Repeated exercise paired with “imperceptible” dead space loading does not alter $\dot{V}E$ of subsequent exercise in humans

S. H. MOOSAVI, A. GUZ, AND L. ADAMS

National Heart and Lung Institute, Imperial College School of Medicine, Charing Cross Campus, London W6 8RP, United Kingdom

Received 17 April 2001; accepted in final form 31 October 2001

Moosavi, S. H., A. Guz, and L. Adams. Repeated exercise paired with “imperceptible” dead space loading does not alter $\dot{V}E$ of subsequent exercise in humans. J Appl Physiol 92: 1159–1168, 2002. First published November 9, 2001; 10.1152/japplphysiol.00358.2001.—We employed an associative learning paradigm to test the hypothesis that exercise hyperpnea in humans arises from learned responses forged by prior experience. Twelve subjects undertook a “conditioning” and a “nonconditioning” session on separate days, with order of performance counterbalanced among subjects. In both sessions, subjects performed repeated bouts of 6 min of treadmill exercise, each separated by 5 min of rest. The only difference between sessions was that all the second-to-penultimate runs of the conditioning session were performed with added dead space in the breathing circuit. Cardiorespiratory responses during the first and last runs (the “control” and “test” runs) were compared for each session. Steady-state exercise end-tidal $P_{\text{CO}_2}$ was significantly lower ($P = 0.003$) during test than during control runs for both sessions (dropping by $1.8 \pm 2$ and $1.4 \pm 3$ Torr during conditioning and nonconditioning sessions, respectively). This and all other test-control run differences tended to be greater during the first session performed regardless of session type. Our data provide no support for the hypothesis implicating associative learning processes in the ventilatory response to exercise in humans.

exercise hyperpnea; classical conditioning; long-term modulation; minute ventilation

EXERCISE HYPERPNEA IN HUMANS cannot be explained solely or simply in terms of feedback modulation of automatic respiratory control. The ventilatory response to moderate exercise remains essentially unaltered by deprivation of peripheral chemosensitivity (37), by congenital lack of central chemosensitivity (27), by deprivation of vagal afferents in heart or heart-lung transplant (4), or by spinal cord transection that deprives patients of afferent information from electrically stimulated exercising muscles (1). The response in humans may therefore be primed by “feedforward” mechanisms that predict ventilatory requirements on the basis of nonmetabolic information. A supraspinal neural source of feedforward drive has been suspected for years. The descending motor command to exercising muscles may also activate efferent respiratory neurons in the brain stem or motor cortex (17). Alternatively, a specific subthalamo locomotor center has been postulated as a potential source of feedforward drive (9).

The present study examines the possibility that feedforward control takes the form of a learned response (29) involving adaptation (long-term modulation) of central neural networks controlling respiratory muscles (28). This assumes that exercise hyperpnea is, in part, an existing “conditioned reflex.” One possibility is that a conditioned reflex originated from an association between nonrespiratory afferent information from the exercising muscles and the reflex respiratory drive at some synaptic site of convergence. Presumably, this would have been formulated early in development and reinforced with a cumulative experience of exercise. The present study indirectly tests for the existence of such a conditioned reflex by repeatedly and systematically altering the level of reflex ventilatory drive associated with a given level of muscular exercise in a controlled laboratory environment.

Experimental evidence for a learned response was first provided by a study in which conscious goats were subjected to an associative learning paradigm involving repeated pairing of exercise with another stimulus to breathe from added external dead space (19). This invoked a reversible hyperventilatory response detected by a drop in the level at which arterial $P_{\text{CO}_2}$ ($P_{\text{aCO}_2}$) was regulated during exercise. Since then, a number of preliminary reports (2, 7, 33) of similar protocols attempted in humans have generated opposing views regarding the existence and significance of plasticity in the ventilatory response to exercise in humans. In the one full publication of which we are aware (12), postconditioning changes in ventilation are reported during the exercise on-transient in humans that were not seen in goats (19). The present study employs treadmill exercise, a relatively small additional dead space, and, most importantly, a within-subject control procedure.

Address for reprint requests and other correspondence: S. H. Moosavi, Physiology Program, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115-6021 (E-mail: smoosavi@hsph.harvard.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Subjects

Twelve subjects (mean age 27, range 22–37 yr), including five women (mean height 1.62, range 1.51–1.72 m; mean weight 63, range 49–87 kg) and seven men (mean height 1.73, range 1.45–1.92 m; mean weight 67, range 45–78 kg), were studied in an exercise laboratory maintained at 20–23°C. Two subjects were light smokers (<5 cigarettes/wk), and one was a regular smoker (>10 cigarettes/day). None of the subjects had a history of cardiorespiratory disease, and all led active lifestyles but were not trained or competitive athletes. Four subjects had participated in one previous experiment in the same laboratory involving treadmill exercise and respiratory measurements. None of the other subjects had previously experienced exercise testing in a laboratory setting. Subjects were informed that the experimenters were interested in monitoring physiological and subjective changes associated with repeated bouts of moderate exercise, but breathing additional dead space was not mentioned. Studies were undertaken with approval of the ethical committee of Charing Cross Hospital, and written consent to participate was obtained.

Exercise Procedure

Each experimental session involved repeated performance of 6-min steady-state runs of moderate-intensity exercise on a motorized treadmill. The treadmill speed was maintained at 3.5 miles/h (~6 km/h) for men and 3 miles/h (~5 km/h) for women. The treadmill slope was always fixed at 10%. These settings approximated an external power output of 80–100 W; the actual workload for each subject would depend on the subjects’ weight and muscle mass relative to weight. Before the commencement of each run, subjects stood astride the treadmill for 1 min in preparation for the exercise; baseline levels of cardiorespiratory variables were recorded during this period. At the end of each run, subjects again stood astride the treadmill and cardiorespiratory measurements were continued for a further minute. Between runs, subjects sat and relaxed for 5–10 min.

Experimental Arrangement

Subjects wore noseclips and breathed through a mouthpiece connected to a heated Fleisch pneumotachograph head (no. 2, P. K. Morgan, Rainham, Kent, UK). The distal side of the pneumotachograph head was linked, via a short length of corrugated tubing (28 mm ID), to an additional external dead space of ~600 ml. This was in addition to the total fixed dead space of the pneumotachograph and tubing (~130 ml). The flexibility of the corrugated tubing allowed subjects to perform moderate exercise without constraining movement, did not add any appreciable resistance to breathing, and was present whether or not the additional dead space was included. Figure 1 shows the experimental arrangement schematically.

Inclusion or Exclusion of the Additional Dead Space

A three-way tap was used to direct warm humidified air, at a constant rate of 200 l/min, through the additional dead space or into the atmosphere. Diversion of airflow through the dead space ensured that the expire of the previous breath was completely cleared before the following inspiration; in this case, the additional dead space was “functionally” excluded. When the airflow was diverted to the atmosphere, each inspirate included ~600 ml of expired air.

Before commencement of exercise, the three-way tap was always set to exclude the dead space. At exercise onset, the three-way tap was turned to include the dead space (“paired” trials) or “sham” turned (i.e., left in its original position) to continue to exclude the dead space (“unpaired” trials).

Cardiorespiratory Measurements

The pneumotachograph provided continuous measurements of respiratory airflow from which respiratory frequency (fR), tidal volume (VT), and minute ventilation (VE) were derived. All values were computed using an automated exercise analysis system (Ergostar, Fenyes and Gut, Basel, Switzerland). End-tidal PCO2 (PETCO2) was measured using a rapidly responding infrared analyzer (model LB2, Beckman Instruments, Fullerton, CA); this measurement was corrected to provide an estimate of PaCO2 on exercise (15). Heart rate (fC) was derived from a three-lead electrocardiogram (CASE, Marquette Electronics, Milwaukee, WI). Arterial O2 saturation (SpO2) was monitored continuously by using a pulse oximeter (Biox 3700, Ohmeda) with an ear probe. Variables were recorded as 30-s averages. Analog signals were recorded on an FM tape recorder (STORE 4, Racal, Hythe, Hampshire, UK) and simultaneously on a chart recorder (Mingograph 800, Siemens-Elema).

Protocol

Subjects spent ~3 h in the laboratory on three separate days (sessions 1, 2, and 3). In seven subjects ≤1 wk separated sessions 2 and 3. Three subjects completed session 3 within 3 wk of session 2; the remaining two did so within 7 wk.
Session 1. During session 1, each subject performed three exercise runs. Throughout session 1, the three-way tap was positioned to exclude the additional dead space. The purpose of this session was to familiarize the subjects with the exercise and test environment.

Session 2. During session 2, subjects completed 8–11 runs. This was the number of exercise runs that they could comfortably manage to complete. The subjects were randomly assigned to group A (n = 6) or group B (n = 6). For group A subjects, this session was the conditioning session in which an unpaired first ("control") run was separated from an identical unpaired last ("test") run by several consecutive paired runs. For group B subjects, this session was the nonconditioning session in which the unpaired control and test runs were separated by several consecutive unpaired runs. The number of paired or unpaired runs separating the test and control runs depended on each subject indicating when they were prepared to perform only a few more exercise runs. However, subjects were unaware of which run would be the last.

Session 3. Session 3 was identical to session 2, except group B subjects now performed the conditioning protocol and group A subjects performed the nonconditioning protocol. Session 3 was terminated after the same total number of runs had been performed as in session 2. Thus the total number of runs performed during the conditioning and nonconditioning sessions was the same within subjects but not necessarily between subjects.

Data Analysis

Only data from sessions 2 and 3 were analyzed. Univariate, full-factorial, repeated-measures analyses of variance (ANOVA) and analyses of covariance (ANCOVA) were performed using SPSS statistical software (release 6.1). Statistical significance was set at P = 0.05. For analyses involving within factors with more than two levels, significant P values were subjected to Greenhouse-Geisser correction; corrected P values are reported. Where significant main effects or interactions of interest were evident, a Fisher’s least significance difference (LSD) statistic (P < 0.05) was calculated to compare specific mean levels.

Test for associative learning effects in the steady-state phase of exercise. For each variable, the exercise steady-state levels (derived from the mean of the last four 30-s average measurements for each run) during each of the test and control runs of the conditioning and nonconditioning sessions were compared using ANOVA with two within and one between factor. The within factors were session (conditioning or nonconditioning) and run (test or control). A significant run by session interaction was required for evidence of an associative learning effect. The between factor was group (A or B). In the event of a significant group effect, the ANOVA was repeated for groups A and B individually. An additional ANOVA was performed on test-control run changes in PETCO2 with gender as a between factor and also by analyzing data from men and women separately.

Test for associative learning effects in the dynamic phase of exercise. To test for associative learning effects in the dynamic phase of the exercise ventilatory response, the above analysis was repeated on the full exercise data set (i.e., each consecutive 30-s average data point) by inclusion of time point as a third within factor (12 levels).

Test for associative learning effects in the exercise on-transient phase. To test for associative learning effects in the exercise on-transient phase, every breath in the 30-s period before and after the onset of exercise during each test and control run was measured from calibrated analog traces. These data were computed to provide breath-by-breath measurements of VE, fiO2, VT, inspiratory time, expiratory time, and PETCO2 that were averaged for all breaths in each 5-s time bin. For each variable, ANCOVA was performed with three within and one between factor. Within factors were session (conditioning or nonconditioning), run (control or test), and time (each 5-s time bin in the 30 s after exercise onset). The between factor was group (A or B). In the event of a significant group effect, ANCOVA was repeated for groups A and B individually. The mean level over six time bins before exercise onset served as the covariate for each run.

RESULTS

Effects of Repeated Paired and Repeated Unpaired Runs on Subsequent Exercise Responses

Exercise steady-state phase. Mean steady-state exercise PETCO2 was lower during test than during control runs by 1.8 ± 2.6 Torr in the conditioning session and by 1.4 ± 1.5 Torr in the nonconditioning session (Fig. 2, top). This was the only variable showing a significant test-control run difference (ANOVA showed a main effect of run: F1,10 = 14.8, P = 0.003). The size of this difference did not depend on whether paired or unpaired runs separated the test and control runs (ANOVA did not detect a significant run-session interaction) but was greater during the session performed first, irrespective of whether this was a conditioning or a nonconditioning session. Thus inspection of Fig. 2 reveals that group A (which performed the conditioning session first) demonstrated greater test-control run differences during the conditioning session, whereas group B showed greater test-control run differences during the nonconditioning session (ANOVA identified a significant group effect on the run-session interaction: F1,10 = 20.7, P = 0.001).

Women maintained lower PETCO2 during steady-state exercise: 37.6 ± 1.0 (SD) Torr during test and control runs compared with 41.4 ± 2.1 Torr for men (ANOVA indicated a significant gender effect: F1,10 = 13.5, P = 0.004). When men and women were analyzed separately, test-control run differences in PETCO2 and VE were significant in men only (ANOVA indicated a main effect of run: for PETCO2, F1,6 = 19.4, P = 0.005; for VE, F1,6 = 9.5, P = 0.022). Even in this subgroup of men, test-control run differences did not depend on whether paired or unpaired runs separated the test and control runs (ANOVA did not detect significant session or run-session interaction).
Table 1 shows the mean steady-state $P_{\text{ETCO}_2}$ arranged in the chronological order of exercise performance, irrespective of session type; a LSD of 1.2 Torr indicates a significant change with order number that is solely due to a reduction between control and test runs of session 1. The median number of days separating the experimental sessions was 6 (range 0–49 days).

### Table 1. Chronologically ordered control and test runs

<table>
<thead>
<tr>
<th>Order of Performance</th>
<th>Session No.</th>
<th>Run</th>
<th>$P_{\text{ETCO}_2}$, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Control</td>
<td>41.5 ± 3.4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Test</td>
<td>38.6 ± 3.4</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Control</td>
<td>39.8 ± 2.5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Test</td>
<td>39.0 ± 2.5</td>
</tr>
</tbody>
</table>

Values are means ± SD in 12 subjects. Steady-state exercise end-tidal $P_{\text{CO}_2}$ ($P_{\text{ETCO}_2}$) was measured during control and test runs of sessions 1 and 2. Means are calculated irrespective of type of session (conditioning or nonconditioning). ANOVA revealed a significant order effect ($P = 0.003$). A least significant difference of 1.2 Torr indicated that the significant change with order number was solely due to a reduction between control and test runs of session 1.

The test-control run change in $P_{\text{ETCO}_2}$ during session 2 (relative to session 1) was unrelated to the number of days separating experimental sessions; no significant linear regressions were observed across subjects of group A, group B, or all subjects combined.

**Dynamic Phase of the Exercise Response**

The dynamic profile of consecutive 30-s average measurements of $P_{\text{ETCO}_2}$ (but not of any other variable) from onset to steady state of exercise was significantly different between test and control runs (ANOVA indicated a run-time interaction: $F_{11,110} = 3.66, P = 0.02, \epsilon = 0.28$). This test-control run difference was also not specifically associated with conditioning sessions (ANOVA did not detect main or interaction effects involving the session factor for any variable). No clear differences were apparent between conditioning and nonconditioning control runs or between conditioning and nonconditioning test runs (Fig. 3).

**Transient responses at exercise onset.** Figure 4 shows the transient response at exercise onset for ventilatory variables averaged across all subjects separately for
test and control runs of the conditioning and nonconditioning sessions. The only significant difference between test and control runs within the first 30 s after exercise onset was that subjects breathed faster and less deep during test runs (ANCOVA detected main effects of run: for $f_R$, $F_{1,9} = 6.48, P = 0.031$; for $V_T$, $F_{1,9} = 6.84, P = 0.028$). This test-control run difference in pattern of breathing in the exercise-on-transient phase was also not specifically associated with the conditioning session (ANCOVA revealed no significant run session interaction for any variable).

The session performed first, irrespective of session type, resulted in greater test-control run differences for $V_E$ in the 30 s immediately after exercise onset (Fig. 5). Thus a greater test-control run difference was observed during the conditioning session for group A and during the nonconditioning session for group B (ANCOVA identified this as a significant group effect on the run-session interaction for $V_E$: $F_{1,9} = 8.75, P = 0.016$).

**Effectiveness of the Extra Dead Space Stimulus Across Repeated Exercise Runs**

Figure 6 shows mean ($n = 12$) steady-state levels of cardiorespiratory variables during each of the first seven runs of the conditioning and nonconditioning sessions. Steady-state exercise levels of $V_E$, $f_R$, and $P_{ETCO_2}$, during runs in which exercise was paired with added dead space were $10.6 \pm 6$ l/min, $2.1 \pm 0.5$ /min, and $1.5 \pm 2$ Torr higher, respectively, than corresponding unpaired runs with no added dead space (ANCOVA indicated main effects of session: for $V_E$, $F_{1,9} = 46, P < 0.001$; for $f_R$, $F_{1,9} = 5.18, P = 0.049$; for $P_{ETCO_2}$, $F_{1,9} = 5.17, P = 0.049$). Differences in mean steady-state $V_T$, $f_C$, and $SPO_2$ during paired and unpaired runs did not achieve significance.

Successive exercise runs were associated with a tendency for steady-state exercise $V_T$ and $P_{ETCO_2}$ to decline and $f_R$ to rise (ANCOVA indicated a main effect of run number: for $f_R$, $F_{5,45} = 4.49, P = 0.02, \epsilon = 0.48$; for $V_T$, $F_{5,45} = 5.10, P = 0.01, \epsilon = 0.50$; for $P_{ETCO_2}$, $F_{5,45} = 6.05, P = 0.001, \epsilon = 0.71$). The tendency for steady-state exercise $P_{ETCO_2}$ to fall with successive runs was primarily a feature of repeated paired runs, rather than repeated unpaired runs (Fig. 6); this run-session interaction just failed to remain significant after Greenhouse-Geisser correction ($F_{5,45} = 2.67, P = 0.07$).
DISCUSSION

The present study finds no evidence for associative learning of the ventilatory response to exercise in humans. Although repeated exercise paired with breathing additional dead space generated changes in subsequent exercise steady-state PETCO2, as well as changes in pattern of breathing at exercise onset, these changes were not specifically associated with the conditioning session. Repeated exercise alone performed by the same subjects on a separate day (the nonconditioning session) generated equivalent changes.

**Interpretation of Changes in Steady-State Exercise PETCO2**

PETCO2 during steady-state exercise was lower during test than during control runs. Although these changes occurred in the absence of measurable changes in steady-state exercise ventilation, it is possible that they reflect changes in alveolar ventilation brought about by altered breathing pattern. However, the ventilatory response during test runs was associated with a tendency to breathe faster with lower VT, a tendency that (although it failed to achieve significance) would promote a lower alveolar ventilation, contrary to that indicated by a lower PETCO2. This inconsistency could be explained, in part, by inadequate correction of exercise PETCO2 in estimation of PaCO2. Other explanations include the possibility that changes in CO2 excretion or physiological dead space had occurred after repeated exercise bouts. Measurements of mixed expired CO2 concentration would have provided useful information on these points, but the design of the breathing circuit in the present study did not allow this to be measured.

**Appraisal of Present Findings With Respect to Previous Reports**

The greatest reductions in steady-state exercise PETCO2 occurred after conditioning trials of session 1 irrespective of whether these trials were paired or unpaired. Our preliminary report (2) had misinterpreted this as a greater propensity for associative learning in those subjects (group A) who experienced fewer unpaired tests before paired trials. The present analysis reveals that test-control differences were equally more pronounced during session 1 performed irrespective of whether this was the conditioning session (in group A) or the nonconditioning session (in group B).

The study in awake goats (19) that prompted our study in humans benefited from direct measurements of PaCO2, eliminating concerns regarding the precision with which PETCO2 can estimate PaCO2 in the face of changing expiratory time. The goats performed twice as many exercise trials as we were able to implement in humans, and the authors ensured that potentially deconditioning extraneous activity between study days was avoided. An associatively learned response was purported on the basis of a larger rest-to-steady-state

Fig. 6. Steady-state levels during successive exercise runs. Steady-state exercise levels of VE, fE, VT, fT, and PETCO2 were measured during the first 7 successive 6-min exercise runs of the conditioning [unpaired (▲) and paired (●)] and nonconditioning sessions [all unpaired (○)]. Each data point is the mean of 4 successive 30-s measurements (last 2 min of each run) averaged across 12 subjects.

*Difference between corresponding conditioning and nonconditioning data points exceeds Fisher's least significance difference statistic derived from analysis of covariance.

ε = 0.70). The LSD statistic indicated that, by run 4, steady-state exercise PETCO2 was not significantly different between paired and unpaired conditions. Steady-state fT and Spo2 did not change significantly with repeated exercise.

Male and female subjects responded differently to the initial paired runs. Although both sexes produced significantly higher VE during paired runs than during unpaired runs (ANCOVA indicated a significant session effect: for men, F1,5 = 35.0, P = 0.002; for women, F1,3 = 15.6, P = 0.03), in men the initial increase was inadequate to maintain PETCO2 at unpaired run levels.

**Subjective Comments**

When subjects indicated that they were ready to terminate a session after a few more runs, this was due to “boredom” or a need to attend to other commitments; excessive fatigue was never indicated. After completion of the entire study, none of the subjects indicated, by volunteered comment or in response to a specific query, that they had been aware of any differences between exercise runs in terms of workload or changes in breathing circuit.
exercise decreases in \(P_{\text{ETCO}_2}\) (and increase in inspiratory ventilation) during the 1- to 6-h period after training compared with pretraining baseline levels, an effect that was repeatable in the same goats months later. A lesser effect was reported when a different group of goats was subjected to repeated exercise alone (34).

Changes from rest to steady-state exercise in our data are summarized in Table 2 for a more direct comparison with the analytic approach in the goat study (9) discussed above. Significant test-control run differences are evident for \(P_{\text{ETCO}_2}\), specifically during the conditioning session in group A. Unlike the study in goats (9), there is no accompanying increase in rest-exercise change in ventilation for any condition. We see a decrease in rest-exercise \(V_T\) for the test run of the conditioning session that is significantly less than that for the control run of the same session or that for the test run of the nonconditioning session. Other studies in humans employing pairings of exercise with dead space (33) or with inspiratory resistive load (30) report small “postconditioning” changes in rest-exercise \(P_{\text{ETCO}_2}\) differences (estimated from \(P_{\text{ETCO}_2}\)), but there is little indication in these preliminary reports of accompanying changes in rest-exercise ventilation. Another recent study in humans that examined changes from a low exercise to a moderate level of exercise (below lactate threshold) found no changes in \(V_{E}, V_{CO_2}\) (\(CO_2\) output), or \(V_{E}-V_{CO_2}\) responses before and after trials paired with extra dead space (7).

The present study is unique, in that the control run paired trials and test run were accomplished on the same day. In virtually all other attempts to generate associative conditioning of exercise hyperpnea, baseline responses were recorded on a separate occasion before training sessions. Although there are potential concerns with both approaches, we reasoned that nonspecific between-day variability would be more difficult to control than a nonspecific within-day variability. Thus we believed that the nonconditioning session provided an adequate within-subject control. The initial response of the day accounted for any differences between control and test runs, irrespective of the type of session.

**No Evidence of Associative Learning in the Transient Ventilatory Response to Exercise**

Our initial expectation was that conditioning effects would occur more readily at exercise onset, since phase I (first 10–15 s) of the exercise ventilatory response in humans is most likely manifested by central neural drive (36). In the present study, the increase in \(f_R\) was accelerated with compensatory deceleration of the increase in \(V_T\) in the first 30 s of exercise onset of the test runs. As with the reduction in postconditioning steady-state \(P_{\text{ETCO}_2}\), these trends were not specific to the conditioning session and cannot be attributed to an associative learning process.

Long-term modulation of transient ventilatory responses to exercise in humans has been reported by Helbling et al. (12) after repeated pairing of exercise with dead space loading. Control data were derived from a separate group of subjects who experienced different test trials as well as different training trials, making interpretation of findings more complex. Although more recent preliminary reports have appeared that claim similar effects with inspiratory resistive loading (14, 38), it remains to be seen how well these studies were controlled to account for nonspecific effects. It is also notable that the same dead space-conditioning paradigm employed in conscious goats (19) was unable to detect long-term modulation of ventilatory responses at exercise onset.

**Effectiveness of the Dead Space Load Employed**

The degree of “long-term modulation” that can be produced is purported to depend on an interaction between the degree of hypercapnia imposed by the dead space load and the number of conditioning trials (32). If it is assumed that this interaction is estimated from the initial hypercapnia generated (since this decreases with successive loadings), the present study could be criticized for employing too small a dead space. The absence of postconditioning changes has been reported, despite a large relative hypercapnia (~10 Torr) associated with the initial dead space load, but it is a preliminary report that requires verification (7). Our rationale for employing a relatively small dead space was that it should remain “imperceptible” to minimize volitional hyperventilation; the problem of human subjects voluntarily “contaminating” conditioned responses is of known concern (8).

**Table 2. Rest-to-exercise changes**

<table>
<thead>
<tr>
<th></th>
<th>Nonconditioning</th>
<th>Conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>(\Delta PETCO_2), Torr</td>
<td>6.5 ± 4.3</td>
<td>5.9 ± 3.8</td>
</tr>
<tr>
<td>(\Delta V_{E}, \text{l/min})</td>
<td>27 ± 10</td>
<td>27 ± 11.5</td>
</tr>
<tr>
<td>(\Delta f_R), min⁻¹</td>
<td>7.6 ± 7.4</td>
<td>8.3 ± 7</td>
</tr>
<tr>
<td>(\Delta V_T), liters</td>
<td>1.08 ± 0.49</td>
<td>1.04 ± 0.62</td>
</tr>
<tr>
<td>(\Delta f_C), min⁻¹</td>
<td>41 ± 10</td>
<td>36 ± 11*</td>
</tr>
</tbody>
</table>

- **Group A (n = 6)**

<table>
<thead>
<tr>
<th></th>
<th>Nonconditioning</th>
<th>Conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>(\Delta PETCO_2), Torr</td>
<td>6.8 ± 5</td>
<td>6.2 ± 3.4</td>
</tr>
<tr>
<td>(\Delta V_{E}, \text{l/min})</td>
<td>30 ± 8.3</td>
<td>29 ± 12.1</td>
</tr>
<tr>
<td>(\Delta f_R), min⁻¹</td>
<td>10.2 ± 7.7</td>
<td>9.1 ± 5.4</td>
</tr>
<tr>
<td>(\Delta V_T), liters</td>
<td>1.15 ± 0.56</td>
<td>1.08 ± 0.49</td>
</tr>
<tr>
<td>(\Delta f_C), min⁻¹</td>
<td>47 ± 11</td>
<td>41 ± 12</td>
</tr>
</tbody>
</table>

- **Group B (n = 6)**

<table>
<thead>
<tr>
<th></th>
<th>Nonconditioning</th>
<th>Conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>(\Delta PETCO_2), Torr</td>
<td>6.2 ± 4.2</td>
<td>5.7 ± 4.8</td>
</tr>
<tr>
<td>(\Delta V_{E}, \text{l/min})</td>
<td>23.9 ± 12</td>
<td>24 ± 12.5</td>
</tr>
<tr>
<td>(\Delta f_R), min⁻¹</td>
<td>5.1 ± 6.7</td>
<td>7.5 ± 8.8</td>
</tr>
<tr>
<td>(\Delta V_T), liters</td>
<td>1.00 ± 0.45</td>
<td>1.00 ± 0.77</td>
</tr>
<tr>
<td>(\Delta f_C), min⁻¹</td>
<td>36 ± 6</td>
<td>32 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SD of the average change from rest to exercise for steady-state \(P_{\text{ETCO}_2}\), minute ventilation (\(V_{E}\)), tidal volume (\(V_T\)), respiratory frequency (\(f_R\)), and heart rate (\(f_C\)) during control and test runs of the conditioning and nonconditioning sessions. *Less than control of same session, P < 0.05; †Lower than nonconditioning test, P < 0.05, (paired t-tests).
Recently, Reed and Coates (24) reported that the ventilatory response for a given workload is significantly raised with concomitant reduction in $\dot{V}E$ after human subjects undergo several daily trials of a breathing task involving isocapnic voluntary hyperventilation at rest. Because this effect was not accountable to respiratory muscle training, which has been reported to reduce $\dot{V}E$ for a given level of exercise (6), the authors propose that this demonstrates a long-term modulation of exercise hyperpnea in humans that is independent of the prior experience of the exercise per se. This would suggest that conditioning trials involving an effective additional dead space (or a breathing task that functionally matches the effect of breathing on a large dead space) is more important than an explicit exercise input (or the need for pairing these inputs).

**Arguments for More Sophisticated Associative Learning Paradigms**

Classical conditioning paradigms are notoriously complex in implementation and interpretation (25). Before rejecting the hypothesis that exercise hyperpnea includes an important learned component, we must be satisfied that the conditioning tool was adequate.

Previous demonstrations of conditioned breathing reflexes in humans are rare and include acquisition of ventilatory responses at rest by the sound of a metronome (16) or an auditory tone (10) after repeated prior association with hypercapnic or hypoxic stimulation. These studies involved at least twice the number of conditioning trials as those completed by the subjects in the present study. The number of trials in the present study was limited by the number of 6-min moderate-intensity exercise runs subjects were willing to perform in a single day. Test and control runs were performed on the same day to avoid the need to restrict subject activity beyond the laboratory, which would have been difficult to achieve. More trials may have been possible by employing shorter durations, particularly for acquisition trials (i.e., it may not be necessary for the paired trials to last $>1$ min).

Mechanisms of plasticity in respiratory control after intermittent stimulation may operate over longer time frames [e.g., intermittent hypoxia may elicit long-term facilitation of respiratory motor output persisting for several hours (22)]. Associative learning effects not evident immediately after acquisition trials (as in the present study) may therefore emerge later. A test run $\geq 1$ h after acquisition trials should be considered in future studies.

If exercise hyperpnea is partly an existing learned response, it is likely to have been forged over decades of experience (in adult subjects); it is more difficult to modify an existing response than to demonstrate emergence of a new one (26). Individuals with extensive recent experience of exercise (and, so, more physically fit) may therefore have been less susceptible to our associative learning protocol. Individual fitness levels were not accounted for in the present study (e.g., by exercising subjects at the same fraction of their maximal $O_2$ uptake). The intense level of exercise required for measurement of maximal $O_2$ uptake would have added substantially to the experience of laboratory exercise by subjects before experimental sessions. However, individual percent predicted maximum heart rates achieved during the control (first) run of the first experimental session did not correlate with test-control changes in exercise $\dot{V}E$ of the conditioning session (our only putative associative learning effect). This suggests that accounting for individual fitness levels would not by itself have significantly influenced the outcome. The range of individual percent predicted maximum heart rates (55–84%) will reflect differences in fitness levels if it is assumed that treadmill exercise at a fixed speed and gradient (with slightly reduced speed for female subjects) imposes a similar relative workload on all subjects.

Existing conditioned responses may be more susceptible to modulation by “operant” (or “instrumental”) paradigms that reinforce or inhibit responses by strategic presentation of rewarding or punishing sensations; such paradigms are more applicable in humans (18). For example, the use of a conditioning stimulus that manipulates breathlessness (an innate “punishing” sensation during exercise) may prove to be a more powerful means of eliciting long-term modulation of exercise hyperpnea in humans. To modify the conditioned reflex, it may be necessary to increase the potency of the additional dead space drive to breathe to an optimal level, which allows the formulation of a behavioral index of the stimulus without generating overt volitional hyperventilation.

**Other Forms of Adaptation in the Present Study**

Adaptation to repeated dead space loading. Short-term potentiation (STP) of the exercise ventilatory response may explain how the slope of the rest-exercise $PaCO_2$ relationship remains the same in the presence of additional respiratory dead space in goats (21). This within-trial STP is believed to occur via a serotonin receptor-dependent mechanism (3) acting at the level of spinal motoneurons (20) but may also depend on other factors (13). The $CO_2$ retention observed during the first paired trial in our male subjects, which is consistent with the results of Ward and Whipp (35), might suggest that STP of the exercise ventilatory response in humans initially fails to fully compensate for the added respiratory dead space. The gradual reduction of $CO_2$ retention with successive paired runs, a phenomenon also reported with a dead space load more than double that employed here (7), may reflect a long-term modulation that sharpens the STP mechanism across trials akin to the functional recovery observed in goats after thoracic dorsal rhizotomy (23, 32). This must remain uncertain, since additional dead space was never presented at rest in the present study. Alternatively, the decline in $\dot{V}E$ with successive exercise runs (with added dead space) may be an ex-
ample of a cerebrally mediated nonassociative adaptive modulation akin to habituation of the respiratory controller (11). Neither can involvement of basic physiological feedback factors, such as a build up of metabolic acidosis with repeated exercise, be entirely excluded from such habituation processes (31).

Putative gender effects. Gender hormones can modulate the serotonergic nervous system by changing extracellular serotonin levels in the central nervous system (5). Serotonergic mechanisms have been implicated in long-term modulation in goats (20, 29). Gender may therefore play a role in associative learning. Gender differences were detected in the present study, but because differences between conditioning and nonconditioning sessions were absent for both sexes, a gender effect cannot by itself account for the absence of associative learning in the present study. However, a more potent STP mechanism may have been active in our female subjects, since less CO2 retention was seen during the first paired run (see above). More female subjects and knowledge of the menstrual phase at the time of testing would have been useful in delineating these effects.

Adaptation to repeated exercise alone. Repeated exercise alone without added dead space resulted in a small decline in steady-state exercise PETCO2 between sessions 1 and 3 but was only apparent in group B, which performed the nonconditioning session first. Thus the test-control run difference for the nonconditioning session in this group matched the test-control run difference for the conditioning session in group A subjects (Fig. 2). Lower test run PETCO2 was a feature of the first experimental session performed and may represent a form of “learning,” or “anticipatory” drive, that is independent of associative stimuli and develops in initial exposures to laboratory exercise, a finding consistent with the fact that subjects were unfamiliar with exercise in a laboratory with a facemask before their first session.

Conclusions

The present results provide no support for the contention that the ventilatory response to exercise in humans is susceptible to long-term modulation by prior association of the exercise drive to breathe with an additional stimulus to breathe. Although the small dead space load used in this experiment is insufficient to elicit long-term modulation of the ventilatory response to exercise, the study by itself does not invalidate the learning theory of the exercise hyperpnea. Further studies are necessary to determine whether a putative learned component may become apparent with greater dead space loading and more sophisticated, presumably more complex, conditioning paradigms.

The authors thank the subjects for their time and cooperation and Drs. R. Banzett, J. Reed, E. Bloch Salisbury, and A. Binks for helpful comments on interpretation of the data and drafting of the manuscript. Dr. K. MaCrae provided much needed statistical assistance.

S. H. Moosavi was supported by a Wellcome Trust Program grant awarded to A. Guz.

REFERENCES


