INFLUENCE OF AIRWAY PRESSURE ON GENIOGLOSSUS ACTIVITY DURING SLEEP IN NORMAL CHILDREN

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ON-LINE DATA SUPPLEMENT

METHODS

Subjects

The subjects were non-snoring, asymptomatic children recruited from community fliers. Exclusion criteria included serious medical conditions, previous adenotonsillectomy, or medications known to effect sleep. The study was approved by the institutional review board at Children's Hospital, Boston. Signed, informed consent was obtained from the parent, and assent from the child.

Protocol

Subjects underwent a single overnight polysomnogram in the supine position, including an intraoral EMG surface electrode. After stable sleep was established, the negative pressure challenge protocol was performed repeatedly in stage 2, stage 4, and REM sleep. Insofar as normal, non-snoring children have a low prevalence of obstructive sleep apnea, baseline polysomnographic data were only obtained from the non-experimental portions of the single night study.

Polysomnography

Each subject underwent an overnight polysomnogram in the supine position including continuous measurement of EMGgg using the intra-oral surface electrode described above. The following parameters were measured: electroencephalogram (C3-A2, O1-A2, F1-A2); right and left electrooculogram; submental electromyogram (EMG); tibial EMG;

surface diaphragmatic EMG; electrocardiogram; end-tidal CO_2 (Novametrix COSMO, Wallingford, CT); arterial oxygen saturation (Novametrix COSMO); and chest and abdominal wall motion (piezoelectric transducers). Mask pressure was measured with a Validyne pressure transducer (Validyne, Northridge, CA) and oronasal airflow with a thermistor. Signals were acquired using a Grass Instruments system (Gamma Software, Grass Instruments, Braintree, MA). Sleep architecture was assessed by standard techniques E1. An obstructive apnea was defined as the presence of chest/abdominal wall motion associated with a reduction of airflow ≥ 80% of baseline, lasting ≥ 2 breaths duration. An obstructive hypopnea was defined as a discernible reduction in airflow associated with either a drop in SpO_2 ≥ 4% or an arousal, lasting ≥ 2 breaths duration. The apnea/hypopnea index (AHI) was defined as the total number of obstructive apneas and hypopneas per hour of total sleep time. An AHI greater than 1 was considered abnormal.

Genioglossal electromyography (EMGgg)

A custom intra-oral mouthpiece electrode was constructed for each subject to measure EMGgg (E2;E3). An impression of the sublingual fossa and teeth was made with a dental-grade, vinyl silicone putty (Splash!; Wennigsen, Germany) using a mandibular tray (Diskus Dental; Munich, Germany) modified to allow impression material to contact the floor of the mouth. The mold is whittled to remove excessive bulk, yet to enclose the lower, front 4-5 teeth. Unipolar surface electrodes are sewn into the inferior surface of the mold using bared 30 gauge, teflon-coated stainless steel wire. The electrodes are situated 3 mm lateral to the frenulum on each side, between the alveolar ridge and the base of the tongue, and are fitted to rest comfortably against the base of the genioglossus. Both unipolar electrodes are referenced to a single forehead ground, thus producing a bipolar recording. The resulting EMG signal is acquired using a Grass Instruments Heritage system (Gamma Software, Grass Instruments, Braintree, MA) and transferred to Spike2 (version 5, Cambridge Electronic Design, Cambridge, UK) for analysis. The signals were amplified, rectified, and integrated on a moving-time-average basis, with a time constant of 200 ms. EMGgg data are presented as a percentage of the EMGgg observed during a maximal tongue thrust maneuver or as a percentage of the baseline sleep period before the negative pressure challenges.

Negative Pressure Challenges

Inspiratory flow was measured in response to negative pressure challenges using previously described techniques (E4;E5). Briefly, the subjects breathed through a nasal mask (Respironics, Pittsburgh, PA) which was attached to a pneumotachometer (Hans Rudolph, Kansas City, MO) and pressure transducer (Validyne Engineering, Northridge, CA). End-tidal PCO_2 was measured from a port in the mask using an infrared capnometer (Novametrix COSMO, Pittsburgh, PA). A chin strap ensured that the mouth remained closed. Pressure and flow signals were acquired simultaneously with a POWERLAB AD converter (Colorado Springs, CO) and GRASS Heritage system (Grass Telefactor, Braintree, MA), before being transferred to SPIKE (Cambridge Electronic Design, Cambridge, UK) analysis software.

The mask pressure was controlled between +3 and -22 cm H_2O using a machine specially modified by Respironics to alternate between positive and negative airway pressure. Subjects were placed on 3 cm H_2O of airway pressure at baseline and permitted to sleep. During the negative pressure challenges, the mask pressure was lowered in 2 cm H_2O increments for 5 breaths, followed by a return to baseline CPAP for 30 seconds. These pressure drops progressed until flow approached zero or a visible electrocortical arousal occurred.

Definitions

AUC EMGgg; area under the curve during inspiration of the genioglossal electromyogram moving time average (MTA). Flow-limited breaths; visible flattening of the inspiratory flow curve on the pneumotachograph tracing. Flow-response breaths; a sudden visible increase in flow during a breath of at least 25% or any breath with increased flow following a flow-limited breath at a particular airway pressure. Instantaneous minute ventilation; minute ventilation calculated on a breath-by-breath basis by multiplying the tidal volume by 60/(inspiratory time + expiratory time). Non-visible EEG arousals; increase in the total power of a particular frequency band (delta, theta, alpha, beta) above the 95 percentile of the baseline power during a 2.56 second interval centered at the start of each inspiration. Visible EEG arousal: a sudden shift in EEG frequency lasting at least 3 seconds beginning during inspiration or the preceding 1 second. Vmin; inspiratory flow during an initial flow-limited plateau.

Data Analysis

Data were evaluated during three intervals; a. Baseline – defined as the 2 - 3 minutes (or at least 40 breaths) prior to the start of the first negative pressure challenge; b.

Negative pressure challenges of progressively increased magnitude, each lasting 5 breaths; c. Post-challenge - the 20 seconds following the completion of the most negative of the pressure challenges not associated with an electrocortical arousal. Each breath during these intervals was analyzed for the following parameters; a. inspiratory EMGgg (peak, mean, AUC, plateau and the EMGgg value at point of flow response); b. Inspiratory flow (plateau, peak); c. Inspiratory time and expiratory time; and d. tidal volume and breath-by-breath minute ventilation. Curves of pressure-flow and pressure-EMGgg measures for challenges with flow-limited breaths (compensated breaths following flow-limited breaths were also included) were constructed using least squares linear regression. The slope of the pressure-flow and pressure-EMGgg curves and the absolute magnitudes of the previously described EMGgg measures were used to characterize the upper airway neuromuscular compensation.

The EEG power spectra ranges (delta 1 – 4 Hz, theta 4 – 8 Hz, alpha 8 – 12 Hz, and sigma/beta 12 – 20 Hz) were analyzed for each artifact-free interval (baseline, negative pressure challenge, post-challenge) using a 2.56-second analysis epoch centered at the start of inspiration. The mean of the forty 2.56-second epochs during the baseline interval was used to determine the control mean ± SD in each spectral range. A breath was defined as associated with a non-visible electrocortical arousal if the total power of any of the spectral ranges exceeded the 95th percentile of baseline. The analysis was performed separately in stage 2 and stage 4 sleep.

Statistical Analysis

Analysis of variance followed by Dunnett's test for multiple comparisons with a single control was used to analyze the following; a. Differences in the EMGgg and flow parameters between baseline and breaths 1 – 5; b. Differences between the EEG parameters at baseline compared to the negative pressure and post-challenge interval. Multiple regression was used to evaluate the AUC EMGgg and peak inspiratory flow (dependent variables) as a function of airway pressure, challenge breath number EEG arousal (none, delta, theta, alpha, beta) and sleep state (independent variables). Individual subjects were considered class variables.

Analysis of variance followed by Tukey's test for multiple comparisons was used to compare the slopes of the regression curves for the pressure-flow and pressure-EMGgg data between the individual breaths. A paired t-test was used to compare differences between sleep stages; Rank techniques were used for data that was not normally distributed (presented as median with range). Otherwise data are presented as mean ± SD. Differences were considered significant if the null hypothesis was rejected at $p < 0.05$. Statistical analyses were performed using SigmaStat (SPSS, Chicago, IL).

RESULTS

Baseline levels of EMGgg activity, air flow and respiratory timing are summarized in Table E1.

REFERENCES

(E1) Rechtschaffen A, Kales A. A manual of standardized terminology: Techniques and scoring systems for sleep stages of human subjects. 1968. Los Angeles, UCLA Brain Information Service/Brain Research Institute.

(E2) Katz ES, White DP. Genioglossus activity in children with obstructive sleep apnea during wakefulness and sleep onset. Am J Respir Crit Care Med 2003; 168:664-670.

(E3) Doble EA, Leiter JC, Knuth SL, Daubenspeck JA, Bartlett D. A noninvasive intraoral electromyographic electrode for genioglossus muscle. J Appl Physiol 1985; 58:1378-1382.

(E4) Marcus CL, Fernandes Do Prado LB, Lutz J, Katz ES, Black CA, Galster P, Carson KA. Developmental changes in upper airway dynamics. J Appl Phys 2004; 97:98-108.

(E5) Marcus CL, Katz ES, Lutz J, Black CA, Galster P, Carson KA. Upper airway dynamic responses in children with the obstructive sleep apnea syndrome. Pediatr Res 2005; 57:99-107.

FIGURE LEGENDS

Figure E1: Raw data segment approximately 10 minutes in length demonstrating two sequential negative pressure challenges during stage 4 sleep. There is no discernible EMGgg response during either challenge and a similar pattern of flow reduction with increasing negative pressures. This example is representative of the qualitative consistency of a given subject's response.

Figure E2: Raw data segment approximately 10 minutes in length demonstrating two sequential negative pressure challenges during stage 4 sleep. There is a discernible EMGgg response during both challenges and a similar pattern of flow reduction and augmentation with increasing negative pressures. This example is representative of the qualitative consistency of a given subject's response.

Figure E3: Raw data segment approximately 10 minutes in length demonstrating two sequential negative pressure challenges during stage 4 sleep. There is a marked EMGgg response during both challenges and a similar pattern of flow preservation with increasing negative pressures. This example is representative of the qualitative consistency of a given subject's response.

Table E1 EMGgg and Respiratory Parameters at Baseline and Post-Negative Pressure Challenges

	Baseline	Post-Challenge			
		0 – 5 secs	5 – 10 secs	10 – 15 secs	15 – 20 secs
AUC Inspiratory EMGgg (percentage of wakeful maximum)					
Stage 2	1.3 ± 0.7	2.3 ± 1.2 *	2.0 ± 1.1*	1.9 ± 0.9*	1.9 ± 1.0*
Stage 4	1.1 ± 0.7	2.0 ± 0.9 *	2.0 ± 1.0*	1.8 ± 0.8*	1.8 ± 0.7*
Inspiratory Time (seconds)					
Stage 2	1.6 ± 0.2	1.3 ± 0.1 *	1.4 ± 0.1*	1.6 ± 0.2	1.7 ± 0.2
Stage 4	1.7 ± 0.2	1.4 ± 0.1*	1.6 ± 0.2	1.7 ± 0.2	1.8 ± 0.2
Expiratory Time (seconds)					
Stage 2	2.2 ± 0.3	1.45 ± 0.2 *	1.62 ± 0.2*	1.71 ± 0.2*	2.0 ± 0.3
Stage 4	2.4 ± 0.3	1.6 ± 0.1 *	1.92 ± 0.2*	2.03 ± 0.3	2.25 ± 0.3
Flow (ml/sec)					
Stage 2	221 ± 31	251 ± 33 *	248 ± 36*	233 ± 29	227 ± 34
Stage 4	209 ± 19	243 ± 26*	229 ± 31	217 ± 24	215 ± 22
Heart Rate (beats/ min)					
Stage 2	65 ± 6	68 ± 8	66 ± 8	66 ± 7	64 ± 7
Stage 4	63 ± 5	67 ± 6	64 ± 7	63 ± 6	62 ± 7

Definitions of abbreviations: EEG = Electroencephalogram; EMGgg = Genioglossus Electromyogram; AUC = Area under the curve. Data are presented as mean ±SD unless otherwise specified. * Indicates a significant difference compared to baseline, $p<0.05$

Figure E1

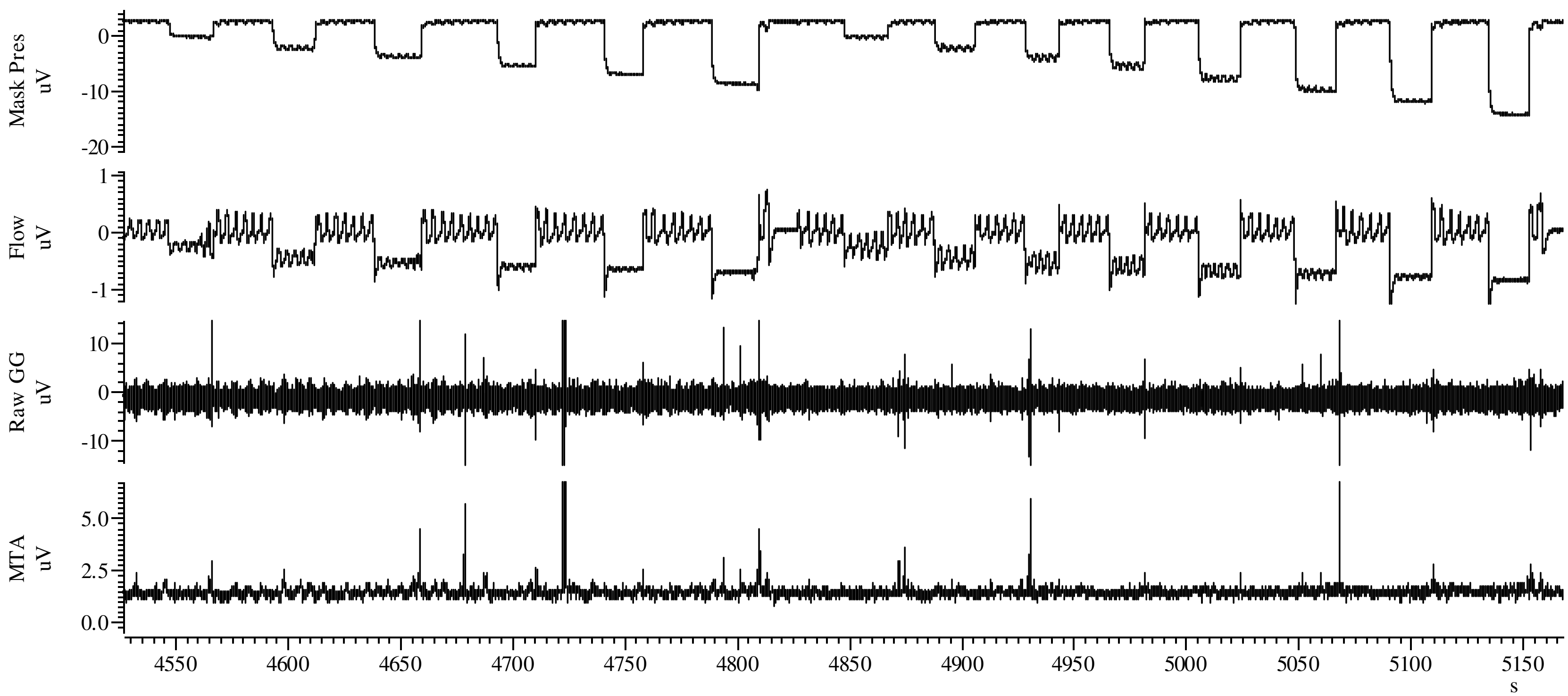

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Figure E2

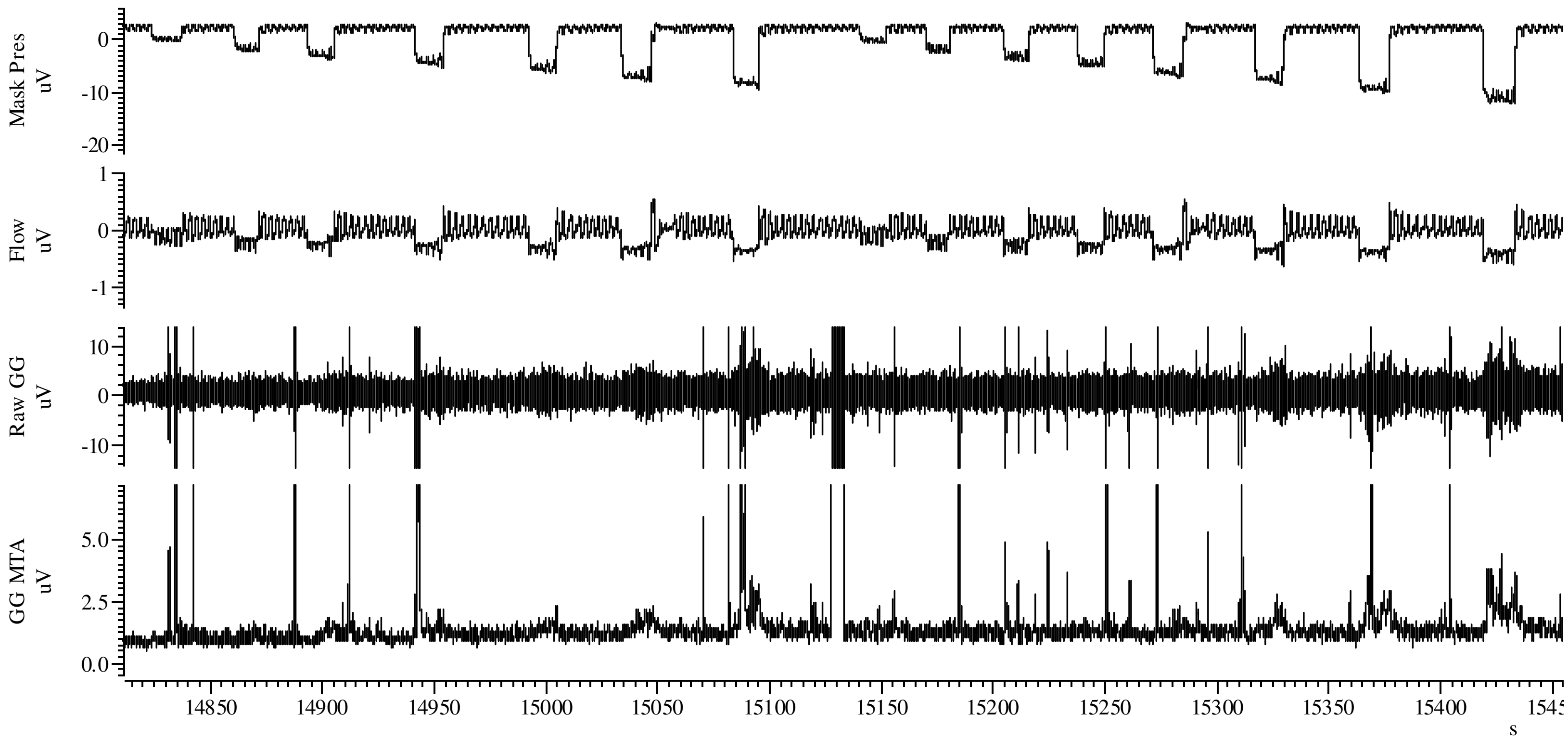

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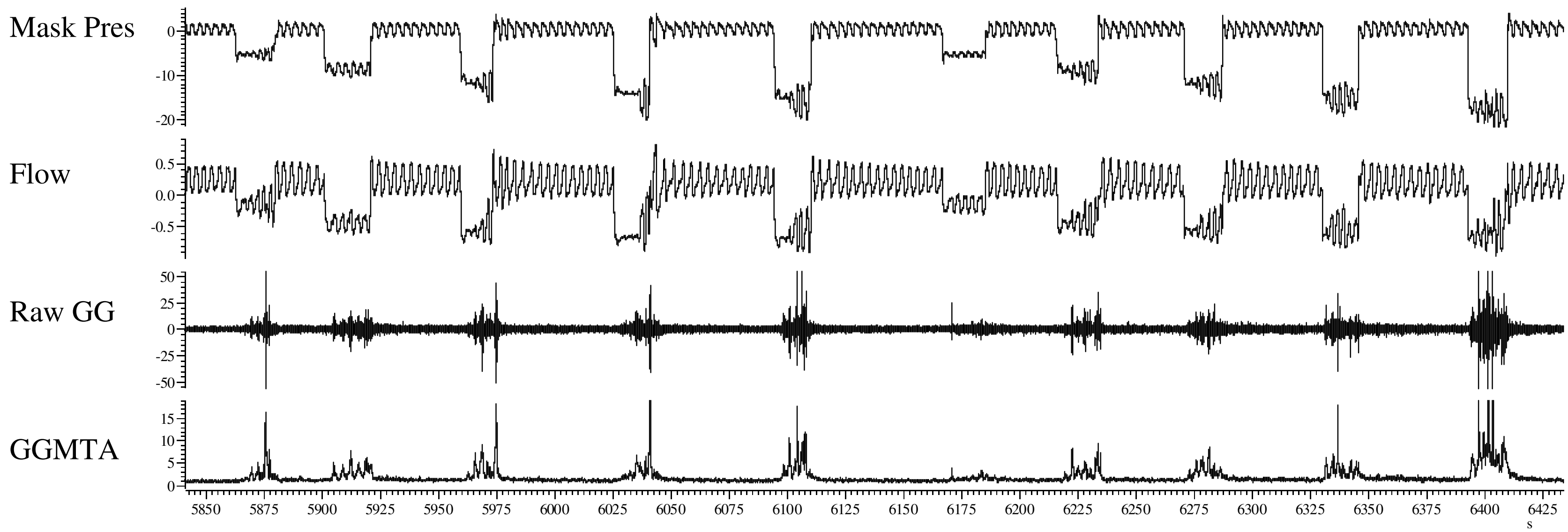
Figure E3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