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RESPIRATORY RESPONSES TO ACUTE INTERMITTENT HYPOXIA AND HYPERCAPNIA IN AWAKE RATS

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This article deals with the recognition of early changes in the breathing pattern, in response to acute intermittent stimuli in awake rats. Two different types of stimuli were given: 9% hypoxia in N_2 and 10% hypercapnia in O_2. Animals were exposed to 3 consecutive cycles consisting of 3-min stimulus period separated by 8-min normoxic recovery intervals. Features of the breathing pattern, such as respiratory frequency, tidal volume, minute ventilation, inspiration and expiration times, peak inspiratory and expiratory flows, were measured by whole body plethysmography. The data were analyzed with the use of pattern recognition methods. We conclude that the overall respiratory changes were rather slight. However, computerized analysis using a *k*-nearest neighbor decision rule (*k*-NN) allowed for a good recognition of the respiratory responses to the stimuli. The misclassification rate (E_r) varied from 5 to 10%. After feature selection, E_r decreased below 1%. The *k*-NN classifier differentiated correctly also the type of intermittent stimulus. Our experimental results demonstrate usefulness of pattern recognition algorithms in studying respiratory effects in biological models.

Key words: *intermittent hypoxia, intermittent hypercapnia, k-NN classifier, pattern recognition*

INTRODUCTION

Intermittent hypoxia or hypercapnia, or repeated exposures to the stimuli lasting minutes to weeks interrupted by normoxia or less hypoxic (hypercapnic) conditions, occurs in various physiological and pathophysiological situations (1-3). Repeated exposures to hypoxia have been examined for both their adverse

and beneficial effects in human and animal models (4-6). Intermittent hypoxia in humans during exercise and sleep is one of the earliest hypoxic manifestations of chronic obstructive and interstitial lung diseases (7). Repeated episodes of intermittent hypercapnia accompanied usually early stages of lung diseases (1, 8). Moreover, several epidemiologic studies demonstrate that sleep-related breathing disorders (SBDs) are an independent risk factor for develop hypertension, probably in consequence of appearing of intermittent hypoxia and hypercapnia, increased sympathetic tone, and altered respiratory control during sleep (7, 9). The SBDs are now recognized as a major and important health public problem. Early diagnosis and treatment of these breathing disorders may help to avoid different health and living complications. So, functional benefits of repetitive exposures to hypoxia have been explored with a therapeutic goal (10, 11) and in endurance training in athletes (12, 13).

Recent results of experimental studies of intermittent hypoxia/ hypercapnia in human and animal models point to a puzzling feature of the respiratory system (14-18). Data consistently show that the respiratory system has various forms of plasticity, defined as alterations in the breathing pattern that develop or persist also after a stimulus has ceased to exist. For example, it is observed that intermittent sensory stimulation may induce long-term alterations, either facilitation or depression, depending on the timing, intensity, and type of stimulation. Kinkead *et al* (14) have suggested that intermittent chemosensory stimulation produces long-term changes in respiratory motor output *via* specific neuromodulatory systems. According to their concept, intermittent hypoxia induces a net long-term facilitation (LTF) of respiratory output mainly *via* the serotonergic system, whereas intermittent hypercapnia elicits depression *via* noradrenergic system. Results of other studies indicated that intermittent hypoxia is a main and substantial feature of obstructive sleep apnea (18-20). Mahamed and Mitchel (18) have proposed new therapeutic strategies to induce and apply of LTF within specific motor pools to increase respiratory muscle activity and upper airway tone during sleep. Dick *et al* (17) have suggested that exposures to hypoxia induce increases in sympathetic activity and hypoxic sensitivity. The increases are coordinated and caused by interactions between the respiratory and sympathetic control systems, both mediated by serotonin. However, the exact determinants of the interaction between LTF and intermittent hypoxia are still unknown and need further studies.

In the present study, changes in the respiratory response to acute intermittent hypoxia and intermittent hypercapnia in awake rats were analyzed. The purpose of the study was to evaluate the feasibility of differentiation between the first and last exposure to the intermittent stimulus and of recognizing the type of intermittent stimulus on the basis of the recorded features describing the breathing pattern. The pattern recognition methods were applied for the purpose (21, 22).

Table 1. Pulmonary variables from a respiratory flow signal.

VARIABLES (features)	SYMBOLS
Breathing frequency	f
Tidal volume, the inspired volume	TV
Minute ventilation, the product of TV and f	MV
Inspiratory time	Ti
Expiratory time	Te
Peak inspiratory flow	PIF
Peak expiratory flow	PEF

MATERIAL AND METHODS

Biological Experiments

The study was approved by a local Ethics Committee. Six experiments were performed in awake adult male Wistar rats, weighing 342-420 g. The animals were placed in a whole body plethysmographic chamber (model PLY3223, Buxco Electronics, Wilmington, NC). The pressure signal from the plethysmograph was amplified (MAX1320 preamplifiers and interface; Buxco Electronics), and integrated by data analysis software (Biosystem XA for Windows SFT3410 v. 2.9, Buxco Electronics). The animals were exposed to intermittent hypoxia (9% O_2 in N_2; denoted as intermittent hypoxia 9%) and hypercapnia (10% CO_2 in O_2; intermittent hypercapnia 10%). The protocol of intermittent ventilatory stimulation consisted of 3 cycles, consisting of 3-min exposures to a given gas stimulus interspersed with 8 min normoxic intervals. *Table 1* demonstrates the list of ventilatory variables which were recorded, or calculated, from the respiratory flow signal, representing 20 breaths of the last minute of each gas exposure.

Statistical analysis

Baseline level (base control) was evaluated before exposures to the intermittent stimuli in each rat, and it was defined as a level of 100%. All values of the variables measured were expressed as a percentage of the baseline value and they were presented as means ±SD. Changes in variables of respiratory pattern were evaluated by the Wilcoxon test. The analysis was performed using a commercial statistical packet (Statistica 5.1, StatSoft, Poland). The analyzed differences were considered as significant when $P<0.05$.

The *k* nearest neighbor (*k*-NN) classifier (21, 22) was used for studying the effectiveness of the recognition and prediction of respiratory responses during the intermittent stimulation of ventilation. The *k*-NN classifier is the most popular and simplest decision rule. It assigns the object x to a class that is most common among its k nearest neighbors in the training set X (e.g., biological data set), where X is the set of objects with the known class membership. As a distance function, the city measure or Euclidean measure is usually used. In the present work, the city measure is applied. The quality of classification is evaluated by the probability of misclassification that can be estimated experimentally by the *leave-one-out* method. It consists in classifying each object x from the training set X by the *k*-NN rule. The *k*-NN operates with the training set decreased by the currently classified object. Thus, to classify the object x the k nearest neighbors are searched for in the set X-$\{x\}$. In such a manner all m objects from the training sets can be tested and the number r

of misclassified objects may be computed. The misclassification rate, $E_r=r/m$, estimates the probability of the assignment to a wrong class. The training set may contain redundant features and features not related to the considered classes. The presence of such features can lead to an increased error rate and, therefore, a feature selection ought to be performed. In case of a small number of features, it is possible to review all feature combinations and to select a feature subset that offers the smallest error rate. In the present study, the procedure of feature selection, to obtain the smallest possible error rate, was performed by the *k*-NN classifier in the whole data set.

RESULTS

The mean values of the features describing the respiratory responses to intermittent hypoxia and intermittent hypoxia, for first and last (third) exposures, are presented in *Table 2*. Changes in the variables were not statistically significant. However, in the last stimulation by intermittent hypoxia, a small increase of f and MV, and a decrease of Ti and Te were observed. For intermittent hypercapnia, MV and its both components (f and TV) slightly decreased, in both exposure and interspersed normoxic periods, so that PIF and PEF were also diminished.

Misclassification rates (E_r) for the differentiation between the first and last exposure to the intermittent stimuli are presented in *Table 3*. E_r for the recognition of the first and last cycle of intermittent stimuli, consisting of the exposure and normoxic recovery periods, were higher for the intermittent hypoxia than

Table 2. Respiratory responses to acute intermittent hypoxia (intermittent hypoxia 9%) and hypercapnia (intermittent hypercapnia 10%).

FEATURES (periods)	intermittent hypoxia 9%		intermittent hypercapnia 10%	
	1st exposure	3rd exposure	1st exposure	3rd exposure
f (exposure) f (normoxia)	160 ±36 97 ±10	189 ±16 87 ±20	149 ±22 86 ±20	135 ±14 73 ±12
TV (exposure) TV (normoxia)	139 ±20 82 ±6	127 ±11 85 ±15	166 ±25 99 ±5	159 ±44 91 ±11
MV (exposure) MV (normoxia)	218 ±19 79 ±10	239 ±6 75 ±26	245 ±29 85 ±16	214 ±53 66 ±10
Ti (exposure) Ti (normoxia)	84 ±24 102 ±7	79 ±17 114 ±20	89 ±21 129 ±9	97 ±10 110 ±24
Te (exposure) Te (normoxia)	57 ±11 107 ±4	50 ±2 121 ±32	56 ±11 120 ±44	62 ±12 138 ±36
PIF (exposure) PIF (normoxia)	157 ±21 84 ±11	152 ±15 77 ±13	165 ±46 79 ±11	140 ±25 63 ±7
PEF (exposure) PEF (normoxia)	152 ±23 95 ±8	178 ±48 105 ±18	289 ±56 87 ±15	241 ±56 79 ±12

Data are expressed as means ±SD (as a percent of the basic level)

Table 3. Misclassification rates (E_r) for recognition of the first and last cycle of intermittent hypoxia (intermittent hypoxia) and hypercapnia (intermittent hypercapnia) during exposure to a stimulus and subsequent normoxic interval (without and with feature selection).

Intermittent stimuli	Without feature selection (E_r)	With feature selection (E_r)
intermittent hypoxia 9%		
Exposure period Normoxic recovery period	0.100 0.092	0.042 0.042
intermittent hypercapnia 10%		
Exposure period Normoxic interval period	0.058 0.050	0.033 0.008

Table 4. Misclassification rates (E_r) for recognition of the type of the intermittent stimulus (intermittent hypoxia-IH *vs.* intermittent hypercapnia-IC), in first and last period (exposure and normoxia), without and with feature selection.

IH *vs.* IC	Without feature selection (E_r)	With feature selection (E_r)
First exposure First normoxia	0.008 0.017	0.000 0.000
Last exposure Last normoxia	0.017 0.008	0.000 0.000

hypercapnia. After the selection of features, most frequently ventilation and its components, E_r still decreased and achieved values below 5% for both stimuli. The smallest error rates were observed for intermittent hypercapnia in both exposure and recovery periods (*Table 3*).

E_r for the recognition of stimulus type, separately for the exposure and normoxic recovery periods are shown in *Table 4*. The *k*-NN classifier was used with the whole set of features, i.e., f, TV, MV, Ti, Te, PIF, PEF, and with a feature selection. Without the feature selection, the misclassification rates for both exposure and normoxic recovery periods were already fairly low, as it amounted to 0.8-1.7%. With the feature selection, the classifier recognized with no mistake the type of intermittent stimulus independently of the cycle (first or last) and of the period (stimulus exposure or normoxic recovery).

DISCUSSION

In the present work, the early respiratory responses to acute intermittent stimuli, consisting of three exposures to hypoxia or hypercapnia, in awake rats and the feasibility of recognition of changes evoked in the breathing pattern, depending on the exposure cycle and stimulus type, were analyzed. The overall breathing changes observed in response to the stimuli were rather small and

statistically insignificant. However, the *k*-NN classifier recognized well whether the respiratory changes were due to the exposure to a stimulus, be it intermittent hypoxia or hypercapnia, or to a normoxic recovery. The classifier also was able to detect a difference in breathing pattern changes caused by the two stimuli; thus it was able to tell which stimulus caused given changes.

Two main approaches are employed in biomedical investigations concerning the effects of intermittent stimuli: (i) to determine the physiological mechanisms of intermittent hypoxia (2, 3, 5, 18, 23-26), and intermittent hypercapnia (1, 8, 27) and (ii) to apply various computer systems, algorithms, or models for analyzing and recognition of the effects during and after intermittent stimuli application (26, 28-32). In practical applications of intermittent stimuli in clinical, rehabilitation, and sports medicine and training (high acclimatization/performance training) it is important to recognize and correctly evaluate the effects that are obtained with the use of different protocols. Computer systems may be helpful to this end. For example, McGuire *et al* (26) analyzed breathing changes in awake rats, which were placed in a computerized pletysmographic chamber. The software provided a breath-by-breath display of ventilatory variables before, during, and after different intermittent hypoxic protocols. In their experiments, 5-min episodes of hypoxia were interspersed with 5-min intervals of normoxia, and MV was monitored up to 60-90 min after the last hypoxic episodes. The authors suggested that there is a certain range of hypoxia that induces ventilatory LTF. In our previous studies (23, 31), a progressive ventilatory augmentation in response to acute intermittent hypoxia (five 1-min exposures to 14% O_2 with 3-min normoxic recovery) was observed in anesthetized, spontaneously breathing rabbits. The augmentation was sustained for at least 30-min after the last exposure. In that study, the pattern recognition algorithms were able to differentiate between the first and last cycle of exposure; the differentiation was best during the normoxic recovery. Hamrahi *et al* (28) studied the regulation of sleep-wake states in response to intermittent hypoxic stimuli in an animal model of obstructive sleep apnea. In the experiments performed in ten rats, a computer detected sleep-wake states and triggered hypoxic (10% O_2) or room air stimuli only during sleep for a 3-h period. Sleep-wake states were also recorded for a 3-h recovery period after discontinuation of stimuli. On average, 69 hypoxic stimuli were applied in each rat over the 3-h phase during sleep. Their results show that sleep-specific hypoxia leads to significant modulation of sleep-wake regulation both during and after application of intermittent hypoxic stimuli. Saastamoinen *et al* (29) presented a computer program for automated sleep depth estimation. They performed a nearest neighbor classification of the EEG recordings of 15 healthy volunteers and evaluated the percentage of agreements between the visual scoring and the automated analysis. The results may be considered successful and the best quality of recognition was obtained in deep sleep stage (from 87 to 93% correct recognitions).

In the present study in awake rats, we demonstrated that exposures to three repetitive bouts of short-term hypoxia or hypercapnia elicit respiratory responses whose character may be well enough recognized by the classifier based on *k*-NN rule (from 90 to 99% correct recognitions).

In conclusion, we agree with the opinion of Zhu *et al* (33) that 'there is increasing interest in applying sophisticated computer-modeling and statistical analysis techniques in health care' and also in experimental research. Those authors showed that the *k*-NN algorithm may be very useful in prediction of rehabilitation. Their study illustrates the potential of a machine-learning (with the use the *k*-NN method) to enhance clinical decision making. Penzel *et al* (32) and Saastamoinen *et al* (29) also presented similar computer methods of sleep recording and analysis in human (healthy or patients) and animal models. The results of the present work corroborate those of our previous studies that point to the usefulness of the pattern recognition approach in different experimental models, such as recognition of diaphragm pathology in anesthetized cats (34), recognition of cycles of intermittent hypoxia (31), or differentiation the strength of a stimulus in anesthetized rabbits (30).

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