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Procedural Memory Trace for Increased Tidal Volume Can be Demonstrated in Healthy Volunteers

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Summary

The minority of healthy subjects can (without previous learning) breathe at exactly double of their resting tidal volume on demand. The aim of the present study was to find whether this estimate of tidal volume value can be learned and at what speed. Basic spirometric values were measured by Oxycon B in 20 healthy volunteers. They learned in one day to breathe at double of the resting tidal volume twice for 3 min. The existence and duration of the memory trace was ascertained four times (three times on the same day, once three weeks later). This engram can be demonstrated on the day of learning after 1 or 2 trials of 3 min each. Three weeks later the memory trace is weak but still present. This fact means that a new breathing pattern can be learned. During learning and tests the subjects hyperventilate but no correlation was seen between learning ability and intensity of hyperventilation.

Key words
Tidal volume – Hyperventilation – Respiratory work – Oxygen consumption – Procedural memory

Introduction

It is well known that human subjects can transiently change their breathing pattern easily. It is also generally assumed that they can learn new breathing patterns with relative ease, but this assumption is substantiated by a relatively few facts. Earlier studies (Swanson *et al.* 1976, Eldridge 1980, Engwall *et al.* 1991) were concerned with very short-lasting memory. The so-called ventilatory afterdischarge is a lasting sign of ventilatory stimulation after extinction of original electrical or nervous stimuli, either in experimental animals or men. It is an event lasting a few seconds which is not identical with learning. Learning is to be understood as a gradual improvement of the quality of an engram (memory trace) the stability of which can be observed for at least a few days.

The aim of the present study was to ascertain a) whether and how rapidly a memory trace for increased tidal volume is formed in man, b) its duration, c) whether there is any relationship between the exactness of reproduction of the learned tidal volume (learning ability) and hyperventilation of the individuals tested, and d) which changes occur in respiratory work during learning.

Material and Methods

Tested subjects

The experiments were carried out on a group of volunteers, 10 men and 10 women. The average age of the men was 40±14 (S.D.) years, their average height 181±6 cm, their average body mass 83.5±8.6 kg and their vital capacity (BTPS, Vitalograph) 4.8±0.6 l. The women were 40±13 years old, their average height 163±9 cm, their average body mass 60.8±14.2 kg and their vital capacity was 3.7±0.8 l. The subjects had no experience with physical training activities, with hathayoga, with singing, with playing wind instruments or with spirometric examination. They were members of the university staff.

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Ventilation measurements were performed in a laboratory room at ambient temperature between 22 and 25 °C. The values of tidal volume (VT), rate of breathing (f), minute ventilation (V'E), oxygen consumption ($Q'O_2$), partial pressures of oxygen and carbon dioxide in end tidal air ($PETO_2$, $PETCO_2$) were ascertained using a face mask and the spirometer Oxycon B (Mijnhardt). The total dead space of the mask and apparatus was 130 ml.

The experiment began with a 10 min control resting period during which the control respiratory variables were recorded. The tested subjects read newspapers during this period and did not think of ventilation. At the end of the resting period each subject was informed about the mean value of VT. Then the subject was asked to breathe for 3 min double VT without a feedback from the oscilloscope screen. This was the second period of experiment. Then, after 3 min of resting breathing a 3 min learning session followed. During this period, the person was asked to breathe with double VT with an optic feedback from the oscilloscope screen. The subject was also advised to think of the VT values and to make an attempt to memorize them. During this phase, the tested subject viewed the screen with a breath-to-breath value of the actual VT and also the learned value of double VT. Immediately after this phase, the subject was also informed about the mean VT value of the last 2 min period.

Table 1. Protocol of the experiments

Period	Aim and description of period	Duration of period
	Control. The subjects read newspapers	10 min
2	Another control. Instruction to breathe double VT without feedback from oscilloscope	3 min
3	Resting period, as in period 1	
4	Learning. Instruction to breathe double VT with feedback from oscilloscope and to memorize the value	
5	Resting period, as in period	3 min
6	The 1st memory test. Instruction to breathe double VT without feedback from oscilloscope	
7	Resting period, as in period 1	
8	The 2nd learning session, as in period 4	
9	Resting period, as in period 1	
10	The 2nd memory test, as in period 6	
11	Resting period, as in period 1	
12	The 3rd memory test, as in period 6	
13	The 4th memory test, as in period 6	3 min

The first 12 periods followed immediately one after the other, period 13 was 3 weeks later.

Memory test

After ventilating at rest, the subjects were asked to remember the double VT value and to breathe at double VT. In this phase they did not see the screen and only afterwards they were told the achieved mean VT value. The criterion of correct response and memory trace formation was a value of double VT±20 %. This limit was chosen on the basis of a pilot study with subjects without learning.

The first phase of learning was in the 17th–19th min. A resting period followed. In the 23rd–25th min memory engram was tested for the first time. Another resting period followed. The second learning phase of reinforcement was in the 29th-31st min. After another resting period, two additional memory tests were performed on the same day, when the subjects were asked to breathe at double VT. Memory tests were performed in the 35th–37th min and in the 41st–43rd min. The last memory test was performed 3 weeks after the first day of learning. It also lasted 3 min. The subject was asked again to breathe at double VT (original value found 3 weeks ago).

Table 1 gives a review of the time schedule. Before the experiment, the subjects were informed about the aim of the study, the time schedule and method. Their informed consent was obtained. The subjects were not limited with respect to coffee consumption, physical exercise or smoking before the experiment. They were, however, mostly non-smokers. All the subjects sat in a comfortable armchair during the measurements.

The means and standard deviations (S.D.) of the respiratory values from the last 2 min of the first control period and also the values of all the other periods were calculated. The reason for not calculating the first minute in all the periods was to eliminate artifacts due to transition of the phases. A statistically significant difference between the values of a given period was ascertained by ANOVA and the t-test. Linear regression was used to evaluate the relationship between hyperventilation and the exactness of VT reproduction. As criteria of hyperventilation not only $PETCO_2$ changes were used, but also increases of V'E. The frequency of responses was evaluated by four-field tables (Documenta Geigy 1963). In all cases the probability level was $p<0.05$.

Results

Control values

The resting VT in all the group of subjects was 0.697±0.144 l, the resting frequency was 15±4 breaths per minute. The resting respiratory values are summarized in Table 2. The resting gas exchange ratio (R) in both sexes was 0.84±0.07. Breathing before learning is denoted as a double VT. After instruction to breathe with double VT without optic feedback, the target value of double VT was 1.394 l, but the obtained value was 5.4±43.7 % lower. Only two out of the 20 subjects tested had a smaller difference from the expected value than ± 20 %.

When learning to breathe at double VT, all persons achieved the VT target value in both learning periods with minimum deviations. In the first and second learning period, the obtained values were by 0.2±6.7 and 0.6±5.1 % lower, respectively.

Memory test

In the first memory test the VT value was found to be by 1.4±22.5 % lower than that expected, in the second memory test it was by 2.5±14.5 % lower than 1.394 l, in the third test it was higher by 1.2±18.9 % and in the fourth test it was higher by 7.4±24.1 %.

After the first learning phase, only 55 % of subjects were able to reproduce double VT correctly (the memory trace lasted at least 3 min). After the second learning phase (after reinforcement) already 85 % of subjects succeeded to be correct (the memory trace lasted 6 min and usually also 3 weeks). All differences were statistically significant against the controls.

Table 2a. Respiratory variables before and during learning sessions (L) and during memory tests (MT).

Period	V'E l . min^-	$PETCO_2$ kPa	(mm Hg)	$Q'O_2$ ml . min^{-1} . kg^{-1}
Control	10.7±2.1	4.75±0.35	(35.6)	3.67±0.86
double VT	15.0±6.2*	4.37±0.39*	(32.8)	3.84±0.55
1st L	15.3±4.5*	4.31±0.39*	(32.3)	3.88±0.55
1st MT	14.0±5.8*	4.25±0.52*	(31.9)	3.72±0.82
2nd L	15.0±5.1*	4.13±0.53*	(31.0)	3.77±0.73
2nd MT	13.6±5.5*	4.21±0.64*	(31.6)	3.72±0.59
3rd MT	14.0±5.7*	4.25±0.63*	(31.9)	3.80±0.83
4th MT	14.7±8.6*	4.42±0.65*	(33.2)	4.03±0.94

*Data are means ±S.D. * significantly different ($p<0.05$) from the control period*

Table 2b. Respiratory variables before and during learning sessions (L) and during memory tests (MT).

Period	f c.min^{-1}	R
Control	15.4±3.8	0.84±0.07
double VT	10.2±3.4*	1.08±0.23*
1st L	11.0±3.4*	1.12±0.23*
1st MT	10.2±3.4*	1.06±0.20*
2nd L	10.8±3.8*	1.05±0.16*
2nd MT	10.0±4.2*	1.00±0.15*
3rd MT	9.9±3.8*	1.01±0.14*
4th MT	9.8±4.0*	1.07±0.16*

*Data are means ± S.D. * significantly different ($p<0.05$) from the control period*

The proportion of correct responses is given in Table 3. This shows the learning ability of tested subjects. No significant relationship was observed between the exactness of the learned value of double VT and the degree of hyperventilation (the two criteria of hyperventilation were described in Methods). The correlation coefficients *r* were low (0.12 and 0.14) and statistically non-significant.

Other observations during experiment

As is evident from Table 2a, the subjects hyperventilated ($PETCO_2$ decreased) after instruction to breathe double VT, during both phases of learning and during all four memory tests.

Furthermore, the gas exchange ratio (R) and oxygen consumption ($Q'O_2$) increased during breathing double VT (Tables 2a and 2b). The increase of $Q'O_2$ was moderate and non-significant, whereas gas exchange ratio increased from 0.84 to the value greater than one. On the other hand, the rate of breathing (f) decreased from the original 15 c.min^{-1} to the values of about 10–11 c.min^{-1} in the periods with increased VT. Both the changes of gas exchange ratio and those of breathing rate were significantly different from the control values.

After each phase of breathing double VT the values of the breathing pattern, gas exchange ratio and $PETCO_2$ returned to control values (data not shown).

Discussion

It was found that only a minority of subjects (10 %) could breathe double VT without a feedback from the screen before learning. The values obtained differed greatly in both directions from the desired value. This observation made possible to test learning in the procedural memory for breathing in man, and this not only concerned short-lasting but also the long-lasting memory. Several investigators (Eldridge 1980, Engwall *et al.* 1991, Swanson *et al.* 1976) studied the afterdischarge – a phenomenon lasting only a few seconds. Freedman and Weinstein (1965) in their studies of the responses of the breathing pattern assumed the participation of short-lasting memory in responses to elastic loading in men, but they did not document this participation.

According to present knowledge, memory can be divided into declarative and non-declarative (Brožek 1991), short-lasting and long-lasting (Moffett *et al.* 1993). The short-lasting memory comprises seconds to minutes, the long-lasting memory endures for hours. Some authors also distinguish immediate memory, lasting only few seconds (Moffett *et al.* 1993). It is not always easy to distinguish in which category and to what extent the short-lasting and long-lasting memory participate. It is also not easy to decide which functional changes are due to reflexes and which due to memory, i.e. experience is no doubt involved.

Memory for breathing belongs to the group of procedural, i.e. non-declarative memories. According to Pokorný (1996) the processes of learning belong to neuroplastic phenomena.

Table 3. Proportion of correct responses of volunteers in tests of memory (MT) before and after the learning sessions.

Conditions	Time after learning sessions	Number of learning sessions	%
Control	none	none	10
1st MT	3 min	1	55*
2nd MT	3 min	2	
3rd MT	6 min	2	
4th MT	3 weeks	2	40*

** significantly different ($p<0.05$) from the control period in 20 subjects.*

It is typical for procedural memory that it improves after repeated connections. This corresponds to our results shown in Table 3. The first part of this study confirmed our assumptions. We demonstrated the possibility of forming a memory trace for the magnitude of VT on demand. After reinforcement this engram lasted at least for 3 weeks when the result was still significant compared with the controls. This finding indicates that a new breathing pattern can be learned.

In another part of the study, we could expect that the individuals, who were more excitable, i.e. with a higher degree of ventilation, will learn a new breathing pattern better than the individuals with lower hyperventilation, because of a higher degree of attention. There was no direct evidence for such a phenomenon. This expectation was thus not confirmed. Electrostimulation of brain areas associated with attention improves learning ability in experimental animals (McGaugh *et al.* 1979). Attention in human subjects leads to hyperventilation (Shea *et al.* 1987). To a certain extent, hyperventilation can indicate a higher degree of concentration. Respiratory alkalosis decreases the level of ionized calcium and thus increases nervous excitability.

During learning and the memory tests all subjects markedly hyperventilated. However, a correlation between hyperventilation and the precision of the response was not found. It is possible, though, that longer lasting experiments, a longer period of learning etc. could have made such a correlation evident. In some individuals, a longer period of learning could result in the disappearance of hyperventilation and chemical control of breathing could come into play. In our study, this control was suppressed as evidenced by the mere fact of hyperventilation.

A further aim of our study was to obtain the data about respiratory work. One of the criteria is oxygen consumption ($Q'O_2$). In our experiments, $Q'O_2$ could be increased under the assumption that the work of breathing will increase with enhanced VT and V'E (Crosfill and Widdicombe 1961, Šmejkal and Felkel 1994), but this increase was not significant compared with the control period (Table 2). This observation is in accordance with the tendency towards optimalization of the work of breathing, which was described earlier (Mead 1960). Apart from respiratory work $Q'O_2$ can also reflect the degree of psychic activation of the individual. Data on oxygen consumption at low levels of minute ventilation do not provide sufficient information about the exact value of respiratory work. It would therefore be useful to supplement them with a physical method.

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