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DISTINGUISHING THE STRENGTH OF HYPOXIC STIMULUS IN INTERMITTENT HYPOXIA

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The intermittent hypoxia (IH) phenomenon is a subject of intensive examinations. In this study we examined whether it could be possible to distinguish the strength of the hypoxic stimulus given in IH cycles on the basis of observed changes in the breathing pattern. We investigated the ventilatory responses to five hypoxic-normoxic cycles (1 min hypoxia/3 min normoxia) in rabbits. Two different hypoxic stimuli were given: gas mixtures of 14% and 11% O_2 in N_2, each one in a separate run of IH. Ventilatory features: frequency (*f*), tidal volume (V_T) and minute ventilation (V_E) were analyzed using the algorithms of the pattern recognition theory. The probability of wrong classification was used as a criterion for the recognition quality evaluation. This probability can be estimated experimentally by calculating the percentage of misclassifications, i.e., an error rate (E_r). When the features were analyzed alone, the V_T offered the lowest misclassification rate of 19.3% and 10.3% in the stimulus and normoxic periods, respectively. However, using the single features measured during the stimulus and recovery phases allowed to decrease the error rate more than 2-fold, achieving 4.3% for V_T. The best results were obtained for both phases associated with single cycles of IH. Suitable feature selection procedures enabled reducing the global misclassification rate to 0.7%. In conclusion, the pattern recognition approach may potentially be useful for controlling the stimulus strength in intermittent hypoxic training.

Key words: *intermittent hypoxia, pattern recognition, ventilatory response*

INTRODUCTION

Intermittent hypoxia (IH) is a subject of intensive studies from the viewpoint of its beneficial and adverse effects. A wide range of techniques and methods is used for this aim. IH triggers molecular, cellular, and physiological or pathophysiological responses (1-2). Studies on IH may contribute to the understanding of health and disease processes. IH is studied in three main areas: (i) clinical pathophysiology; (ii) experimental (human, animal or cell culture) models; and (iii) IH training.

Clinical studies show that recurrent sleep apnea provides a natural form of intermittent hypoxia, which is manifested by intermittent and recurrent pauses in breathing during sleep (3-5). These factors lead to decreased oxygen saturation, sleep fragmentation and excessive daytime sleepiness, and evoke other symptoms such as poor concentration or fatigue. Patients with chronic IH caused by recurrent apneas have a greatly increased risk for developing systemic hypertension, myocardial, and brain infarctions, neurocognitive deficits and even psychosocial dysfunctions. It is estimated that approximately one in four men and one in ten women have at least five apneas or hypopneas in each hour of sleep (3), but the number of sleep apneas per hour is dependent on the hypoxic ventilatory sensitivity. It has been observed that patients freshly diagnosed obstructive sleep apnea exhibit augmented hypoxic ventilatory response, whereas those suffering from apneas for nearly 20 years had depressed hypoxic ventilatory sensitivity (4). Epidemiological studies have shown that 2-3% of the sleep apnea occurs in children, 3-7% in middle-aged adults, and 10-15% in the health elders (above 65 years old) (5).

Neuroplasticity of respiratory output, induced by IH (6-8), might be beneficial in endurance training (9-11), prevention and treatment of various ailments (12-15), and in acclimatization before going to high altitude (16, 17). The clinical use of IH training is, as a therapeutic modality, useful in chronic lung diseases, bronchial asthma, hypertension, Parkinson's disease or emotional disorders (14, 15). Levine (17) presented that IH training can be divided into two different strategies: (i) providing hypoxia at rest with the primary goal of stimulating altitude acclimatization; and (ii) providing hypoxia during exercise, with the primary aim of enhancing the training stimulus. The ideal IHT protocol for increasing the hypoxic ventilatory responses is not yet known.

A good control system seems of importance for monitoring the effects of the hypoxic training, especially when IH is used as a therapeutic method. Such a system may be based on a computerized classification approach. In the present study we investigated whether it would be possible to distinguish, using the statistical pattern recognition theory (18), from the ventilatory effects of IH the differing strength of the hypoxic stimulus used. The results indicate that the pattern recognition algorithms can be useful in recognizing the pattern of IH depending on the stimulus strength.

MATERIAL AND METHODS

Biological experiments and measurements

The study obtained approval from a local Ethics Committee. Biological procedures were similar to those published previously (19, 20). The ventilatory response to five hypoxic-normoxic cycles was analyzed in the adult rabbits, which were anesthetized and spontaneously breathing. Two different hypoxic stimuli were applied: gas mixtures of 14% and 11% O_2 in N_2; each one in separate 6 experiments. The study protocol consisted of intermittent 1 min hypoxic (Stim) phases interspersed with 3 min recovery (Rec) phases. Ventilatory variables: minute ventilation (V_E), its frequency and tidal volume (f, V_T) were analyzed for five sequential breaths at the end of each phase cycle. These variables were expressed as a percentage change from the baseline level, i.e., before the first hypoxic episode.

Data analysis with the use of k-NN classifier

The possibility of distinguishing the stimulus strength in IH was investigated with the use of the pattern recognition theory and object classification approach (18). This statistical elaboration deals with the classification of objects and it supplies several algorithms for construction of a classification rule, i.e., a classifier. To construct the classifier, a set of objects with known class membership, called a training set, is required. Information contained in the training set is used for obtaining a decision rule that allows assigning the class to the new object that is to the object from outside the training set with unknown membership. The most powerful approach to the classifier construction is based on a distance function. The objects are considered as points in the feature space. Thus, the more similar the two objects are the closest distance is between the points that represent them in the feature space. For the sake of convenience, the objects will be identified by points, which represent them in the feature space. The most powerful classification rule is a *k* nearest neighbor rule (*k*-NN). It assigns a new object to the same class in which the majority of objects are among its *k* nearest neighbors found in the training set. The classification error rate depends also on the features. Some features available in the reference set can be redundant or not related with the considered classes. They may increase the error rate. For this reason, it is reasonable to select such a feature subset of the complete feature set, which promises the lowest misclassification rate. The ratio $E_r=r/m$ is the error rate that estimates the probability of misclassification, where *r* is the number of misclassified objects and *m* objects can be tested (i.e. the size of the data set used as the reference set for the *k*-NN rule).

In the present study, the classifier based on the known *k* nearest neighbor rule (*k*-NN) was applied. Each of the measured variables: V_E, f, and V_T was treated as an object, i.e., as a point or a feature vector. The different repetitive hypoxic exposures were defined as two classes depending on the hypoxic stimulus strength, i.e., 14% hypoxia (Class 1) and 11% hypoxia O_2 (Class 2). The values of variables, i.e., features characterizing the breathing pattern, were gathered as an experimental data set, which consisted of 300 objects, which in half originated from 14% hypoxia and in the other half from 11% hypoxia. Moreover, 150 objects belong to Stim phase and another 150 to the Rec phase; there were 60 points gathered for each cycle. This data set was treated as the training set and used for construction and validation of the classifier that can be applied for recognizing the two different hypoxic stimuli on the basis of V_E and its components f, V_T. The probability of wrong classification, estimated experimentally by an error rate E_r was used as a criterion for the classifier quality evaluation.

RESULTS

The ventilatory responses (features: V_E, and f, V_T) to short five hypoxic-normoxic cycles, of 14% (Class 1) and 11% (Class 2) hypoxia, was considered in the experimental model of IH in rabbits. The results of distinguishing them by *k*-

NN classifier are presented in *Table 1* and *Table 2*. The error rates (E_r) were computed for the classifier operating with single features V_E, f and V_T (*Table 1*), with the set of all variables, and with the selected feature set (*Table 2*).

When the features were analyzed alone (*Table 1*), then tidal volume offered the lowest misclassification rate of 19.3% and 10.3% in the stimulus and recovery phases, respectively. However, the classification based on these features measured in both phases allowed decreasing the errors E_r more than 2-fold, and E_r achieved only 4.3% for the V_T component. The set of all ventilatory features {f, V_T, V_E} offered the misclassification rate of 9.3% and 20.3% during exposures and after them, respectively. The errors decreased even to 1% if the recognition of the stimulus strength of IH was based on the set {f, V_T, V_E} in both hypoxic-normoxic phases (*Table 2*).

The selection of a feature set consisting of the f, V_T components, measured during the stimulation periods, or containing the single feature V_T, measured in the recovery phases, was sufficient for good distinguishing of the strength of IH, with the rates of 9.0% and 10.3%, respectively (*Table 2*). However, the classification performed with the use of ventilatory components measured in both phases decreased the error rate to 0.7%. The *k*-NN rule gave also a very good distinguishing power in each single cycle of IH (*Table 2*).

Table 1. Misclassification rates (E_r) for single ventilatory features (V_E, f, V_T) used for recognizing two different strength of IH stimuli: Class 1 – 14% hypoxia and Class 2 - 11% hypoxia.

	E_r (%)		
	V_E	f	V_T
Stimulus phases, Stim	21.0	41.3	19.3
Recovery phases, Rec	30.0	27.3	10.3
Stim & Rec phases	9.3	12.3	4.3

Table 2. Misclassification rates (E_r) for the feature set {f, V_T, V_E} and the selected feature combination used for the recognition between the two different strength of IH stimuli: Class 1 – 14% hypoxia and Class 2 - 11% hypoxia.

	E_r (%)	
	Without feature selection	With feature selection
Stimulus phases, Stim	9.3 {f, V_T, V_E}	9.0 {f, V_T}
Recovery phases, Rec	20.3 {f, V_T, V_E}	10.3 {V_T}
Stim & Rec	1.0 {f, V_T, V_E}	0.7 {f, V_T}
Single cycle:		
1	0.0	0.0
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	1.7	0.0

DISCUSSION

This work sought to determine whether the statistical pattern recognition theory could distinguish between two hypoxic stimulus strengths, 14% hypoxia (Class 1) and 11% hypoxia (Class 2), on the basis of early ventilatory responses to five-cycle hypoxia-normoxia runs. The *k*-NN classifier enabled a very good recognition of the two classes considered with a fraction of mistakes below 1% for the ventilatory features measured in both stimulus (hypoxic) and recovery (normoxic) phases. The stronger of the two stimuli elicited more intensive ventilatory responses during repetitive exposures. An important finding is that a very effective recognition was achieved using only two features, f and V_T. Generally, it would be possible to analyze, in the presented manner, the influence of other stimuli of various kind and strength on physiological variables. An analysis of ventilatory data based on *k*-NN rule was also performed in our previous studies. It has been shown that the *k-NN* rule applied for the defined strength of the hypoxic stimulus (14% hypoxia) with the features $\{f, V_T, V_E\}$ allowed recognizing IH cycles (five classes) with an error rate of 15.0% (19). It has also been demonstrated that minute ventilation progressively increased with each next hypoxic-normoxic cycles, and the increases were driven by both ventilatory components, f *and* V_T. Moreover, the increase in ventilation persisted for up to 30 min after the last hypoxic episode, a sign of long-term facilitation (20). In summary, it seems that the presented approach may be a good method to test or compare many options of different IH protocols, especially in IH training. The pattern recognition methodology may be applied to recognize not only different stimuli strength or cycle numbers or phases of IH (as presented above), but also other parameters of repetitive hypoxic exposures. In other words, the classification algorithms may be helpful in: (i) preparing IHT protocols for individuals; (ii) verification of the effects of strategies used for IH training.

Computerized classification approach has been used for data analysis of sleep studies in animals (21, 22) and humans in health and disease (23, 24) for more than 35 years. These investigations mainly concerned the visual and automatic analyses of normal and disturbed sleep stages, as based on EEG, EOG, EMG, and other recordings. Conventional sleep parameters obtained as a result of visual sleep stage scoring poorly reflect sleep disruption caused by, e.g., apneas. Witting et al (22) proposed a classification algorithm that recognizes sleep-wake states on the basis of EEG and EMG signals. The validation of the computer system was done by visual classification. Their experiments performed on four rats demonstrated a good agreement of both evaluations (kappa coefficient correlation of 0.77). Costa-Miserachs et al (21) described a five step method of an automated sleep-wake staging in the rat, which was also helpful to differentiate the animal conditions. Data from 15 rats were used to develop and verify this approach. Ten of them were used to create a training set and to develop a classifying algorithm and the other five animals for its validation. This way of analysis has given a global agreement of 94.3% between human and automatic scoring in the hippocampal electroencephalographic signal, delta and theta waves, and EMG recordings. Saastamoinen et al (23) found that the EMG parameters turned out to be most useful in showing differences in sleep depth between 16 healthy controls and 16 obstructive sleep apnea patients. They found a parameter combination that offers the best separation between the two diagnostic groups. In addition, Saastamoinen et al (25) presented an amplitude-independent method for continuous-scale, sleep depth estimation, and obtained the following results for agreement for the nearest neighbor classification in 15 healthy volunteers: 69.7-77.0% in awake, 41.3-43.1% in light sleep, 88.5-93.1% in deep sleep, and 44.7-51.5% in REM stages. These investigators argue that the computer systems developed to use classification methods are simple and have a high performance and reliability.

The pattern recognition approach gives a freedom of choice and of definition of classes (e.g., studied groups, processes or conditions), features (e.g., physiological variables, signals or other parameters) and classification rules, which makes it enticing. Generally, the best recognition is characterized by a small misclassification rates, e.g., Costa-Miserachs et al. (21) obtained only 5.7% wrong classifications of their system classifying sleep stages in rats, and Saastamoinen et al (25) obtained 6.9% misclassifications (for *k*-NN classifier) in healthy subjects.

IH models and training protocols greatly differ in cycle duration, number of hypoxic episodes, stimulus strength, and measured variables (2). These differences have an impact on the assessment of whether IH is beneficial or harmful (2, 14, 17). The knowledge about IH and the areas of its application remains incomplete and, therefore, await further explorations. We conclude that the pattern recognition approach may be useful in the assessment of the effects of intermittent hypoxia, which is a promising field of research.

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REFERENCES

1. Prabhakar NR, Fields RD, Backer T, Fletcher EC. Intermittent hypoxia: cell to system. *Am J Physiol Lung Cell Mol Physiol* 2001; 281: L524-L528.
2. Neubauer JA. Physiological and pathophysiological responses to intermittent hypoxia. *J Appl Physiol* 2001; 90: 1593-1599.
3. McNicholas WT, Ryan S. Obstructive sleep apnoea syndrome: translating science to clinical practice. *Respirology* 2006; 11: 136-144.
4. Prabhakar NR, Peng Y-J. Peripheral chemoreceptors in health and disease. *J Appl Physiol* 2004; 96: 359-366.

5. Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: population health perspective. *Am J Respir Crit Care Med* 2002: 165: 1217-1239.
6. Peng Y-J, Prabhakar NR. Reactive oxygen species in the plasticity of respiratory behavior elicited by chronic intermittent hypoxia. *J Appl Physiol* 2003; 94: 2342-2349.
7. Gozal E, Gozal D. Respiratory plasticity following intermittent hypoxia: developmental interactions. *J Appl Physiol* 2001; 90: 1995-1999.
8. Mitchell GS, Baker TL, Nanda SA et al. Intermittent hypoxia and respiratory plasticity. *J Appl Physiol* 2001; 90: 2466-2475.
9. Roels B, Thomas C, Bentley DJ et al. Effects of intermittent hypoxic training on amino and fatty acid oxidative combustion in human permeabilized muscle fibers. *J Appl Physiol* 2007; 102: 79-86.
10. Hinckson EA, Hopkins WG, Downey BM, Smith TBRJ. The effects of intermittent hypoxic training via a hypoxic inhaler on physiological and performance measures in rowers: a pilot study. *J Sci Med Sport* 2006; 9: 177-180.
11. Truijens MJ, Toussaint HM, Dow J, Leavine BD. Effects of high-intensity hypoxic training on sea-level swimming performances. *J Appl Physiol* 2003; 94: 733-743.
12. Burtscher M, Pachinger O, Ehrenbourg I et al. Intermittent hypoxia increases exercise tolerance in elderly men with and without coronary artery disease. *I J Cardiol* 2004; 96: 247-254.
13. Serebrovskaya TV, Swanson RJ, Kolesnikova EE. Intermittent hypoxia: mechanisms of action and some applications to bronchial asthma treatment *J Physiol Pharmacol* 2003; 54 Supp I: 35-41.
14. Serebrovskaya TV. Intermittent hypoxia research in the former Soviet Union and the commonwealth of independent states: history and review of the concept and selected applications. *High Alt Med Biol* 2002; 3(2): 205-221.
15. Harrison CC, Fleming JM, Giles LC. Does interval hypoxic training affect the lung function of asthmatic athletes? *New Zealand J Sport Med* 2002; 30: 64-67.
16. Kinsman TA, Gore CJ, Hahn AG et al. Sleep in athletes undertaking protocols of exposure to nocturnal simulated altitude at 2650 m. *J Sci Med Sport* 2005; 8: 222-232.
17. Levine BD. Intermittent hypoxic training: fact and fancy. *High Alt Med Biol* 2002; 3: 177-193.
18. Jóźwik A. Practical approaches to statistical pattern recognition. *Biocyber Biomed Eng* 2002; 22: 207-221.
19. Sokołowska B, Jóźwik A. Statistical evaluation of ventilatory patterns in response to intermittent hypoxia in the rabbit. *J Physiol Pharmacol* 2005; 56 Supp4: 203-207.
20. Sokołowska B, Pokorski M. Ventilatory augmentation by acute intermittent hypoxia in the rabbit. *J Physiol Pharmacol* 2006; 57 Supp4: 341-347.
21. Costa-Miserachs D, Portell-Cortes I, Torras-Garcia, Morgado-Bernal I. Automated sleep standing in rat with a standard spreadsheet. *J Neurosci Meth* 2003; 130: 93-101.
22. Witting W, van der Werf D, Mirmiran M. An on-line automated sleep-wake classification system for laboratory animals. *J Neurosci Meth* 1996; 66: 109-112.
23. Saastamoinen A, Oja H, Huupponen E, Varri A et al. Topographic differences in mean computational sleep depth between healthy controls and obstructive sleep apnoea patients. *J Neurosci Meth* 2006; 157: 178-184.
24. Penzel T, Conradt R. Computer based sleep recording and analysis. *Sleep Med Rev* 2000; 4: 131-148.
25. Saastamoinen A, Huupponen E, Varri A et al. Computer program for automated sleep depth estimation. *Comp Meth Program Biomed* 2006; 82: 58-66.

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