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Generational Variability in The Patterns of Motor Activity Circadian Rhythm in The Rats

T. Cambras* and A. Díez-Noguera

Departamento de Ciencias Fisiológicas Humanas y de la Nutrición
Laboratorio de Fisiología Animal
Facultad de Farmacia
Universidad de Barcelona
08028 Barcelona

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Motor activity circadian rhythm of 32 rats belonging to three consecutive generations of rats from a laboratory strain, has been studied. This rhythm has been registered from the day of weaning (at 21 days) to at least 30 days after. The pattern of this rhythm has been analyzed by means of a Fourier analysis, by which the daily power spectrum of each rat was obtained. Based on the mean power spectrum of each animal, the variance among the different families and generations has been calculated. Results show that the variance increases in a statistically significant way with the succeeding generations. It can be suggested that in the third generation, the characteristics of the progenitors are dispersed, confirming the genetic character of the rhythm and suggesting a multigenic character for the transmission of the circadian rhythm of motor activity.

Key words: Circadian rhythm, Inheritance, Motor activity, Rat.

Circadian rhythms in living systems have an inheritable nature. Although circadian clocks can be influenced by environmental conditions, the contribution of environmental influences have been recognized to be minor in comparison to that of the endogenous circadian system (7). Several experiments have been carried out to test the genetic nature of the circadian rhythms. Thus, comparative studies have been performed amongst different kinds of animals, i.e. insects (4,5) and rodents (1-3) proving that rhythm characteristics are defined by genes. Different mutants of a fruit fly *(Drosophila Melanogaster)* have been found to show different periods, each one characteristic of one mutation (4). In rodents, inbred strain comparisons have been performed to analyze food and water consumption (8), and catecholamines (6). There have also been found differences among the rhythms of albino rats compared with pigmented animals, possibly due to genetic factors (9). The pattern of the motor activity rhythm has been

* To whom all correspondence should be addressed.

proven to have an inheritable nature since different strains of rats show different patterns of this rhythm (2, 11). Non-inbred strains of rats, quite often born and mated in the same laboratory, are the most common animals used in the experimentation. Thus, it would be interesting to know how the rhythm patterns change, if they do, across the different generations of rats.

The purpose of this work is to study the variability among the patterns of motor activity rhythms of animals belonging to three consecutive generations within the same strain of rats, in order to examine the constancy of the pattern of the motor activity rhythm, and its possible hereditary nature.

Materials and Methods

Thirty-two rats belonging to three different generations of the same strain (Sprague-Dawley) were used. The first generation was comprised of 5 animals from the same family (family 1). The animals of the second generation (family 2 and family 3 with 5 rats in each) were reproduced by the mating of one female with two different males of family 1 consecutively. The animals of the third generation (family 4 with 8 rats, and families 5 and 6 with 5 rats) were reproduced by mating different animals of family 3.

All the animals were born and reared under constant conditions (constant light, LL, at 300 lux) in order to determine the endogenous rhythm of each animal. When rats were 21 days old they were isolated in individual cages and motor activity was recorded by means of an optical detecting system which used crossed-infrared beams. Motor activity data was recorded every 30 minutes for a period up to 30 days after the day of weaning (34, 45, 78, 55, 40 and 45 days respectively for families 1 to 6). All the animals of the same family were registered at the same time. The endogenous period of the motor activity rhythm, thau, was calculated by the periodogram of SOKOLOVE and BUSHELL (10). Taking the thau of each animal as the period of the fundamental harmonic, a Fourier analysis was applied to motor activity data, and a power spectrum with 20 harmonics was calculated for each day and for each rat. Then, the mean power spectrum of each animal, characteristic of the pattern of its own rhythm, was calculated by averaging the power spectra of the days between day 24 (when the circadian rhythms were considered to be well defined) and the last day of registration. The mean power spectra thus calculated reflects the pattern of the motor activity circadian rhythm when the animal is adult.

As each power spectrum has been calculated with 20 harmonics, the mean one for each rat can be considered as a point in a 20-dimensional space. Here, the variation and the variance of each group of points were calculated. Dispersion was determined by considering the distance between points of each group and its center.

Results and Discussion

In Figure 1 the dispersion ellipses of the three generations are shown. The variances obtained for the points grouped according to the three generations were: 31.43 (G1, $n = 5$), 31.33 (G2, $n = 10$), 79.6 (G3, $n = 17$). Using an analysis of variance, the differences among the second and the third generation calculated in this way, were no significant ($p = 0.068$).

Then, variances for the elements grouped according to the 6 families were calculated: 31.43 (F1, $n = 5$), 19.01 (F2, $n = 5$), 17.06 (F3, $n = 5$), 92.34 (F4, $n = 8$), 83.94 (F5, $n = 4$), 66.13 (F6, $n = 5$). Variation among the animals of each generation was calculated taking into account the intra-familiar variances. Variances for the second and third generations were as

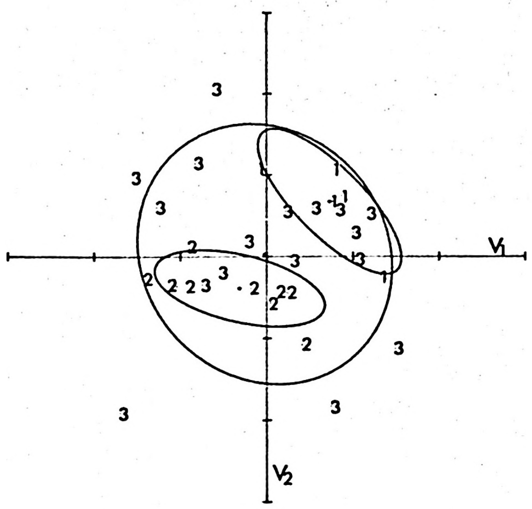

Fig. 1. *Two dimensional projection of all individual points (spectra) in the plane of the two first reduced dimensions.*
The number of each point indicates the generation to which each animal belongs. The ellipses are the two dimensional standard deviation interval for each generation.

follows: 16.03 (G2, n = 10), 69 (G3, n = 17). An analysis of variance showed a statistically significant difference (F = 4.30, d.f. = 14/8, $p < 0.05$) between the second and third generation. The intrafamiliar variance for the first and the second generations was calculated to be 19.29. This result was compared with the variance of the third generation, using the Snededor F test. Here, there was a statistically significant difference (F = 3.57, d.f. = 14/12, $p < 0.05$). The graphic representation of the dispersion is shown in Figure 1. These results show that variance increases among the animals of the third generation compared with the elements of the first and second generations. In the second generation, there was a concentration of the characteristics of the animals belonging to the first generation: variance was smaller. The increase of variance in the families of the third generation can be due to a dispersion of the characteristics of the rhythm of the progenitors. As variance increases, the motor activity pattern may be considered to be due to more than one gene (a multigenic character) dispersed and mixed in the successive generations. When experiments with animals of the same non-inbred strain are performed, the changes of the different parameters due to the different families or generations should be taken into account to avoid a false interpretation of data.

Resumen

Se estudia el ritmo circadiano de actividad motora de 32 ratas pertenecientes a tres generaciones consecutivas. El patrón del ritmo circadiano se analiza a través de un analisis de Fourier, obteniéndose el espectro de potencia diario para cada animal en base al cual se calcula la varianza entre los espectros de los animales de las distintas muestras y generaciones. Los resultados muestran cómo la varianza aumenta de manera estadísticamente significativa a través de las generaciones. En los elementos de la tercera generación, las características del ritmo de los progenitores están dispersas, confirmando el carácter genético del ritmo de actividad y sugiriendo un carácter multigénico para la transmisión del patrón circadiano de actividad.

Palabras clave: Ritmo circadiano, Herencia, Actividad motora, Rata.

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