# Cluster Analysis of Extracardiac Control of the Heart Rate in Random-Bred Albino Rats at Rest

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Heart rate variability was examined in random-bred albino rats at rest. The rats were clusterized according to activity of autonomic contour of heart rate control. Combination of factor and cluster analyses enhanced informative value of spectrum parameters of the heart rate variability in nonlinear rats grouped by initial neurovegetative status. The contours of central and autonomic regulation describe the most general features of the control influences that realize modulating influences at the heart level via sympathetic and parasympathetic control pathways. Their activity is comprehensively assessed by the normalized power spectrum of variations in the heart rate.

**Key Words:** *heart rate variability; factor analysis; cluster analysis; autonomic control; random-bred rats*

Analysis of heart rate variability (HRV) is widely used in clinical and experimental work. The method of variation pulsometry is employed to examine the age-related peculiarities of vagosympathetic control of rat heart, as well as the effects of desympathization [4,5], stress [2], and other factors on the cardiac function. Power spectrum analysis of HRV revealed specificity of autonomic control in Wistar and August rats [6] and clarified the physiological nature of the wave structure of the heart rate [11,12]. However, most experimental papers use routine approach to analyze the group-average HRV indices, which neglects the initial state of the control systems in the individual animals. The need for individual approach in assessment of HRV data in experiments with animals is discussed [7-9]. The studies on rabbits demonstrated importance of preliminary selection of the animals by the parameters of variation pulsometry [8]. However, information value of HRV spectrum analysis for assessment of autonomic control was called into question [7].

We found no published data on individual peculiarities of HRV in the random-bred rats. Logically, we examined the initial HRV indices in a group of the random-bred albino rats in view to reveal heterogeneity of the group in the matter of activity of the autonomic and central control contours. In addition, we tried to assess the information value of the time- and frequency-domain HRV indices in the grouping of animals by the initial state of their extracardiac control pathways.

## MATERIALS AND METHODS

Experiments were carried out on 15-week random-bred albino rats of both sex ($n$=186) kept on unrestricted food and water diet under standard vivarium conditions. ECG was recorded in non-narcotized rats using miniature clamp electrodes connected to a Varycard firmware complex (Ramena). The rats were not restricted. Instead, they were placed in a compact box by 4-5 species, which prevented intensive motion but did not limit the choice of postures.

The off-time analysis of *R—R* intervals was performed with an ISKIM6 software. The accuracy

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in measurements of *R--R* intervals was 1 msec. The cardiac indices were calculated by 300 *R—R* intervals in any ECG record. The following indices were obtained: heart rate (HR), variation range (DX), mode amplitude of cardio intervals (AMo), root mean square of successive differences (RMSSD) of *R—R* intervals, standard deviation of normal-to-normal *R—R* intervals (SDNN) in the analyzed dynamic series, autonomic balance index (ABI) calculated as a ratio of AMo to ($\Delta X$). The strain index was calculated by modified Baevsky formula with due account for the width of histogram class (7.8 msec) used to determine AMo: $SI=(AMo/2\times\Delta X\times Mo)\times(50/7.8)\times1000$. Power spectrum analysis of HRV was carried out in the ranges of high frequencies (HF, 0.9-3.0 Hz), low frequencies (LF, 0.32-0.9 Hz) and very low frequencies (VLF, 0.10-0.32 Hz). In addition, we calculated the total spectrum power was also determined (TP, msec2), the normalized spectrum powers in selected ranges (HF%, LF%, and VLF%), and centralization index (CI).

Mathematical analysis of entire data file was performed with cluster and factor analyses with the help of a Statistica 6.0 software. Significance was assessed by Student's *t* test.

**TABLE 1**. Parameters of Heart Rate in Rats with Low and High Activity of Autonomic Control Contour (*M*±*SD*)

| Index | Cluster 1 (group 1, *n*=78) | Cluster 2 (group 2, *n*=108) |
|---|---|---|
| ΔX, msec | 44.70±14.63 | 23.10±6.98** |
| SDNN | 8.60±2.54 | 4.500±1.357** |
| TP, msec$^2$ | 60.590±37.996 | 15.440±7.538** |
| HR, min$^{-1}$ | 332.70±32.39 | 340.00±23.71 |
| RMSSD | 6.170±2.798 | 3.290±1.429** |
| AMo, % | 37.520±8.831 | 58.240±13.404** |
| SI, rel. units | 17.050±8.831 | 53.430±31.032** |
| ABI | 0.950±0.412 | 2.930±1.704** |
| TP, msec$^2$ | 60.590±37.996 | 15.440±7.538** |
| HF, abs, msec$^2$ | 10.110±10.031 | 2.830±3.517** |
| LF, abs, msec$^2$ | 11.760±12.085 | 2.450±1.966** |
| VLF, abs, msec$^2$ | 13.460±20.275 | 3.440±2.643** |
| CI | 4.510±4.761 | 3.030±1.129* |
| HF% | 29.190±19.148 | 32.490±17.905 |
| LF% | 31.500±13.873 | 27.500±10.281 |
| VLF% | 39.300±17.609 | 40.000±16.932 |

**Note.** *$p<0.01$, **$p<0.001$ compared to cluster 1.

## RESULTS

The primary analysis of HRV indices in rats performed with the methods of descriptive statistics revealed a broad scatter of data indicating various degrees of activity in the control pathways to the heart at rest. The use of cluster and factorial analysis met the requirements to reduce a great number of variables (heart rate indices) to minimal set of the leading factors responsible for variety of selected data and carrying information on the activity of subdivisions and the structural levels of autonomic nervous system in respect to the heart rate control.

For cluster analysis of data file, we selected the indices TP, SDNN, and ΔX (Fig. 1). According to physiological interpretation, they reflect the general level of the control influences and result from activity of sympathetic and parasympathetic control pathways to the heart [1]. These indices are strongly correlated.

Standardization with k-mean clustering divided the initial data into 2 large clusters comprising 78 and 108 rats. The first cluster comprised the rats with enhanced HRV, while the second cluster was composed of rats with low HRV (Table 1). A high HRV at the state of relative rest results from enhanced activity in the autonomic regulation contour [1], while its significant moderation attests to weakening of autonomic control and subordination of the cardiac pacemaker to central influences [1,2]. Consequently, the first cluster was defined as the group of animals with a high activity in autonomic regulation contour (group 1), while the second cluster was referred to as the animal group with a low activity in this control pathway.

These two groups significantly differed in majority of other indices of variation pulsometry and spectrum analysis with the exception of normalized power spectra and HR (Table 1). The latter showed that the defined clusters partially overlapped by the index of HR. In other words, resting HR in group 1 rats is formed under enhanced activity of parasympathetic control pathway, low values of AMo, strain index, and ABI accompanied by enhanced powers of all HRV spectrum components. By contrast, in group 2 rats resting HR is characterized with a high level of sympathetic influences and low HRV power spectrum components, which reflects a pronounced centralization in the control of the heart rate [1,2,10].

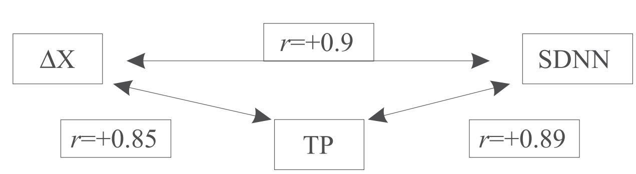


**Fig. 1**. Indices describing the control contours of the heart.

**TABLE 2**. Factorial Analysis of HRV with High and Low Activity of Autonomic Control Contour

| Index | Croup 1 | | Group 2 | |
|---|---|---|---|---|
| | factor 1 | factor 2 | factor 1 | factor 2 |
| HF% | -0.880549 | -0.398645 | -0.939952 | -0.292164 |
| LF% | 0.052620 | 0.997631 | 0.065580 | 0.996644 |
| VLF% | 0.914686 | -0.321934 | 0.941902 | -0298205 |
| CI | 0.829598 | 0.124438 | 0.926931 | 0.159892 |
| Expl. Var | 2.303021 | 1.273311 | 2.634190 | 1.193150 |
| Prp. Totl | 0.575755 | 0.318328 | 0.658547 | 0.298288 |

Modern views on physiological nature of spectrum components in variable heart rate [1,3,12] explains the absence of the differences between both groups in the values of normalized power spectrum by the fact that both adrenergic and cholinergic mechanisms of the heart rate control are involved in the work of autonomic and central regulatory pathways. To delineate their role in separation of the rats into two groups by HRV indices, the rats with a high and low activity of autonomic regulation contour were analyzed by the factorial methods.

Only 4 indices were selected as initial variables: HF%, LF%, VLF%, and CI. Analysis of major components with orthogonal rotation by Varimax normalized method revealed 2 factors, which in total were responsible for 89% and 95% variations in the data (Table 2).

The factorial loads of factor 1 determine the values of VLF% and CI, which according to the reported data [1,3] result from activity of suprasegmental ergotropic structures. A strong negative relation was revealed between factor 1 and the index of relative activity of parasympathetic control pathway to the heart, HF% [1,3,12]. These data interpreted factor 1 as a value reflecting the sympathetic influences. Factor 2 strongly correlated only with LF%, which is an indicator of chronotropic component of baroreflex [1]. Since realization of this reflex depends mainly on the increment in vagal activity, we conventionally considered factor 2 as reflecting the parasympathetic influences. The matrices of factorial analysis indicated identity of the internal structure of the relations between the nervous pathways controlling the heart rate in both groups. Thus, the contours of central and autonomic regulation reflect the most general features of the controlling influences, which realize their effects to the heart via adrenergic and cholinergic pathways.

The cluster analysis of rat groups with different activity in autonomic regulation contour by individual factorial values calculated for the factors of sympathetic and parasympathetic influences, revealed 3 clusters in each group (Table 3). Within terminology routinely used to characterize the tone of autonomic nervous system [3], the subgroup with dominating respiratory waves in HRV power spectrum can be referred to as “vagotonic”, because HF variations in this subgroup results from predominantly parasympathetic influences on the heart rate [1]. In contrast, the subgroup with do-

**TABLE 3**. Parameters of Heart Rate Variability in Rats with Different Types of Autonomic Control: Cluster Analysis Data Yielded by Individual Factor Values Calculated by Individual Factors of Sympathetic and Parasympathetic Influences (*M*±*SD*)

| Index | Group 1 | | | Group 2 | | |
|---|---|---|---|---|---|---|
| | RWD (*n*=8) | SWD (*n*=29) | VSWD (*n*=41) | RWD (*n*=24) | SWD (*n*=37) | VSWD (*n*=47) |
| HF% | 71.71±8.63 | 37.35±8.66** | 15.13±5.27**+++ | 60.65±12.83 | 27.25±8.47** | 22.35±8.02**+ |
| LF% | 16.25±5.39 | 28.18±8.72** | 38.82±15.12**++ | 19.63±5.31 | 38.47±8.02** | 22.65±4.93*+++ |
| VLF% | 12.03±7.14 | 34.46±9.59** | 48.05±16.72**+++ | 19.72±10.14 | 34.27±10.09** | 54.98±7.90**+++ |
| CI | 0.41±0.18 | 1.91±0.86** | 7.15±5.26**+++ | 0.700±0.139 | 3.14±1.74** | 4.13±1.99**+ |

**Note.** RWD — respiratory wave domination in HRV spectrum; SWD — slow wave domination in HRV spectrum; VSWD — very slow wave domination in HRV spectrum; *$p$<0.05, **$p$<0.001 compared to RWD; +$p$<0.05, ++$p$<0.01, +++$p$<0.001, compared to RWD in primarily selected clusters 1 and 2.

minating of very slow waves should be termed as "sympaticotonic" based on the data about the relation between VLF and the changes in sympathetic activity [1]. Finally, the subgroup with dominating slow waves should be baptized as "normotonic", because the rats of this subgroup demonstrated roughly equal contribution of HF and VLF to variability of the heart rate, the percentage of LF in total spectrum power being appreciably high (Table 3).

Thus, among the examined random-bred rats, 41.9% animals were characterized with a high activity in autonomic regulation contour, while low activity of this pathway was revealed in 58.1% rats, which where additionally characterized with enhanced activity in the central regulation contour affecting the heart rate at rest. Analysis of contribution of spectrum components into variability of the heart rate showed that pronounced domination of VLF (sympathicotonia) characterized 47% rats, while 35% rats demonstrated a high contribution of LF into HRV (normotonia). A high contribution of HF into HRV (vagotonia) was observed only in 17% rats.

The present data corroborate the views on heterogeneity of random-bred albino rats in the matter of autonomic control of the heart rate. This heterogeneity is manifested both in different activity of regulation contours (autonomic and central) and in various degree of involvement of cholinergic and adrenergic pathways into the control of the heart rate. These findings should be taken into consideration in planning some experimental studies (for example, in the study of stress reactivity, development of adaptation to physical loads, *etc*.).

Our study revealed significant informativity of HRV spectrum indices, which explains quite comprehensively the diversity of a random sampled animals in respect to autonomic control of the heart rate. These indices provide the tool to assess neurovegetative status with due account of a high activity of central and autonomic regulation contours. The results of cluster analysis of HRV indices and the reported data [3,7,8,10] confirm that the spectrum methods of HRV analysis can assess the initial neurovegetative status not only in humans, but also in animals of various species. In its turn, this indicates a common outline in the structure of multilevel system of autonomic control of hemodynamics in mammals.

## REFERENCES

1. R. M. Baevskii, G. G. Ivanov, L. V. Chireikin, *et al., Vestn. Aritmol*., **24**, 1-23 (2001).
2. R. M. Baevskii, O. I. Kirillov and S. Z. Kletskin, *Mathematical Analysis if Heart Rate Variability during Stress* [in Russian], Ìoscow (1984).
3. *Autonomous Disorders: Clinics, Diagnostics, and Treatment*, Ed. A. M. Vein [in Russian], Ìoscow, (2003).
4. T. L. Zefirov and N. V. Svyatova, *Byull. Eksp. Biol. Med.*, **123**, No. 6, 703-706 (1997).
5. N. I. Ziyatdinova, A. L. Zefirov, and T. L. Zefirov, *Ibid.*, **132**, No. 6, 616-618 (2002).
6. T. N. Kirillina, M. A. Usacheva, and L. M. Belkina, *Ibid.*, **142**, No. 10, 376-381 (2006).
7. K. Sh. Nadareishvili, I. I. Meskhishvili, D. D. Kakhiani, *et al.*, *Ibid.*, **134**, No. 12, 657- 659 (2002).
8. K. Sh. Nadareishvili, I. I. Meskhishvili, D. D. Kakhiani, *et al.*, *Ibid.*, **138**, No. 9, 306-310 (2004).
9. K. V. Sudakov, *Zh. Nevropatol. Psikhiatr.,* No. 2, 4-12 (2005).
10. N. I. Shlyk, *Heart Rate and Central Hemodynamics during Physical Activity in Children* [in Russian], Izhevsk (1991).
11. H. Inagaki, M. Kuwahara, and I. Tsubone, *Exp. Anim.,* **54**, No. 1, 61-69 (2005).
12. C. A. Murphy, B. P. Sloan, and M. M. Myers, *J. Auton. Nerv. Syst.*, **36**, No. 3, 237-250 (1991).