Material and methods

Sleep recording

Conventional polysomnographic recordings were completed according to published recommendations [12]. They consisted of in-lab continuous acquisition of electroencephalogram, electrooculogram, submental electromyogram, arterial oxyhemoglobin saturation by transcutaneous pulse oxymetry, naso-oral airflow with thermistor, nasal pressure with nasal canula, chest and abdominal movements by impedence plethysmography (Respitrace™, Ambulatory Monitoring Inc., Ardsley, NY), electrocardiogram, and breath sounds. Sleep position was continuously assessed by the attending technician with an infrared camera. All variables were digitally recorded (Sandman Elite™ System, Mallinckrodt, Kenilworth, NJ).

Measurement of UA pressures

Silver cup electrodes were placed on the mid-clavicular line in the seven to eight right and left intercostal spaces for surface recording of the right and left costal diaphragmatic EMG activities (Biopac System/Biopac, Santa Barbara, CA). Pressure and flow recordings were completed as previously described [8-11]. After local anesthesia (xylocaine 2 % spray) of one nostril, a pressure-tipped catheter (model CT/S X1058, Gaeltec, Hackensack, NJ) was passed through one nostril and its position was marked when its tip was 2 cm below the soft palate and 4 cm down in the oropharynx (figure 1). A plastic nasal stent was placed in the anterior nares to prevent nasal collapse. A continuous positive airway pressure nasal mask was then placed over the nose with the catheter passing through one drilled hole. Occlusion of the mask opening during maximal

inspiratory efforts was used to assess its airtightness. A second catheter was passed through another opening of the mask to measure pressure inside the mask. A pneumotachograph (model 112467-3850A, Hans Rudolf, Kansas City, MO) connected to the mask was used to obtain instantaneaous flow. Pressures and flow were digitally recorded at 2000 Hz sample rate (Digidata 1320, Axon Instrument, Forster City, CA). Subjects were seated in a comfortable armchair with a 60 degree inclination. Measurements were obtained during exclusive nasal breathing.

Phrenic nerve stimulation procedure

BAMPS was performed with two Magstim 200 stimulators (Magstim, Whitland, Dyfed, UK), connected to two 90º handle 45-mm eight-shaped coils, according to previously described technique [8 – 11, 13]. Each stimulating coil was positioned anterolaterally over the anatomical landmark of the phrenic nerve in the neck by a high precision multipositional support consisting of two articulated arms (MAN 143; Manfrotto Trading, Bassano del Grappa, Italy) (figure 1). The optimal position and orientation of the coils was determined separately for each side. The two stimulators were triggered by a timer driven by the changes in flow direction, the 1 ms twitches being delivered 2 seconds after expiratory onset. BAMPS intensity was individually set at a power output sufficient to induce clear flow-limited twitches as well as at a 20 % higher intensity.

Analysis

Sleep and respiratory variables were scored according to standard criteria [12]. All EMG, flow and pressure tracings were recorded on a microcomputer (Axoscope software 9.0;

Axon Instruments). Twitch-induced breaths were considered flow-limited when instantaneous flow ($\dot{V}_I$) plateaued or decreased despite a persistent increase in driving pressure (P_d). A P_d versus $\dot{V}_I$ relationship including flow value ranging from 0 to $\dot{V}_{\mathrm{Im}in}$ allowed to model the UA dynamic response of flow-limited twitches with a polynomial regression model of the form $\dot{V}_I = k_1 P_d + k_2 P_d^2$ as previously described [8 -11]. Solving the last equation for $\dot{V}_I = 0$ results in $P_d = -k_1/k_2$ and provides a value of the driving pressure at which the UA will be closed. As a consequence, the k_1/k_2 ratio describes UA closing pressure (the less negative the k_1/k_2, the higher the UA collapsibility). Polynomial model fitting and determinations of k_1 and k_2 values were performed semi-automatically using custom-made software (JMP 7.0; SAS Institute, Cary, NC).