How to engage medical students in chronobiology: an example on autorhythmometry

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Rol de Lama, M. A., J. P. Lozano, V. Ortiz, F. J. Sánchez-Vázquez, and J. A. Madrid. How to engage medical students in chronobiology: an example on autorhythmometry. Adv Physiol Educ 29: S160–S164, 2005; doi:10.1152/advan.00005.2004.—This contribution describes a new laboratory experience that improves medical students’ learning of chronobiology by introducing them to basic chronobiology concepts as well as to methods and statistical analysis tools specific for circadian rhythms. We designed an autorhythmometry laboratory session where students simultaneously played the role of researchers and experimental subjects. During this session, which lasted 24 h, students recorded their own arterial pressure, heart rate, oral temperature, forced expiratory flow, glucose tolerance, muscular strength, reaction time, and sensorimotor coordination at regular intervals and also took the Horne and Östberg test, after which they analyzed their own data. Furthermore, to gather information from subjects under normal sleep and eating schedules, some students acquired data at home. To guide and help students with their work, a dedicated web page was implemented with scientific references, cosinor analysis software, and other valuable information. All these “raw” data were combined into a single database that students could use to evaluate whatever aspect of the data they seemed fit. A number of suggestions were offered to them as guidance. Students were then instructed to write a scientific article on the subject they had chosen. The experience was highly rewarding for both instructors and students alike. In view of the high level of absenteeism in Spanish universities and the fact that 93% of the students attended the exam and 95% of these passed, the experience was considered a great success.

Methods

In summary, besides introducing our students to the scientific method in a way that fosters their initiative, the main objectives of these sessions were as follows: 1) using the Internet to gather information, clear up doubts about procedures and the use of equipment, download software for time-series analysis, and send processed data to the instructors; 2) applying statistical analysis, either classic or specific for chronobiology research, to the data they had obtained; 3) learning to use spreadsheet and graphing software; 4) getting used to search reference databases; 5) writing a scientific paper; and 6) applying chronobiological knowledge.

MATERIAL AND METHODS

Course Description

At the University of Murcia Medical School, Chronobiology is a one-term course that is taught in the second year. A total of 68
volunteer students (39 women and 29 men) from 20 to 25 yr old took part in the exercise. All of them had access to the Internet, either at home or at the university. Furthermore, five women and seven men from this group also agreed to take an oral glucose overload test to evaluate morning-evening differences. All participants had to sign an informed consent before the exercise, and the study was approved by the Ethics Committee of the University in accordance with the principles of the Declaration of Helsinki.

**Equipment**

After the corresponding training period, students were provided with the following materials: 1) digital thermometers; 2) spirometers (Spirotest, Riester) for lung capacity measurements over a range of 1,000–7,000 cm³, with their corresponding single-use mouthpieces; 3) spirometers (Datospir Peak-10, Sibelmed) for peak expiratory flow measurements, with their corresponding single-use mouthpieces; 4) automatic wrist blood pressure and pulse monitors [Omron RX-1, Omron Healthcare Europe; heart rate monitors (Polar S610i, Polar Electro) were also used, the main advantage being the ability to acquire and store data over long periods for subsequent analysis with a personal computer]; 4) dynamometers (Grip-A, Takei Scientific Instruments) with a measuring range of 0–100 kg to evaluate muscular strength on both hands; 5) 500 ml (1 kcal/ml) of diet for enteral nutrition (Nutrison, Nutricia; experimental subjects received 2,000 kcal distributed into sixteen 125-ml doses); and 6) blood glucose monitors (Acutrend sensor, Roche Diagnostics) with a lancing device and test strips (Accu-Check, Roche Diagnostics).

Students also needed access to a computer connected to the World Wide Web to use the reaction time and sensorimotor coordination measuring programs.

**Other Resources**

A specific section of the chronobiology group web page (http://www.um.es/cronobio), developed by J. P. Lozano, was implemented to provide students information regarding manuscript preparation, references, laboratory sessions, software, and on-line lab sessions (freely available at http://www.um.es/cronobio/cronolab). Besides, the University of Murcia has developed a Virtual Campus, named Servicios Universidad de Murcia Abierta (SUMA; in English, Services of the Open University of Murcia), available at https://suma.um.es/suma, with restricted access for students and staff of the university. All course information was also posted in SUMA, so students could decide which site to visit depending on their Internet connection speed and the number of people connected at any given time.

**Protocol**

Before the experimental work proper, students were given an introductory talk with a view to motivate them, an explanation of the objectives and the data collection procedure, and a training session on the use of the equipment and the software required to analyze temporal series as well as a talk on how to write a scientific paper. Physiological parameter determinations were complemented with the Horne and Östberg questionnaire (3), a morningness-eveningness test to determine the relationship between each student activity rhythm and the natural light-dark cycle to classify them in terms of their activity habits (i.e., to make homogeneous groups according to their biological rhythm phase).

**Experiments and data collection. AUTORHYTHMOMETRY.** Students were divided into groups of three to five subjects, one of which was appointed as team coordinator and the person responsible for the material provided. All coordinators were summoned to the Physiology Laboratory to perform the first set of determinations under the supervision of an instructor and where they were subjected to a predefined protocol for measuring oral temperature, systolic and diastolic pressure, heart rate, peak expiratory flow, lung vital capacity, hand grip strength, reaction time, and sensorimotor coordination every 2 h during 24 h. Reaction time was measured as the time required to react to a visual stimulus (a color change like that of a traffic light). Sensorimotor coordination was evaluated using a test in which subjects were required to mouse click a target as fast as they could. During the test, a series of points would appear on the computer screen, each one becoming visible after the previous one had been successfully clicked. The number of hits within 30 s was used as the score for a single test trial, and a mean of five trials was used for calculations. These two latter tests are available at http://www.um.es/cronobio/cronolab. Left and right hand grip strength was determined as the mean of two trials on each hand. Coordinators were sleep deprived and ate isocaloric liquid food to avoid the masking effects of sleep and meal time. Between determinations, all data were entered into a computer. Afterward, coordinators received a briefcase with all the necessary material, and they became in turn the instructors of their respective groups, which had to perform the same determinations at home except that they ate and slept normally. Once they had collected the data, the students had to apply a cosinor analysis (7) (a mathematical-statistical method of describing a rhythm by determining by least-squares technique the cosine curve best fitting to the data) to calculate the midline estimating statistic of rhythm (mesor; the value midway between the highest and the lowest values of the cosine function best fitting to the data), amplitude (the difference between the maximum and the rhythm-adjusted mean (mesor) of the best fitting curve), acrophase (the crest time of the cosine curve best fitting to the data), and significance level of the rhythm. All these data were sent to the instructors, who reviewed them and prepared an Excel data sheet freely accessible at the web page.

**SLEEP-WAKE CYCLE.** During 2 wk and to compute their total time of sleep, students recorded the time they went to sleep and woke up every day as well as any daytime naps they may have taken. Data were processed as previously described.

**EFFECT OF ADMINISTRATION TIME ON GLUCOSE TOLERANCE TEST.** Twelve healthy students volunteered for this study. Half of them took the test in the morning (8:00) and the other half in the evening (20:00). Before the test, both groups fasted for 10 h. Glucose plasma levels were measured just before and 30, 60, 90, and 120 min after glucose intake. After 2 days, the times at which both groups performed the test were exchanged. Data were sent to the instructors to be reviewed and prepared as described in AUTORHYTHMOMETRY.

The database was checked to correct any potential typing errors and to ensure that the units used to express the different parameters were homogenous. To test some of the hypotheses that were proposed (see below), the experimental subjects had to be arranged into subgroups according to their chronotypes as determined by the Horne and Östberg questionnaire. However, because extreme chronotypes were rarely represented, the five categories provided by the test were regrouped into just three (morningness, eveningness, and neither type), with extreme morningness and extreme eveningness being included with the morningness and eveningness chronotypes, respectively. According to this, 11.1% and 31.4% of the students displayed a morningness and eveningness chronotype, respectively, whereas 57.4% fell into the “neither type” category. This redistribution of experimental subjects according to their chronotypes also had the effect of decreasing the large standard deviation of the data due to the different life styles, thus providing relatively homogeneous study populations on which to test a variety of hypotheses.

**Data processing and reporting of results.** Once all data were collected and reviewed, students were asked to use the database to explore and test any hypothesis they wanted and then present their results as a formal scientific article. During this process, it was essential to maintain fluid communication through the Internet.

As guidance, we proposed that they address any of the following issues:
Evaluate the sleep-wake habits of university students. How much time do they sleep, when do they go to sleep, whether or not they change their routine during the weekend, and, if so, whether this would be similar to a shiftwork in terms of stress.

- Does the time of the day influence the tolerance to an oral glucose overload?

- Is there any relation between the Horne and Ostberg questionnaire score and the acrophase of the different physiological parameters evaluated?

- Does the oral temperature rhythm determine the acrophase of other parameters (marker rhythm)? Make a correlation analysis between the temperature acrophase and the rest of the acrophases. Can you write an equation to predict the acrophase of these parameters from that of the temperature?

- Feeding and sleeping masking effect. Compare the circadian rhythms parameters of subjects exposed to a constant routine (no sleep, liquid food every 2 h) with those who performed the experiment at home (normal sleep and eating time table).

Students, however, were encouraged to investigate any other hypothesis they wanted provided it could be tested using the available data.

Briefly, as any standard scientific article, their written report had to include: 1) a title page including the names of the authors (up to 5) and working address (Department of Physiology, University of Murcia); 2) a summary of up to 250 words with 5–6 keywords; 3) an introduction providing the essential background information required to understand the interest of the study as well as the objectives they pursued; 4) the materials and methods used, including how they analyzed the data; 5) a results section with figures and tables as required, 6) a discussion of their results in the context of previous studies; 7) a reference list; and 8) their acknowledgements, if the authors wished to acknowledge the help of any person or institution with some aspect of their work. To help them organized the article, students were given a detailed description, similar to that provided by Krilowicz et al. (4), of what issues should be included on each section. All these instructions were given to the students in a written document during the first practical session.

Fig. 1. Reaction time (in s) and sensorimotor coordination (expressed as the number of hits within 30 s) versus the time of the day. The graph pools together data from all students that attended the 24-h determination session at the Physiology Laboratory. Data are expressed as means ± SEM.

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Fig. 2. Cosinor analysis of sensorimotor coordination for the morningness chronotype. Top: x-y plot showing the fitted rhythm (dotted line) superimposed on the raw data (solid line) and the midline estimating statistic of rhythm (mesor; horizontal line). Bottom: polar representation of the rhythm in the form of a 24-h clock where the hand points to the acrophase, the hand length is proportional to the rhythm amplitude, and the circle represents the combined error for amplitude and acrophase. This was taken from one of the papers presented by the students.

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Evaluation

For evaluation purposes, we used a number of criteria such as hypothesis originality, pertinence of the data subset selected for the hypothesis, data presentation, use of data analysis tools specific for chronobiological research, writing style, relevance of the citations, etc. This exercise accounted for 80% of the grade assigned to the experimental part of the course, with the remaining 20% depending on the student attendance to practical sessions; this was in turn combined with the grade they obtained on the theory exam to determine their final grade on the course.

RESULTS AND DISCUSSION

Students presented a total of 21 scientific papers addressing a variety of issues. These included the effect of the time of the day on the glycemic response \( (n/H_11005) \), the relationship between chronotypes and the different physiological parameters evaluated \( (n/H_11005) \), how the temperature acrophase affects that of other rhythms \( (n/H_11005) \), and the masking effect of feeding \( (n/H_11005) \). Furthermore, 10% of these papers included a combination of two or more objectives.

Thus the autorhythmometry data acquired and analyzed by the students confirmed that almost all physiological parameters selected followed a circadian rhythm, with oral temperature, hand grip strength, reaction time, and sensorimotor coordination displaying the most robust and highest amplitude rhythms. As an example, Fig. 1 shows a conventional representation of the reaction time and number of hits at the sensorimotor coordination test at different times of the day, displaying a clear reaction time increase around 4–6 AM, not surprisingly accompanied by a concurrent decrease in the number of sensorimotor coordination hits. When chronotypes are considered and a specific time-series analysis applied, as shown in Fig. 2 for the morningness group, a much more clear and significant rhythm emerged \( (P < 0.001, \text{acrophase at } 4.09 \text{ hours}) \).

Also, some students confirmed that the morningness–eveningness nature of an individual influences the acrophase of some parameters, with the morningness chronotype displaying a 1- to 2-h phase advance compared with the eveningness one, as exemplified in Fig. 3 for oral temperature. Still others observed that glucose tolerance decreases in the afternoon, as shown in Fig. 4, and they came to understand how some patients could be identified as “false” diabetics if they were tested in the afternoon. Thus students not only represented and analyzed temporal series and became acquainted with chronobiology terminology but they were also able to provide an adequate interpretation and discussion of their results.

Furthermore, all students used the Internet, and 76% also used SUMA, both during the experiments and while writing their papers. In fact, visits to our web page during the course amounted to \( /H_11022 \). In general, they applied their previous knowledge of statistics (ANOVA, Student’s \( t \)-test) as well as...
recently learned statistical tools to analyze temporal series (cosinor analysis, phase maps).

We found this experience to have several further advantages, one of them being that the student’s data could be used as class material for Chronobiology lectures, specially on subjects such as hormonal rhythms, mental and physical performance rhythms, and shiftwork, thus establishing a very clear relationship between the theory and practical content of the course. Another advantage worth mentioning is that measuring their own physiological parameters greatly increases the students interest and motivation for what is generally considered as a secondary subject for medical sciences. In fact, many students were amazed to find out that those variables usually defined as “constant” showed predictable rhythms. This means that in their future professional practice, they will never forget that time may be a very important parameter to consider when making a diagnosis or treating a medical condition.

In view of the high level of absenteeism at Spanish universities and the facts that 93% of the students attended the theory exam, that 95% of them passed, and that 4.5% obtained an A+ grade, we consider the experience to be a great success with very positive effects on the students’ motivation and performance in the course. As an example, this compares quite favorably with Human Physiology, another subject taught by our department at the medical school, where only 58.5% of the students attended the exam, 60.4% of them passed, and only 1.9% obtained the highest grade.

In conclusion, this experience was highly enjoyable and rewarding for both instructors and students. Not only did our students experience by themselves circadian physiology and were able to apply chronobiology concepts, but they also became acquainted with the methods and statistical tools used to analyze circadian rhythms.

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REFERENCES