special emphasis on the relationship between psychological challenges and the electrical activity of the heart (4,7,13). The effects of mental stressors on cardiac electrogensis are mediated by the autonomic nervous system, with different stressors producing different shifts of autonomic balance, toward either a sympathetic or a parasympathetic prevalence (6,11,15,23). A dominance of sympathetic activity, as induced by a number of stressful stimuli, was shown to enhance the risk of cardiac tachyarrhythmias, especially when acting on an altered cardiac substrate. Conversely, a prevalence of parasympathetic tone has often been regarded as a protective mechanism against arrhythmia vulnerability (17,19,20,28,32).

Despite the wealth of information available about the impact of psychologically stressful stimuli on the autonomic control of the heart, two important issues have been so far poorly addressed. First, the face validity of the experimental stress model. What has been documented so far was mostly ob-

1Requests for reprints should be addressed to Andrea Sgoifo, Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Parco Area delle Scienze 11/A, 43100 Parma, Italy. E-mail: sgoifo@biol.unipr.it
tained in stress contexts, which bear little or no relationship with the animal everyday life challenges. Therefore, we were interested in testing the influence of more naturalistic stress situations like aversive social confrontations, which can be well mimicked in the rat in laboratory conditions, and which are known to induce severe neuroendocrine responses (2,10,21). Second, the need of a detailed documentation about stress influences on autonomic balance and cardiac electrical stability in healthy animals, with no underlying cardiovascular pathology, either spontaneous or experimentally induced. This issue is particularly important when one of the goals of a research project is to find out early markers of risk for severe cardiac arrhythmias.

In this article, ECG signals were obtained from healthy rats exposed to a social challenge (social defeat) and to other non-social stress stimuli (restraint, shock-probe test, swimming) by means of a chronically implanted telemetry system (3), which allows reliable recordings from freely behaving animals.

In the different challenging situations, values of so called “time-domain indexes of heart rate variability” were quantified, which represent a valid tool for indirect and noninvasive evaluation of autonomic control over cardiac electrical activity (23,26). In addition, the incidence of different types of cardiac arrhythmias was measured to verify which stress situation is more detrimental for cardiac electrical stability.

MATERIALS AND METHODS

All procedures in this study were approved by the Committee on Animal Bioethics of the University of Groningen, The Netherlands.

Animals and Housing

We used 40 wild-type male rats (Rattus norvegicus), originally derived from the Agricultural University of Wageningen (The Netherlands) and bred in our department under conventionally clean conditions. The wild-type animals used in these experiments belonged approximately to the 20th generation of laboratory breeding.

Animals were housed in unisexual groups of five individuals, from weaning until the onset of experiments (6 months of age), in clear Plexiglas cages measuring 60×30×30 cm. Additional males were used as resident dominants in the social stress test (“resident–intruder test”), where they were vigorously attacked (1,14); 2) restraint test (RES, n = 10), in which the experimental animal is introduced into a black polyester tube (i.d. 6 cm, length 20 cm), closed at both ends by removable partitions provided with holes (16,23); 3) shock-prod test (SHO, n = 10), in which the animal was presented an electrified probe in its own cage, according to the procedure described by de Boer and colleagues (5,12); 4) swimming test (SWI, n = 10), in which the animal was introduced into a black polyester circular pool (diameter 140 cm, height 35 cm, depth of the water 30 cm) where it was forced to swim in order to float.

After the test, each animal was returned to its own home cage for recovery. All experimental sessions were performed in the light phase between 1000 and 1300 h.

ECG Data Acquisition and Processing

The pulse-modulated signal at the output of the receiver was simultaneously routed to two IBM-compatible computers. One PC was provided with a software package developed in our lab (CARDIA) for real-time acquisition and analysis of R-R intervals. R-waves were converted into pulses using a threshold circuit. Pulse times were measured with <0.5 ms accuracy. R-R pulse intervals were expressed as heart rate (beats per min), displayed on line and stored for subsequent analysis. The second PC contained the LABPRO data acquisition system (Data Sciences, St. Paul, MN), which was used only for monitoring, storage, and visual inspection of ECG waves. The following ECG parameters were quantified: 1) the mean R-R interval duration (RR, ms); 2) the standard deviation of RR (SD, ms); 3) the ratio between SD and RR (SD/RR, “coefficient of variance”); 4) the “root mean square of successive RR differences” (r-MSSD, ms) (26). Although SD and SD/RR estimate the overall HR variability and, therefore, include the contribution of both branches of the autonomic nervous system to heart rate control, the r-MSSD specifically quantifies the influence on HR variability of the parasympathetic input to the heart (25–27). Generally speaking, increased sympathetic or decreased parasympathetic tone are reflected in decreased values of variability indices, while decreased sympathetic or increased vagal nervous system activity are reflected in increased values of HR variability parameters (26). Finally, the incidence of the most common arrhythmic events, such as ven-
Mean RR interval duration and RR variability measures were performed after removal of RR intervals surrounding arrhythmias.

Statistical Analysis

From the ECG recordings, R-R interval parameters (mean R-R, SD, SD/RR, and r-MSSD; see ECG data acquisition and processing) were quantified as means of 15-min periods for baseline, test, and recovery conditions. The occurrence of arrhythmias was expressed as the number of events per each 15-min recording period. Means of baseline, test, and recovery values for R-R interval parameters and arrhythmias in each stress situation were compared via two-way ANOVA, with “type of stressor” as between-subject factor (four levels) and “recording period” as within-subject factor (three levels). Delta (Δ) values for each animal and for all RR interval parameters were calculated as the difference between 15-min test and 15-min baseline values. Means of Δ values in the four stress conditions were compared via one-way ANOVA.

Further post hoc analyses on all electrocardiographic parameters were performed by means of Scheffe’s test. Values for these parameters were always expressed as mean ± standard error of the mean (SE). Statistical significance was set at $p < 0.05$.

RESULTS

R-R Interval and R-R Interval Variability

Table 1 reports the values of R-R interval and R-R interval variability indexes in the four stress conditions. The average R-R interval was significantly decreased during the test compared to baseline in all stress conditions (DEF: $p < 0.01$; RES: $p < 0.01$; SHO: $p < 0.05$; SWI: $p < 0.01$). However, Δ values (difference between test and baseline) for social defeat were significantly lower (i.e., more negative) than those for the

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RR INTERVAL AND RR INTERVAL VARIABILITY INDEXES DURING BASELINE, TEST, AND RECOVERY PERIODS IN RATS EXPOSED TO SOCIAL DEFEAT, RESTRAINT, SHOCK-PROBE TEST, AND SWIMMING</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stressor</th>
<th>n</th>
<th>Period</th>
<th>RR, ms</th>
<th>SD, ms</th>
<th>SD/RR</th>
<th>r-MSSD, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defeat</td>
<td>10</td>
<td>Baseline</td>
<td>168.9 ± 4.7</td>
<td>12.5 ± 1.3</td>
<td>0.074 ± 0.007</td>
<td>3.36 ± 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>114.9 ± 1.4*</td>
<td>3.4 ± 0.2*</td>
<td>0.029 ± 0.001*</td>
<td>1.77 ± 0.06*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recovery</td>
<td>132.4 ± 4.1*</td>
<td>9.3 ± 1.3</td>
<td>0.069 ± 0.007</td>
<td>1.99 ± 0.12*</td>
</tr>
<tr>
<td>Restraint</td>
<td>10</td>
<td>Baseline</td>
<td>168.5 ± 6.1</td>
<td>12.0 ± 1.4</td>
<td>0.071 ± 0.009</td>
<td>2.80 ± 0.57</td>
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<tr>
<td></td>
<td></td>
<td>Test</td>
<td>138.5 ± 4.5*</td>
<td>15.8 ± 1.4</td>
<td>0.114 ± 0.011</td>
<td>3.13 ± 0.41</td>
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<tr>
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<td></td>
<td>Recovery</td>
<td>155.5 ± 4.9</td>
<td>11.7 ± 1.5</td>
<td>0.075 ± 0.008</td>
<td>2.62 ± 0.33</td>
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<td>Shock probe</td>
<td>10</td>
<td>Baseline</td>
<td>176.2 ± 7.7</td>
<td>12.3 ± 1.6</td>
<td>0.070 ± 0.009</td>
<td>2.85 ± 0.22</td>
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<tr>
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<td></td>
<td>Test</td>
<td>150.5 ± 3.3</td>
<td>15.1 ± 1.7</td>
<td>0.100 ± 0.017</td>
<td>2.50 ± 0.16</td>
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<tr>
<td></td>
<td></td>
<td>Recovery</td>
<td>172.5 ± 2.1</td>
<td>14.1 ± 1.5</td>
<td>0.082 ± 0.009</td>
<td>2.70 ± 0.16</td>
</tr>
<tr>
<td>Swimming</td>
<td>10</td>
<td>Baseline</td>
<td>172.1 ± 2.7</td>
<td>11.4 ± 0.7</td>
<td>0.067 ± 0.005</td>
<td>2.68 ± 0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>148.2 ± 0.9*</td>
<td>14.4 ± 0.9*</td>
<td>0.097 ± 0.006*</td>
<td>2.28 ± 0.12</td>
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<tr>
<td></td>
<td></td>
<td>Recovery</td>
<td>156.6 ± 1.9*</td>
<td>7.3 ± 0.3*</td>
<td>0.047 ± 0.002*</td>
<td>2.30 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE. RR = average R-R interval; SD = standard deviation of RR; SD/RR = coefficient of variance; r-MSSD = root mean square of successive R-R interval differences. Two-way ANOVA revealed significant effects of: 1) period for RR ($F = 0.79, p < 0.001$); SD/RR ($F = 5.8, p < 0.01$); and r-MSSD ($F = 4.7, p < 0.02$); 2) type of stressor for RR ($F = 26.6, p < 0.001$), SD/RR ($F = 12.9, p < 0.001$), and r-MSSD ($F = 10.3, p < 0.01$); 3) period × stressor interaction for RR ($F = 4.8, p < 0.001$), SD ($F = 10.8, p < 0.001$), SD/RR ($F = 12.2, p < 0.001$), and r-MSSD ($F = 2.8, p < 0.02$).

*p < 0.01 and †p < 0.05, significant differences vs. baseline corresponding values, Scheffe’s test.
nonsocial stressors \((p < 0.05, \text{Fig. 1})\), indicating that the effect of social challenge on heart rate was stronger. Recovery values were still significantly reduced compared to baseline in DEF and SWIM animals \((\text{Table 1}, p < 0.01)\), whereas the effect of SHO and RES on heart rate was limited to the test period.

As reported in Table 1, R-R variability measured as standard deviation \((\text{SD})\) was significantly reduced in the test period in DEF animals \((p < 0.01)\), whereas it was significantly increased during swimming \((p < 0.02)\) and only slightly modified \((\text{increased, nonsignificant variations})\) in the other stress conditions. As for average R-R interval, \(\Delta\) values for SD (Fig. 1) were significantly lower in DEF compared to RES, SHO, and SWI rats \((p < 0.01)\). In the recovery phase, SD was back to baseline in all stress conditions, except for SWI, in which it was significantly reduced compared to baseline and test values \((\text{Table 1}, p < 0.01)\). The coefficient of variance \((\text{SD/RR})\) was affected by the stressors in a similar way as the SD. It was significantly reduced during the test in DEF rats \((\text{Table 1}, p < 0.01)\), while it was increased in the other stress contexts \((\text{SWIM}, p < 0.01; \text{RES}, p < 0.05; \text{SHO}, p = NS)\). \(\Delta\) values for SD/RR (Fig. 1) were significantly lower in DEF as to RES, SHO, and SWI \((p < 0.01)\). SD/RR was back to baseline after each challenge, except for SWI \((\text{recovery values significantly higher compared to baseline and test}, p < 0.01; \text{Table 1})\). The root-mean-square of successive RR differences \((\text{r-MSSD}, \text{index of vagal activity})\) was significantly reduced during the test only by defeat \((p < 0.01)\), this effect being still evident also in the recovery phase \((p < 0.01)\) (Table 1). The nonsocial stressors did not produce significant changes in r-MSSD, neither in the test nor in the recovery phase. Accordingly, \(\Delta\) values of r-MSSD for DEF rats were lower compared to those of RES, SHO, and SWI rats \((\text{respectively}, p < 0.05, p = 0.07, \text{and} p = 0.08; \text{Fig. 1})\). The values of SD, SD/RR, and r-MSSD measured in the four stress conditions suggest that the changes were not only larger in the social context but also of a different nature: sympathetic-vagal control was shifted toward a marked sympathetic dominance in rats exposed to defeat, whereas autonomic balance was substantially maintained in the nonsocial stress contexts.

Cardiac Arrhythmias

In all stress conditions, the most recurrent arrhythmias were ventricular premature beats (VPBs), either isolated or as salvos (two or three consecutive VPBs), and their incidence in baseline, test, and recovery periods is reported in Fig. 2. VPBs were significantly more frequent in rats exposed to DEF \((p < 0.01)\), with an average occurrence of about two ectopic beats per min of test recording. The incidence of VPBs was larger in SWI compared to SHO and RES rats \((p < 0.01)\), the latter two groups showing substantially no rhythm disturbances. Noteworthy, a much higher incidence of VPBs was found in SWI rats compared to the other groups in the recovery phase \((p < 0.01)\).

DISCUSSION

In this study on healthy wild-type rats, acute electrocardiographic responses to social defeat are described and compared with those observed in nonsocial aversive contexts, namely restraint, shock-probe test, and swimming. The social challenge produced a much larger increment of heart rate and a higher incidence of cardiac (ventricular) arrhythmias. Time-domain heart rate variability measurements \((\text{SD, SD/RR, and r-MSSD})\), a tool for indirect and noninvasive evaluation of autonomic control of cardiac electrical activity, confirmed that during defeat a strong sympathetic activation takes place, and that the sympathovagal control is markedly shifted in the direction of a sympathetic dominance \((23,25)\). On the contrary, in rats exposed to nonsocial stressors, although significant heart rate accelerations were also found, heart rate variability during the test was either increased or unchanged as compared to baseline, suggesting that autonomic balance was substantially maintained.

A valid objection to this conclusion could be that the different electrocardiographic responses observed between social and nonsocial stress conditions are simply the consequence of different levels of catecholaminergic activation. Recently, Koolhaas and colleagues provided a detailed review of the activation of the sympathetic–adrenomedullary system and of the pituitary–adrenocortical axis produced in rats by a number of stressful stimuli, including those used in this study \((10)\). Indeed, social defeat proved to be a particularly severe stressor with respect to plasma catecholamine and corticosterone elevations. However, swimming test induced similar increments of norepinephrine and epinephrine in venous blood. Despite this similarity in catecholaminergic responsivity, the two stressors turned out to be markedly differentiated in heart rate variability indices. All these parameters were reduced during defeat test compared to baseline, whereas they were either increased \((\text{SD and SD/RR})\) or unchanged \((\text{r-MSSD})\) during the swimming test. Noteworthy, episodes of II degree atrioventricular block, known to be a marker of heightened parasympathetic activity, were often observed in rats during swimming \((\text{on average}, 10.9 \pm 4.7 \text{events})\) but never during defeat test \((\text{personal observations})\), suggesting that a robust vagal antagonism to sympathetic activation was present only in swimming rats. In addition, with the exception of shock-probe test, the reduction in heart rate variability pa-
rameters (SD, SD/RR, and r-MSSD) we observed in the first 3 min of test recording was similar in the different stress conditions (defeat, swimming, restraint; unpublished data). Therefore, the overall larger heart rate response and the much lower values of heart rate variability during social defeat cannot be explained simply in terms of higher sympathethic input to the heart, but also in terms of a concomitant lack of vagal rebound to sympathetic activation.

A possible explanation of the different autonomic responsiveness observed in the 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 that show that biochemical, physiological, and/or pathological changes in an organism do not seem to be caused by the noxious nature of the stressor but by the ability or inability of the individual to control or deal with it (5,29). 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 that compares the consequences of controllable versus uncontrollable yoked electric foot/tail shock (31). In particular, it has been shown that control versus lack of control over a stressor has a dramatic impact on subsequent changes in behavior and physiology. Although our measurements were limited to the very acute outcome of stress exposure, it can be hypothesized that the much stronger shift of autonomic balance toward a sympathetic dominance observed during social defeat represents the physiological reaction to a far more uncontrollable stimulus than shock-probe test, restraint, or swimming.

The much higher occurrence of ventricular arrhythmias we found in defeated rats confirms the generally accepted view of an association between low values of heart rate variability (indicative of sympathetic dominance) and increased cardiac electrical instability (25,32). One may argue that the incidence of ventricular premature beats during defeat (on average, about 30 events per animal), although much higher than during the other nonsocial challenges, may be not pathologically relevant. It must be taken into account, however, that the experimental animals were young adult normal individuals in which there was virtually no occurrence of ventricular premature beats in baseline conditions.

An interesting finding is the relatively high occurrence of ventricular premature beats in the recovery phase following swimming. Actually, this period was also characterized by reduced values of heart rate variability (SD and SD/RR) compared to baseline. In addition, a sustained somatomotor activity due to intensive grooming behavior was systematically observed, associated with very high levels of circulating catecholamines (18), which might explain such an occurrence of rhythm disturbances.

In conclusion, the rat autonomic response to the social stressor defeat (at least as far as cardiac electrical activity is concerned) is more sympathetically dominated and more prone to enhance ventricular vulnerability than that produced by nonsocial stressors such as restraint, shock-probe test, and swimming. On the other hand, the association between higher HR variability and lower incidence of arrhythmias during these nonsocial challenges is in accordance with the generally accepted view of a protective role of the vagal input to the heart against tachyarrhythmias (32).

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