Online Supplement for:

TERMINATION OF RESPIRATORY EVENTS WITH AND WITHOUT CORTICAL AROUSAL IN OBSTRUCTIVE SLEEP APNEA.

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METHODS

Subjects:

20 CPAP-treated (>3 months) patients with OSA were recruited as part of a large study that aims to characterize the pathophysiologic phenotypes of OSA. The main study involves 3 over-night sleep studies performed in a random order: 1) a standard clinical polysomnography, 2) a study of respiratory control and 3) a detailed muscle/airway physiology study. Only data from the physiology study night were used for this analysis. No subject smoked and all subjects were free of neurologic, cardio-respiratory and sleep disorders other than OSA. The sample size of 20 subjects was based on prior arousal studies which showed 10-14 subjects were required for statistical significance in within subjects designs (E1, E2).

Measurements and Instrumentation: Subjects arrived at the laboratory approximately 2 hours before their usual bedtime having not eaten for 4 hours. After a medical history and physical examination subjects were instrumented with 2 EEG (C3-A1, Oz-A2), left and right EOG and submental EMG (EMG_{SUB}) electrodes for measurement of sleep stage and detection of arousals. Following this, a nasal decongestant (0.05% oxymetazoline HCl) was delivered to both nostrils and the more patent nostril was anaesthetised (4% Lidocaine HCl). A pressure tipped catheter (MCP-500, Millar, Houston, TX) was then advanced through the anaesthetized nostril until it was located near the epiglottis (1.5-2cm below the base of the tongue) for epiglottic airway pressure measurement (P_{EPI}). Local anaesthetic was then applied under the tongue and to the soft palate near the pterygoid hamulus. Once surface anaesthesia to light touch was achieved, fine wire EMG electrodes (AS 765-36 O.D. 71μm; Cooner Wire Company, Chatsworth, CA with 2mm Teflon removed from the

tip, inside 25 Gauge needles) were inserted into the body of the genioglossus and tensor palatini muscles. Genioglossal wire electrodes were placed per-orally, 3-4 mm lateral to the frenulum and to a depth of approximately 15mm. Tensor palatini electrodes were inserted approximately 2mm posterior to the pterygoid hamulus, at a 45° angle along the lateral surface of the medial pterygoid plate, to a depth of approximately 12mm. The two wires in each muscle were referenced to a surface ground electrode to produce a bipolar EMG recording. Both EMGs were amplified and filtered (30-1000Hz, CP511 Grass Instruments, West Warwick, RI).

The subject then lay supine on a bed and a nasal mask (Gel Mask, Respironics, Murrysville, PA) with pneumotachograph (model 3700A, Hans Rudolf Inc, Kansas City, MO) and differential pressure transducer (Validyne Corp., Northbridge, CA) was applied for measurement of airflow. End-tidal CO_2 ($PETCO_2$) was monitored at the nares (Capnograph monitor, BCI, Waukesha, WI) and mask pressure (P_{MASK}) recorded (Validyne Corp., Northbridge, CA).

EEG, EOG, EMG_{SUB}, ECG and arterial oxygen saturation (SaO_2) data were recorded on a Nihon Khoden (Tokyo, Japan) digital recording system and passed in real time to a Spike 2 data acquisition system (1401*plus*, CED, Cambridge, UK) on which all signals were recorded. Genioglossus and tensor palatini EMG's were recorded at 1000Hz; EEG, EOG and submental EMG at 250Hz; and all other signals at 125Hz.

Protocol: When subjects were comfortable, they performed several manoeuvres 3 times each to allow maximum EMG activity to be recorded. The manoeuvres included swallowing, taking deep breaths, protruding the tongue against the teeth as hard as

possible, and sniffing. The EMGs were then rectified, averaged with a moving time window of 100ms and scaled between maximum activity and electrical zero. 5 minutes of resting wakefulness was then recorded before the patient was placed on CPAP at their prescribed level. A further 5 minutes of resting wakefulness was recorded before the lights were turned off and the subject was allowed to fall asleep.

Once stable NREM sleep was achieved, the CPAP level was suddenly dropped during expiration to a lower level for 3 minutes unless a full awakening (>15s alpha) occurred, in which case the pressure was returned to the prescribed level (during inspiration) until stable sleep resumed. The amount of reduction in CPAP was adjusted such that a range of severities of respiratory events were induced. At least 3 minutes of sleep on the prescribed CPAP level separated CPAP drops. Pressure drops continued in all sleep stages until the subject could no longer sleep or approximately 40 drops had been performed, after which all equipment was removed and the subject was allowed to go home or sleep in the laboratory without the equipment until morning.

Data and Statistical Analysis: A trained sleep technician, blinded to the study hypotheses, staged the sleep and marked arousals according to the Rechtschaffen and Kales (20) and ASDA criteria (1). The technician was instructed to pay close attention to the timing of arousals and to mark start and end times of arousals as accurately as possible. Custom written, semi-automated software was used to extract the physiologic information on each breath for 1 minute before, during and 1 minute after every CPAP drop in NREM sleep (REM was not analysed due to marked breathing variability and missing data in some subjects). The specific variables extracted

included breath timing, inspired tidal volume (V_T), minute ventilation (V_I), mean (V_T/T_I) and peak inspiratory flow (PIF), mean arterial blood oxygen saturation (SaO_2), P_{MASK}, inspiratory P_{EPI} nadir, plus peak and tonic EMG activity of the genioglossus (EMG_{GG}) and tensor palatini (EMG_{TP}) muscles.

Respiratory events were defined as a period of flow limitation that ended with a sudden increase in airflow. Objective criteria were used to identify the breath on which a respiratory event was terminated. Specifically, the PIF on the breath that terminated the event was at least 50% larger than the breath immediately prior and at least 80% of the level observed on fully therapeutic CPAP (shown by arrows in Figure 1). Each event termination was then allocated to one of 3 types: ASDA Arousal, No ASDA Arousal or CPAP Increase according to the following criteria: If CPAP was constant and an ASDA arousal was scored within one respiratory cycle of the event termination, then the event was considered to be terminated with an ASDA arousal. If CPAP was constant but there was no scored arousal for at least 2 breaths after event termination, the event was designated a No ASDA Arousal type. Finally, if no arousal was scored for 2 breaths, but the increase in airflow occurred when CPAP was increased, then it was designated a CPAP Increase event. CPAP Increase events were examined as a control because of the ongoing debate regarding whether respiratory events that are terminated without clear cortical arousal actually have subtle arousals present or not. For this same reason heart rate changes at the termination of events were also assessed. Examples of each of these 3 event termination types in one subject are shown in Figure 1.

Physiologic data were averaged in each event type for 5 breaths before and after the termination of respiratory events as long as CPAP remained low (in ASDA Arousal and No Arousal events) and the subject remained asleep (for No ASDA Arousal and CPAP Increase trials). Due to the marked between subject variability in baseline activity of the genioglossus and tensor palatini muscles, each was expressed as a percent of that subjects mean activity across all analysed breaths. This was done such that baseline differences in muscle activity were not obscured. Heart rate data were compared for 20 beats before and after the termination of respiratory events. Cardiovascular and respiratory data were then compared between event termination types with a 3 way, repeated measures ANOVA (breath or heart beat number as the repeated factor). When significant main effects were found, Student's t-tests were used for post-hoc analyses.

Secondary respiratory events were considered to occur when a second period of flow limitation that was terminated by a sudden increase in PIF (same criteria for initial events) occurred during the 3 minutes of reduced CPAP (see Figure 4 top panel for an example). The proportion of ASDA Arousals and No Arousals that were followed by a second respiratory event were compared with a paired Student's t-test. Furthermore, the physiologic characteristics of secondary respiratory events were compared to initial events by comparing the last 2-3 breaths during the first respiratory event after sudden CPAP drop (when compensatory responses were most marked) to the 2-3 most flow limited breaths during secondary respiratory events (Figure 4). In all cases the breath immediately before the sudden flow increase that terminated the event was not analysed to avoid contamination by EMG increases associated with the arousal/event termination. The secondary events were then separated into whether an

ASDA Arousal or No Arousal was present between the initial and secondary events. 2-way repeated measures ANOVA were then used to compare respiratory data from initial and secondary respiratory events, and between ASDA Arousal and No Arousal events.

RESULTS

16 subjects had complete data. One subject did not have OSA (AHI=3.5), in 2 subjects CPAP was increased immediately following event termination in an attempt to optimise sleep/data for the main study and the final subject did not have any events terminated without ASDA arousal. The anthropometric data for the remaining 16 subjects are shown in Table 1. In addition, ECG analyses were excluded in two subjects, one of whom had a wandering atrial pacemaker and the other who had frequent premature ventricular contractions. The intramuscular TP EMG electrodes became dislodged at some point during the night in 3 subjects. As the exact time of dislodgment is unknown, TP data were discarded for the whole night in these subjects leaving 13 subjects for TP analyses.

Arterial oxygen saturation (SaO_2), duty cycle (T_I/T_{TOT}) and expiratory times (T_E) for different event types in all 16 subjects included in the main analysis are shown in Figure E1. Arterial oxygen saturation fell significantly during events ($p<0.001$) however there were only trends toward differences between event types and for interaction effects ($p=0.051$ and 0.069 respectively). Duty cycle was significantly lower for CPAP increase events than either ASDA Arousal or No Arousal events ($p<0.001$), but no interaction effect existed ($p=0.164$), indicating that over time duty

cycle changes similarly for all event types. Expiratory time was also significantly longer for CPAP increase events (p=0.002) but changed similarly over time in all 3 event types (p=0.5 for interaction effect).

Sub-analysis of events matched for severity and duration.

Respiratory events that were terminated with ASDA Arousal in the main analysis were more severely flow limited (lower ventilation and lower peak flows) and had more negative epiglottic pressures than events terminated with No Arousal. Thus, some of the differences observed after event termination in the main analysis may have been a result of the events being more severe prior to ASDA Arousal. To investigate this possibility we conducted a sub-analysis of ASDA Arousal and No Arousal events that were matched for severity (average ventilation during CPAP drop within 1L/min) and duration of event (number of breaths since CPAP drop within 2 breaths). The results of this analysis are presented in Figure 4 in the main paper as well as Figure E2 in this repository.

No significant ANOVA interaction effects existed for any variable indicating that the physiological changes were similar for both ASDA Arousal and No Arousal event types over the 10 breath period. However, a significant ANOVA main effect for arousal type was observed for TP activity (p=0.031) indicating that the activity of the TP was statistically higher when all breaths were averaged. This effect was due to TP having a more sustained increase after arousal.

REFERENCES

E1 Jordan AS, Eckert DJ, Catcheside PG, McEvoy RD. Ventilatory response to brief arousal from non-rapid eye movement sleep is greater in men than in women. Am J Respir Crit Care Med 2003;168:1512-1519.

E2 Jordan AS, McEvoy RD, Edwards JK, Schory K, Yang CK, Catcheside PG, Fogel RB, Malhotra A, White DP. The influence of gender and upper airway resistance on the ventilatory response to arousal in obstructive sleep apnoea in humans. J Physiol 2004;558:993-1004.

Figure E1

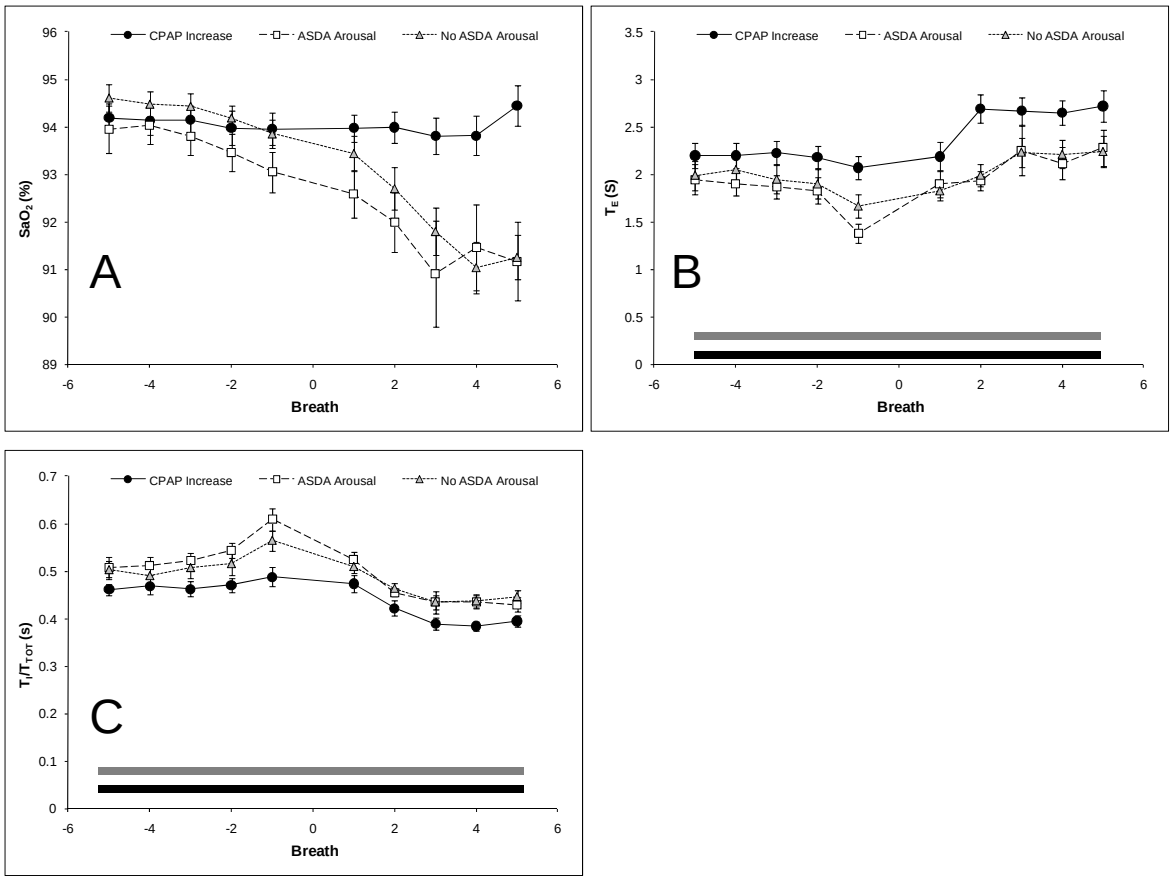

Figure E1: Physiologic changes following respiratory event termination with and without ASDA arousal from sleep or by sudden CPAP increase.

Arterial oxygen saturation (SaO_2), expiratory time (T_E) and duty cycle (T_I/T_{TOT}) for 5 breaths before and after respiratory event termination by suddenly increasing the continuous positive airway pressure (CPAP Increase), or with (ASDA Arousal) or without (No Arousal) arousal. See online repository text for ANOVA results. Post-hoc differences are shown by the bars.

- p<0.05 CPAP Increase versus No Arousal
- p<0.05 No Arousal versus ASDA Arousal
- p<0.05 CPAP Increase versus ASDA Arousal

Figure E2

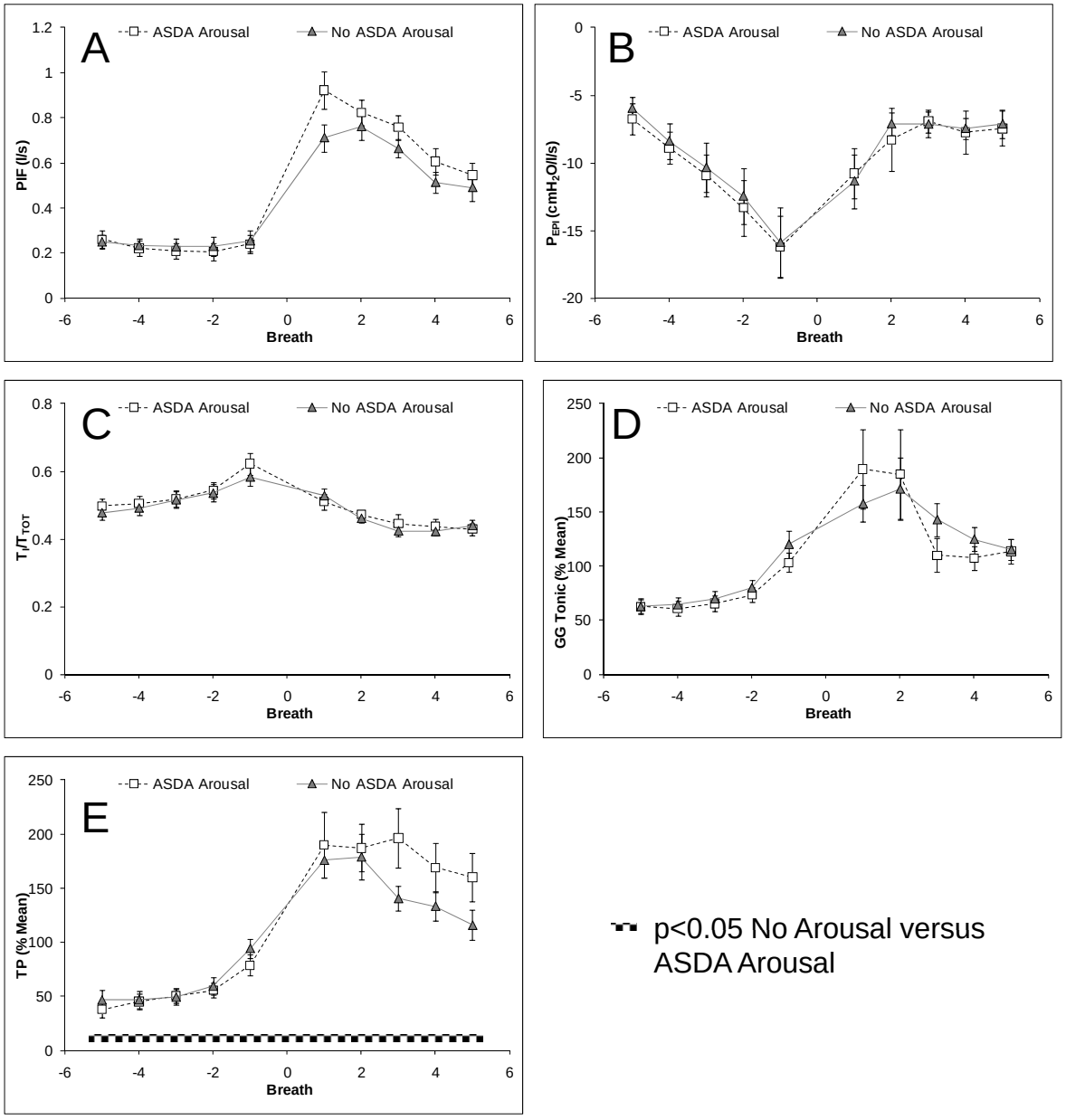

Figure E2: Physiologic changes following respiratory event termination with and without ASDA arousal from sleep in events matched for severity and duration of hypoventilation.

Peak inspiratory flow (PIF), epiglottic pressure (P_{EPI}), expiratory tonic genioglossus (GG) and tensor palatini (TP) muscle activity for 5 breaths before and after respiratory event termination with (ASDA Arousal) or without (No ASDA Arousal) arousal. No significant differences (ANOVA main or interaction effects) existed between arousal types for any variable except TP which was higher in ASDA Arousal than No Arousal (significant ANOVA main effect for arousal type). Ventilation and peak inspiratory GG responses are presented in Figure 4 of the main paper.