

## Effects of Spontaneous Swallows on Breathing in Awake Goats

Thom R. Feroah<sup>1,2</sup>

H.V. Forster<sup>1</sup>

Carla G. Fuentes<sup>1</sup>

Ivan M. Lang<sup>3</sup>

David Beste<sup>2</sup>

Paul Martino<sup>1</sup>

L. Pan<sup>4</sup>

Tom Rice<sup>2</sup>

Departments of Physiology<sup>1</sup>, Pediatrics<sup>2</sup>, Medicine<sup>3</sup>  
Medical College of Wisconsin,  
Zablocki VA,  
Department of Physical Therapy<sup>4</sup>,  
Marquette University,  
Milwaukee WI

Running title: Effects of Swallowing on Breathing

Send correspondence to:

H. V. Forster, Ph.D.  
Medical College of Wisconsin  
8701 Watertown Plank Road  
Milwaukee, WI 53226  
Phone: 414 / 456-8533 Fax: 414 / 456-6546

**Abstract**

The effects of spontaneous swallows on breathing prior to, during, and after solitary swallows were investigated in 13 awake goats. Inspiratory ( $T_I$ ) and expiratory ( $T_E$ ) time and respiratory output ( $V_T$  and  $DIA_{Peak}$ ) were determined from inspiratory airflow and peak diaphragmatic activity. The onset time for 1,128 swallows was determined from pharyngeal muscle electrical activity. During inspiration, the later the swallowing onset, the greater increase in  $T_I$  and  $V_T$  while there was no significant effect on  $T_E$  and  $DIA_{Peak}$ . Swallows in early expiration increased the preceding  $T_I$ , reduced  $T_E$ , while later in expiration, swallows increased  $T_E$ . Following expiratory swallows,  $T_I$  and  $V_T$  were reduced while minimal changes in  $DIA_{Peak}$  were observed. Phase response analysis revealed a within-breath, phase-dependent effect of swallowing on breathing, resulting in a resetting of the respiratory oscillator. However, the shift in timing in the breaths following a swallow was not parallel, further demonstrating a respiratory phase-dependent effect on breathing. We conclude that in the awake state, within and multiple breaths effects on respiratory timing and output are induced/required in the coordination of breathing and swallowing.

Indexed Terms: Respiration, Deglutition, Pharyngeal Muscles, Diaphragm, Electromyography

## Introduction

Both breathing and swallowing are continual ongoing events in mammals, although at different frequencies. As a result of the shared use of the upper airway, it is extremely important that the motor pattern generators associated with breathing and swallowing are tightly coupled to provide effective coordination for cleansing and removing secretions from the lower airways<sup>(3, 8, 13)</sup>. A loss of coordination between these pattern generators is associated with dysphasia, weight loss, coughing, and pulmonary aspiration leading to pneumonia. However, the exact neural and physiologic nature of the interconnections between the swallowing and breathing pattern generators are unknown. Nevertheless, the functional relationships between these generators have been investigated for decades.

While the frequency of spontaneous swallows during the different phases of respiration varies among species, swallowing has been generally reported to inhibit breathing in human, dogs, rabbits, and cats<sup>(1, 2, 5, 7, 8, 22, 23, 25, 29)</sup>. For example, swallows during inspiration or expiration are reported to increase the phase of respiration in which it occurred. In the few studies that measured respiratory output (e.g., tidal volume, and electrical activity of the diaphragm and laryngeal abductors), swallowing reduced inspiratory output<sup>(11, 23, 24)</sup>. In addition, the observation of swallows during the transition between inspiration-expiration or expiration-inspiration are infrequent<sup>(18, 22)</sup>, but if observed they disruptive respiratory timing in humans<sup>(27)</sup>.

The neural substrate that generates and coordinates the motor patterns for swallowing and breathing are located in the dorsomedial and ventrolateral brainstem<sup>(1, 4, 13, 16)</sup>. To understand the interaction between these pattern generators, several interpretative models have been applied, mostly using anesthetized or decerebrate models with superior laryngeal nerve stimulation<sup>(7, 19, 27)</sup>. Few studies have examined this interaction on the swallowing and respiratory pattern generators in unanesthetized animals and human<sup>(20, 23, 24, 25)</sup>.

The aim of this study was to investigate in unanaesthetized, awake goats the effect of spontaneous swallows on respiratory output and timing. We hypothesized that a swallow in either phase of respiration would produce a within breath effect to increase respiratory timing, enough to cause a phase delay but not a resetting of the respiratory rhythm. We also hypothesized that swallows would not have an effect on the

timing or tidal volume of the breath before the swallow, but would reduce the tidal volume if the swallow occurred during inspiration.

## Methods

### *Animals*

Nine adult goats of various breeds that were used in this study received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” formulated by the National Research Council, 1996. The study protocol was approved by the Institutional Animal Care Committee of the Medical College of Wisconsin.

### *Surgical Procedures*

Surgery was performed to implant chronic electromyographic electrodes in the diaphragm (EMG<sub>DIA</sub>), thyropharyngeus (EMG<sub>TP</sub>), posterior cricoarytenoid (EMG<sub>PCA</sub>), and thyroarytenoid (EMG<sub>TYA</sub>) muscles. In four goats, a tracheostoma was created at the level of the 8<sup>th</sup> tracheal ring to isolate the upper airway. The animals received an intravenous injection of ketamide (Ketaset) and xylazine (12:1 ratio, 15 mg/kg) for induction of anesthesia prior to intubation. Following intubation for mechanical ventilation, general anesthesia was maintained with 1.5 – 2.5 % halothane in oxygen (sufficient to eliminate the withdrawal reflex and any signs of pain.)

EMG electrode placement has been previously described <sup>(10)</sup>. Briefly, teflon-coated 32-gauge stainless steel bipolar microelectrodes were inserted into the muscles defined above. For implants into airway muscles, a midline incision was made on the ventral surface of the neck from the hyoid bone to 4 cm below the thyroid cartilage, exposing the lateral aspect of the pharynx. The EMG<sub>TP</sub> electrode was sewn into the thyropharyngeus midway between the posterior midline of the pharynx and the insertion on the thyroid cartilage 0.5 cm below the cranial laryngeal nerve. To implant the EMG<sub>PCA</sub>, a small window was made at the inferior edge of the thyroid cartilage, and the tissue was dissected between the anterior wall of the esophagus and the posterior wall of the cricoid cartilage to expose the posterior cricoarytenoid. For placement of the EMG<sub>TYA</sub> electrode, a U-shaped window was made in the lateral wall of the thyroid cartilage to expose the thyroarytenoid. The EMG<sub>SP</sub> electrode was sewn into the SP muscle close to its

disappearance under the hypopharyngeus. The wires were looped in the subcutaneous layer of connective tissue and exited on the lateral ventral surface of the neck. For the EMG<sub>DIA</sub>, a lateral thorocotomy was performed between the 9<sup>th</sup> and 10<sup>th</sup> ribs midway between the sternum and spine. The diaphragm electrodes were implanted in the costal portion of the diaphragm and exteriorized next to the incision. Following the above instrumentation, the carotid arteries were elevated bilaterally so that they could be easily catheterized for monitoring of arterial blood pressure during the experimental studies. A 5 cm segment of each carotid artery was elevated subcutaneously and sutured in place.

For at least 24 h following surgery, laboratory personnel frequently inspected the animals. The animals received daily intramuscular antibiotics (ceftiofur sodium, 2 mg/kg) and their rectal temperature, eating habits, and behavior were monitored daily. These measures indicated that the goats were in good health and fitness during recovery from surgery and the subsequent experimental period.

#### *Methods of Measurement and Experimental Design*

For measurements of airflow in the animals without a tracheostoma, a tight-fitting, custom facemask was connected to a one-way breathing circuit. In the animals with a tracheostoma, airflow measurements were made via an 8 french cuffed tracheostomy tube that was inserted into the trachea, cuff inflated, and connected to a one-way breathing circuit with a two-way non-rebreathing valve (model 2600, Hans Rudolph, Inc.). A pneumotachograph was connected in-line on the inspiratory side of non-rebreathing valve and connected to a differential pressure transducer to measure inspiratory airflow only. The proximal ends of the EMG wires were connected via microclips to a Grass Recorder for signal processing and recording. The EMG signals were filtered at a bandpass of 3-500 Hz. A carotid artery was catheterized at least 2 days prior to initiation of experimental studies. Arterial blood pressure was monitored during all studies by connecting the arterial catheter via a Statham blood pressure transducer to a Grass Recorder. The airflow, raw EMG signals and arterial blood pressure were sent to a CODAS computer data acquisition system at a sampling rate of 250 Hz for display, digital recording, and analysis.

Prior to initiating data collection, the goats were acclimated for several hours a day for a week in a stanchion where they were connected to the recording system. On the days the goats were studied, respiration, upper airway EMG activity, and blood pressure were continuously monitored for a minimum of 2 hours. Chewing and other movement artifacts were eliminated from the analysis. All goats were studied in the awake state in the prone recumbent position. Respiratory rate and arterial blood pressure were normal throughout the experimental period indicating that the goats were in good health, relaxed and free of pain.

#### *Data Analysis*

Respiratory airflow and EMG signals were processed and analyzed (WinDaq, DATAQ Instruments). Raw EMG data were full wave rectified and passed through a moving time averager (time constant of 0.1 sec) to obtain an integrated EMG signal. The integrated EMG<sub>TP</sub>, EMG<sub>PCA</sub>, and EMG<sub>TYA</sub> signals were analyzed to obtain the occurrence of swallowing. The absolute time of a swallow was determined at the peak of a 10-fold increase in the integrated EMG<sub>TP</sub> and EMG<sub>TYA</sub> activity with a 200 millisecond duration and without signs of movement artifacts. The start of a swallow was then set at 0.15 seconds before peak EMG<sub>TP</sub> activity. Even though the TP muscle is activated in the later part of the recruitment order of pharyngeal muscles, the onset of activation is within 0.2 seconds of the initiation of the oro-pharyngeal phase of swallowing<sup>(8, 9, 18)</sup>. In this study, spontaneous swallows were considered as non-feeding swallows that were initiated reflexively by the accumulation of oropharyngeal secretions. These secretions were presumably sensed by afferent receptors in the laryngeal mucosa innervated by the trigeminal, glossopharyngeal, and vagal nerves<sup>(1, 8, 12, 13)</sup>. None of the swallows analyzed were related to mastication.

For the analysis of ventilation, a two-state computer algorithm (inspiration/expiration) was used for the automatic detection of the breath-by-breath calculations for tidal volume ( $V_T$ ), duration of inspiration ( $T_I$ ), and duration of expiration ( $T_E$ ) from the airflow signal. Peak EMG<sub>DIA</sub> activity ( $DIA_{Peak}$ ) was obtained for each breath. In this two-state computer algorithm, inspiration was determined when flow exceeded zero respiratory flow by 0.01 L/sec for a minimum duration of by 0.2 sec. The start of

expiration was determined when a pause in the  $EMG_{DIA}$  activity and in the inspiratory flow longer than 0.35 sec was detected. Although this period was shorter than the reported deglutition apnea in animals and humans<sup>(5, 17, 22, 23, 24, 27, 30)</sup>, we felt it was a conservative estimate of the establishment of expiratory cycle. In addition, our observations in goats led us to the use of these criteria, because deglutition apneas, if present during inspiration, were very short. Therefore, we believe that the determination of the phase of breathing was accurately and reliably determined. The absolute time for the start of inspiration for each breath was recorded along with the time of each swallow. The total duration of a breath ( $T_{Tot}$ ) was calculated as the sum of  $T_I$  and  $T_E$ . The inspiratory duty cycle ( $T_I/T_{Tot}$ ) and the ratio of  $V_T/T_I$  calculated for each breath.

Three types of analysis of the effects of swallowing on breathing were made. First, to examine the general effects of swallowing on breathing, swallows were categorized into one of four phases of ventilation (Figure 1): expiration ( $SW_E$ ), the transition from expiration to inspiration ( $SW_{EI}$ ), inspiration ( $SW_I$ ), and the transition from inspiration to expiration ( $SW_{IE}$ ). The effects of  $SW_E$ ,  $SW_{EI}$ ,  $SW_I$ , and  $SW_{IE}$  on  $T_I$ ,  $T_E$ ,  $T_{Tot}$ ,  $V_T$ ,  $T_I/T_{Tot}$ ,  $V_T/T_I$ , and  $DIA_{Peak}$  (expressed as a percent of control) on the breath prior (n-1), during (n), and following (n+1) the swallows were evaluated. Second, the Gamma Analysis ( $\gamma$ ) evaluated whether the time of occurrence of a swallow, either within inspiration ( $\gamma_I$ ) or expiration ( $\gamma_E$ ), had an effect on the breath prior to or during a swallow<sup>(23, 24)</sup>. For this analysis, five consecutive breaths were evaluated, with a solitary swallow in the fourth breath (Figure 2A and 2B). In this analysis, a breath was considered to start with expiration followed by inspiration. Swallows with movement or augmented breaths within the set of five consecutive breaths were eliminated from this analysis. Averages for  $T_I$ ,  $T_E$ ,  $V_T$ , and  $DIA_{Peak}$  were calculated from the first, second, and fifth breaths of the set of five breaths and used as control values. The value for  $\gamma_I$  was calculated as the time from the beginning of inspiration to the onset of the solitary swallow, expressed as percent of the control  $T_I$ . The value for  $\gamma_E$  was calculated as the time from the beginning of expiration to the onset of the solitary swallow, expressed as percent of the control  $T_E$ . Values for  $T_I$ ,  $T_E$ ,  $V_T$ , and  $DIA_{Peak}$  for the previous breath (n-1) and the breath during (n) the swallow were expressed as a percent of its control value and plotted against  $\gamma_I$  or  $\gamma_E$ .

Finally, to evaluate the effect of swallows on the oscillations of the respiratory pattern generator, a phase response analysis (similar to the analysis of biological oscillators) was performed<sup>(26, 27, 31)</sup>. In this analysis, six consecutive breaths were evaluated, with a solitary swallow in the fourth breath. The old phase ( $\phi$ ) was defined as the time from the beginning of inspiratory flow of the fourth breath to the onset of the swallow (Figure 3A). The subsequent co-phases ( $\theta$ ) were defined as the time from the onset of swallow to the beginning of inspiratory flow of breath five ( $\theta_{n1}$ ) and six ( $\theta_{n2}$ ), and to the end of expiration of breath six ( $\theta_{n3}$ ). The co-phase for breath prior to the swallow ( $\theta_{n-1}$ ) was calculated as the time from the beginning of the swallow backward to the beginning of inspiratory flow of breath three. Movement or augmented breaths in any of these breaths eliminated the set from analysis. Ventilatory values from breaths one, two, and six of the set were averaged and used as controls. All values of  $\phi$  and  $\theta$  were expressed as a fraction of control values. Values of  $V_T$  and  $DIA_{Peak}$  in this analysis were calculated as a percent of control values.

Following the above definitions of  $\phi$  and  $\theta$ , the effect of swallowing on the respiratory rhythm can be assessed, as previously presented by Paydarfar et al.<sup>(27)</sup>. As defined in Figure 3A, the normalized measures of  $\phi$  and  $\theta$  would equal one ( $\phi + \theta = 1$ ) if swallowing had no effect on rhythm at any time during the respiratory cycle, and the slope of  $\theta_{n1}$  vs.  $\phi$  would be  $-1$ . Furthermore, as demonstrated in Figure 3B, the effect on any subsequent breaths ( $n$ ) would be anticipated by the equation,  $\theta_n = -\phi + n$  with a similar slope. A phase advance or delay in the onset of breathing would be anticipated if swallowing has an effect on the respiratory rhythm generator to shorten or lengthen inspiration or expiration. If swallowing had more than a transient effect on the respiratory rhythm, then a permanent shift or resetting of the subsequent start of ventilation would occur. To evaluate the possible phase shift in the phase response curves, the difference in the onset from the previous breath ( $\theta_{n1}-\theta_{n-1}$ ,  $\theta_{n2}-\theta_{n1}$ ,  $\theta_{n3}-\theta_{n2}$ ) for each swallow in all the animals was calculated and averaged every 0.05 interval of  $\phi$ . Given sequential breaths and the above equation for  $\theta_n$ , a parallel shift in the  $\theta_n$  curves would result in a difference of one. A phase delay would result in an increase in the difference between  $\theta_n$  curves relative to the previous swallow, while a phase advance would result in a decrease in the difference. To estimate the potential phase shift of the breath prior to the swallow, the difference between  $\theta_{n-1}$  and a non-affected

old phase ( $\theta_{Ideal}$ ) with a slope of  $-1$  was calculated and averaged every  $0.05$  interval of  $\phi$ . In this later comparison ( $\theta_{n-1}-\theta_{Ideal}$ ), the expected difference would be zero if there was no difference between the curves for any value of  $\phi$ .

The phase transition from inspiration-to-expiration (IE) for the breaths with swallows was estimated by calculating the half-cycle period relative to a specific old phase that began at the start of inspiration. The co-phase ( $\eta_{IE}$ ) in this analysis was calculated as period of time from the start of the swallow to the start of expiration. The average phase transition was then estimated for the breaths with swallows by solving for the relationship of  $\phi$  vs.  $\eta_{IE}$  using a third order polynomial equation for an intercept of zero.

### *Statistical Analysis*

The average rate of swallowing and its standard deviation was calculated for each goat, and a Kolmogorov-Smirnov test was performed to test whether the rate of swallowing significantly deviated from Gaussian distribution ( $P < 0.05$ ). For the categorization of swallows, a Student's t-test was performed on the means of the percent change of  $T_I$ ,  $T_E$ ,  $T_{Tot}$ ,  $V_T$ ,  $T_I/T_{Tot}$ ,  $V_T/T_I$ , and  $DIA_{Peak}$  for the breaths prior to, during, and following the swallows, compared to no change (100%) within  $SW_E$ ,  $SW_{EI}$ ,  $SW_I$ , and  $SW_{IE}$  ( $P < 0.02$ ). A comparison was also made using a one-way analysis of variance (ANOVA) between the means of the percent change for the breaths prior to, during, and following the swallows within  $SW_E$ ,  $SW_{EI}$ ,  $SW_I$ , and  $SW_{IE}$  ( $P < 0.02$ ).

In the Gamma Analysis, a linear regression analysis was performed on  $T_E$ ,  $T_I$ ,  $V_T$ , and  $DIA_{Peak}$  (expressed as a fraction of its control) on the previous breath, and the breath in which the swallow occurred was evaluated for  $\gamma_I$  and  $\gamma_E$  ( $P < 0.02$ ). The slopes and intercepts were compared to determine if the time of occurrence of the swallow ( $\gamma_I$  or  $\gamma_E$ ) during inspiration or expiration had an effect on respiratory parameters from the breath prior to and during the swallow.

In the evaluation of the phase shift, the mean value of  $\theta_{n1}-\theta_{n-1}$ ,  $\theta_{n2}-\theta_{n1}$ , and  $\theta_{n3}-\theta_{n2}$  at every  $0.05$  interval of  $\phi$  was tested to determine if it differed significantly from a hypothetical value of one using a

Student's t-test ( $P < 0.02$ ). Similarly, the mean of  $\theta_{n-1} - \theta_{\text{Ideal}}$  was compared to a value of zero using a Student's t-test ( $P < 0.02$ ).

## Results

During expiration, the normal phasic respiratory activity of  $\text{EMG}_{\text{TP}}$  was inhibited just before its recruitment for a swallow (Figure 1B, *a*). Commonly associated with the swallows during expiration was a small burst of activity in the  $\text{EMG}_{\text{DIA}}$  (Figure 1B, *b*). During late expiration, swallows were associated with a short a burst of inspiratory airflow, usually termed a “swallow breath” or *Schluckatmung*. However, if a swallow occurred during the first third of expiration, inspiratory flow was not seen. During inspiration, phasic  $\text{EMG}_{\text{SP}}$  activity was inhibited at the onset of  $\text{EMG}_{\text{TP}}$  swallow activity (Figure 1B, *c*). A short inhibition, or pause, in  $\text{EMG}_{\text{DIA}}$  activity occurred midway through  $\text{EMG}_{\text{TP}}$  swallow activity (Figure 1B, *d*). In all goats (with an intact upper airway or with a tracheostomy), a short pause (less than 0.1 sec) and a significant decrease in inspiratory airflow were seen during swallows following increases in  $\text{EMG}_{\text{TP}}$  activity and decreases in  $\text{EMG}_{\text{DIA}}$ .

The average rate of swallowing for all the goats was  $2.88 (\pm 1.22)$ , swallows per minute; Table 1). Goats with tracheotomies had an average of rate  $1.86 (\pm 0.31)$ , swallows per minute), while goats with intact airways presented with an average rate of  $3.3 (\pm 1.9)$ , swallows per minute). On average, 12.4% of the swallows from each goat were not used in the analysis due to movement artifacts or the presence of more than one swallow within a breath. The average duration of the moving time average of a swallow in the TP signal was  $0.25 (\pm 0.03)$  sec. No pharyngolaryngeal or swallowing dysfunction was observed in any goat in this study.

### *Effects of Spontaneous $\text{SW}_{\text{E}}$ , $\text{SW}_{\text{EI}}$ , $\text{SW}_{\text{I}}$ , and $\text{SW}_{\text{IE}}$ on Breathing*

In this analysis, shown in Figure 4, a total of 1,128 solitary swallows met the criteria for evaluation during the observation of 7,573 breaths. In the  $\text{SW}_{\text{E}}$  analysis (317 swallows, 28% of total),  $T_{\text{E}}$  was significantly increased in the breath prior to, during, and following the swallow. In contrast,  $T_{\text{I}}$  decreased during the inspiration following the  $\text{SW}_{\text{E}}$ . As a result,  $T_{\text{Tot}}$  was increased in the breath prior to,

during, and following the swallows despite the decrease in  $T_I$  following the swallow. The inspiratory duty cycle,  $T_I/T_{Tot}$ , was decreased in the subsequent breath following  $SW_E$  due to the decrease in  $T_I$  and increase in  $T_E$ . The  $V_T$  just prior to and following  $SW_E$  were significantly reduced, as well as  $V_T/T_I$ . Similarly,  $DIA_{Peak}$  activity was decreased in the inspiration prior to and following the  $SW_E$ . Therefore, swallows during early and mid expiration had an immediate affect on timing during expiration (increasing) and the subsequent inspiration (decreasing), while having a wider effect over time by decreasing respiratory output, as indicated by  $V_T$  and  $DIA_{Peak}$  on the breath prior to and after the swallow.

Swallows during the EI transition (296 swallows, 26% of total) provided a slightly different pattern (Figure 4). Immediately following  $SW_{EI}$ ,  $T_I$  was significantly decreased, while no significant changes were found in  $T_E$  or  $T_{Tot}$  in the breath prior to, during, or following the swallow. A small increase in  $T_I/T_{Tot}$  was observed in the breath following  $SW_{EI}$ . Following  $SW_{EI}$ ,  $V_T$  was significantly decreased and then increased in the subsequent breath. No changes in  $V_T/T_I$  were seen, demonstrating that  $V_T$  and  $T_I$  decreased proportionally in the immediate inspiration but then increased in the subsequent breath. In contrast,  $DIA_{Peak}$  was not significantly altered in the breath prior to, during, or following  $SW_{EI}$ . Therefore, swallows during the EI transition solely affected the subsequent inspiratory timing and, consequently,  $V_T$ , but not total output as suggested by  $DIA_{Peak}$ .

In the  $SW_I$  analysis (507 swallows, 45%),  $T_I$  was greatly increased during  $SW_I$ , while no effect was seen in  $T_I$  in the breaths before or following (Figure 4). Swallows during  $SW_I$  had no affect on  $T_E$ . As a consequence,  $T_I/T_{Tot}$  was significantly increased. Tidal volume was slightly increased in the breath with a swallow and in the subsequent breath, while a significant decrease in  $DIA_{Peak}$  was observed during  $SW_I$ . However,  $V_T/T_I$  was significantly decreased during the inspiratory swallow, primarily as a result of the increases in  $T_I$ . These results suggest that swallows during inspiration affected respiratory timing but not output.

Because so few swallows were observed during the IE transition (8; 0.01%), great variability and lack of significant changes were observed in these data.

*Within-breath Effects of Swallows: Gamma Inspiratory and Expiratory Analysis*

In the Gamma Inspiratory Analysis, the slope of the  $T_I$  vs.  $\gamma_I$  relationship for the  $n$  breaths was significantly different ( $P < 0.01$ ) from zero and from the  $n-1$  breaths (Figure 5B, Table 2). In addition, the estimated y-intercept of the  $T_I$  vs.  $\gamma_I$  relationship was significantly above control ( $P < 0.02$ ). However, the observed  $T_I$  did not show a linear increase from the estimated intercept but, rather, an abrupt increase around 0 to 15  $\gamma_I$ . In contrast, no differences were found in the  $T_E$  vs.  $\gamma_I$  relationship either for the breaths prior to ( $n-1$ ) or during ( $n$ ) the swallow (Figure 5A and Table 2). Swallows during early inspiration tended to reduce  $V_T$  as demonstrated by the significantly reduced y-intercept of the  $V_T$  vs.  $\gamma_I$  relationship for  $n$  breaths ( $P < 0.02$ ), while swallows in the later part of inspiration increased  $V_T$ , as indicated by the significant positive slope of the  $V_T$  vs.  $\gamma_I$  relationship ( $P < 0.01$ ; Figure 5C, Table 2). In contrast, the slope of  $DIA_{Peak}$  vs.  $\gamma_I$  was not significantly different from zero for both the  $n$  and  $n-1$  relationships. However, a significant reduction in the y-intercept of the  $DIA_{Peak}$  vs.  $\gamma_I$  was seen for the  $n$  breaths (Figure 5D, Table 2). Primarily because of the lengthening of  $T_I$  above control in the  $n$  breaths (Figure 5B), values greater than control (100%) were observed for  $\gamma_I$ .

In the Gamma Expiratory Analysis ( $\gamma_E$ ), swallows during expiration had a different effect on the respiratory pattern generator. In the  $n-1$  breaths, the slope of the  $T_E$  vs.  $\gamma_E$  relationship, although significantly greater than zero, was small (Figure 6A, Table 3). However, in the  $n$  breaths, swallowing during expiration had a significant effect on  $T_E$ , although there was considerable variation in the data. The slope of the  $T_E$  vs.  $\gamma_E$  relationship (0.42) was significantly greater than zero and significantly different from the slope of the  $n-1$  breaths ( $P < 0.01$ ), while the y-intercept was significantly less than the control (71.42%;  $P < 0.01$ ; Figure 6A, Table 3). In contrast, the slope of  $T_I$  vs.  $\gamma_E$  relationship for  $n-1$  breaths was significantly less than zero (-0.10,  $P < 0.01$ ), with an y-intercept that was significantly greater than control (109.8,  $P < 0.02$ ). However, the y-intercept for the  $T_I$  vs.  $\gamma_E$  relationship for the  $n$  breaths was significantly less than control (93.8%;  $P < 0.01$ ; Figure 6B, Table 3), while the slope was not significantly different from zero. Therefore, while swallows during expiration demonstrated a moderate affect on  $T_E$  a cycle before the swallow, these results demonstrate a strong *within-phase* dependency of  $T_E$  on the timing of the onset of swallow, which is not shown in the categorical analysis. For the effect of expiratory

swallows on  $T_I$ , the data suggests the later the swallow occurs in expiration, the less effect it has on the previous  $T_I$ , while, in contrast, the following inspiratory period is attenuated irrespective of when the swallow occurred in the previous expiration. Similar to what was observed in  $\gamma_I$  analysis above, the increases in  $T_I$  and  $T_E$  produced  $\gamma_E$  values greater than 100%.

The slopes of the  $V_T$  vs.  $\gamma_E$  for both the  $n-1$  and  $n$  breaths were not significantly different from zero. However, only the y-intercept for the  $n$  breaths relationship was significantly less (88%) than control ( $P < 0.02$ ; Table 3). In contrast, neither the slopes nor the intercepts for either  $DIA_{\text{peak}}$  vs.  $\gamma_E$  relationships were significant (Figure 6, Table 3). These results suggest that total respiratory output was not altered, but the overall attenuation of  $V_T$  was similar to the decrease in inspiratory flow.

#### *Effect of Swallows on the Respiratory Phase Response and Output*

The occurrence of spontaneous swallows that meet the criteria for the phase response analysis (427) demonstrated a bimodal distribution (Figure 7A), with approximately 53% occurring during inspiration (average IE transition  $0.47 \pm 0.006$ ; see arrow in Figure 7A) and 47% during expiration. The  $\theta_{n-1}$  curve in Figure 7B illustrates the effect of swallowing on respiratory timing in the breath before the swallow. A linear regression analysis of the  $\theta_{n-1}$  curve found a slope of  $-1.01 (\pm 0.01)$ , with a y-intercept of  $-0.004 (\pm 0.001)$  and a correlation coefficient ( $R^2$ ) of 0.97, indicating that swallowing had no effect on the timing of the previous cycle regardless of the phase of onset in the respiratory cycle. In contrast, the flattening of the  $\theta_{n1}$  curve between the  $\phi$  values of 0.1 and 0.3 reflects a phase delay in the respiratory timing produced by increases in  $T_I$  with swallows during inspiration. The slope of the  $\theta_{n1}$  curve after the IE transition ( $\approx 0.4$ ) to a  $\phi$  value of 0.7 was approximately  $-1.23$ . This slope was more negative than that found for the  $\theta_{n-1}$  curve over the same period indicating that swallows during this period may have produced a phase advance in the respiratory rhythm. This latter finding is supported by the tendency of  $T_E$  to decrease early in the  $T_E$  vs.  $\gamma_E$  relationship (Figure 6A). After  $\phi$  values of 0.7, the  $\theta_{n1}$  curve again flattens and runs parallel with its expected intersection with zero. This flattening of the  $\theta_{n1}$  curve and its extension beyond  $\phi$  values of 1.0 is a result of both an increase in  $T_E$ , delay in the onset of the next cycle and a comparable decrease in the subsequent  $T_I$ . These later observations are also seen in the  $\gamma_E$  analysis

in the  $T_E$  above 90% and in the generally depressed  $T_I$  vs.  $\gamma_E$  relationship in the breaths with swallows (Figure 6 and Table 3). A parallel shift between the  $\theta_{n1}$  and  $\theta_{n2}$  and  $\theta_{n3}$  curves, except between  $\phi$  of 0.9- to-1.2 where an upturn in  $\theta_{n2}$  and  $\theta_{n3}$ , suggests a slight phase delay. These results clearly support a type 1 or a weak resetting of the respiratory rhythm generator.

The changes in  $T_I$  relative to  $T_E$  during a respiratory cycle due to swallowing is shown in the graph of  $T_I/T_{TOT}(n)/T_I/T_{TOT}(c)$  (Figure 7C). In this figure, the  $T_I/T_{TOT}(n1)/T_I/T_{TOT}(c)$  curve increases above control during the inspiratory section of the phase response plot. This increase is explained by progressive increases in  $T_I$ , with swallows during inspiration having no affect on  $T_E$  (Figure 5). However, following the IE transition ( $\approx 0.4$ ), the cause of the increased  $T_I/T_{Tot}$  is due to an overall shortening of  $T_E$  relative to  $T_I$ , as shown in the  $\gamma_E$  analysis (Figure 6). Thereafter, the eventual return to control levels of the  $T_I/T_{TOT}(n1)/T_I/T_{TOT}(c)$  curve is due to the relative lengthening of  $T_E$  compared to a general shortening of  $T_I$  (Figure 6). The effect of swallows on  $T_I/T_{Tot}$  is limited to the  $n1$  breaths, as  $n2$  and  $n3$  curves are observed at control levels.

In Figure 8, the effect of swallows on  $V_T$  is seen during expiration ( $\phi \sim 0.8-1.5$ ), where the  $V_T(n)/V_T(c)$  curve is lowered during the inspiration following the swallow ( $n2$  curve). Subsequent compensation due to a reduction in  $V_T$  was not seen in the  $n3$  curve. A modest decrease in  $DIA_{Peak}(n)/DIA_{Peak}(c)$  is observed at different sections of the  $n1$  and  $n2$  curves. The  $n1$  curve is slightly depressed near the IE transition ( $\phi \sim .35$ ), while the  $n2$  curve is depressed in the later part of expiration ( $\phi \sim 0.8$ ; Figure 8, Panel B). In contrast to the categorical data (Figure 4) and the within-breath data (Figures 5 and 6), these latter data show that swallowing has a limited, temporal perturbation affect on respiratory output.

Figure 9 presents the differences in  $\theta_{n-1}-\theta_{Ideal}$ ,  $\theta_{n1}-\theta_{n-1}$ ,  $\theta_{n2}-\theta_{n1}$ , and  $\theta_{n3}-\theta_{n2}$  every 0.05  $\phi$  interval. No significant difference was found for  $\theta_{n-1}-\theta_{Ideal}$  at any value of  $\phi$  ( $P < 0.02$ ), thus supporting the observation that the timing of the previous breath was unaltered. In contrast,  $\theta_{n1}-\theta_{n-1}$  differed significantly from the predicted difference of 1 at several points along the  $\phi$  axis ( $P < 0.02$ ). The increase in  $\theta_{n1}-\theta_{n-1}$  from 0.15 to 0.40 of  $\phi$  reflects a phase delay in the  $\theta_{n1}$  curve due solely to increases in  $T_I$ . Thereafter, from a  $\phi$  value of 0.40 to 0.60, the  $\theta_{n1}-\theta_{n-1}$  curve progressively decreased. Although fewer

swallows were observed during this phase of ventilation, this decrease was due to the mixed and varied effects of swallows to: 1) increase  $T_I$  just before the swallow (phase delay), and 2) decrease  $T_E$  (phase advance) in early expiration. From a  $\phi$  value of 0.65 to 0.85, the difference between  $\theta_{n1}$  and  $\theta_{n-1}$  was not significantly different from either from 1 or the expected difference if swallowing had no effect on respiration. Thereafter,  $\theta_{n1}-\theta_{n-1}$  significantly increased due to the effect of swallows late in expiration to increase  $T_E$  or phase delay the onset of the next breath ( $P < 0.02$ ). A significant decrease ( $P < 0.2$ ) in the  $\theta_{n2}-\theta_{n1}$  curve between a  $\phi$  of 0.9 and 1.1 is a result of the effect of swallows during later part of expiration to also decrease the subsequent  $T_I$  and produce a phase advance of the next respiratory cycle.

## Discussion

Our data suggest that spontaneous swallows have a phase-dependent effect on respiratory timing and output in the awake state. Changes in breathing prior to and after a swallow further suggest that the interaction between the respiratory and swallowing pattern generators exists beyond the apparent, immediate, all-or-nothing event of swallowing.

### *The Interaction between Swallowing and Breathing*

The distribution of swallows during the phases of ventilation differs among animals, infants, and adults. In unanaesthetized<sup>(18, 20)</sup> and anesthetized<sup>(18, 19, 20)</sup> animals, 80 to 95% of the spontaneous swallows occur during inspiration. In human infants, spontaneous swallows are reported to be equally distributed during expiration and inspiration<sup>(30)</sup>. In unanaesthetized and anesthetized adults, spontaneous swallows are primarily produced during expiration<sup>(5, 23, 24, 27)</sup>. These studies suggest that swallowing may be coupled to specific phases of ventilation. In goats, a biphasic occurrence of swallows during the phases of ventilation was observed, with the least number of swallows occurring during the IE transition and early expiration. That over half of the swallows occur during inspiration suggests a well-coordinated, anatomically efficient means to minimize aspirations during a phase of ventilation that is normally at high risk, at least in human adults. In goats, the epiglottis overlaps the soft palate<sup>(10)</sup>, which has been shown to allow a bolus to circumvent the glottis during an inspiratory swallow and pass into the esophagus<sup>(22)</sup>. Based on this anatomy, Negus<sup>(22)</sup> proposed that airflow would continue during a swallow. A similar

anatomical mechanism has been proposed to occur in infants during inspiratory swallows that not only minimizes aspirations but also allows for an uninterrupted in airflow <sup>(6; 28)</sup>. However, in goats, we observed an interruption of airflow with inspiratory swallows similar to that found in infants by Wilson et al. <sup>(30)</sup>. We attribute this interruption to a decrease in DIA activity (observed in this study) and upper airway closure due to constrictor activity <sup>(10)</sup>. Therefore, we hypothesize that in goats the overlapping of the soft palate and epiglottis allows for inspiratory swallows with a low risk for aspirations, but does not allow for separate air and liquid channels.

While both the presence of swallows during EI transition and inspiration and the absence of pharyngolaryngeal problems suggest a benign interaction between the respiratory and swallowing functions, the lack of spontaneous swallows during the IE transition and early expiration is curious. In awake humans, Paydarfar et al. <sup>(27)</sup> observed the highest occurrence of spontaneous swallows during the IE transition and the lowest during EI transition, suggesting a very different relationship between the swallowing and respiratory pattern generators in humans, dogs, monkeys, and goats. Several studies <sup>(19, 27)</sup> have shown that stimulation of peripheral afferents during IE transition has a disruptive effect on respiratory timing, which may be the neurophysiologic basis for the reduced occurrence of spontaneous swallows during transition in these animals.

Less well investigated has been the effect of spontaneous swallows on respiratory timing and total output. Studies in decerebrate cats showed that swallows increased the duration of the current and subsequent respiratory cycle <sup>(21)</sup>. In awake rabbits, swallows during expiration significantly increased  $T_E$  and the preceding  $T_I$ , while during inspiration, swallows significantly increased  $T_I$  and the subsequent  $T_E$  <sup>(20)</sup>. These swallows did not significantly affect  $T_I$  or  $T_E$  in the breaths that followed. In unanaesthetized humans, both spontaneous and water-induced swallows during expiration increased  $T_E$  and  $T_{TOT}$ , as well as,  $V_T$  immediately following the swallow <sup>(23)</sup>. Spontaneous swallows during inspiration reduced  $T_I$ ,  $V_T$ , and the following  $T_E$ . In the subsequent breath,  $V_T$  was increased. A similar response was found in stimulated swallows that occurred during inspiration, except that  $T_{TOT}$  was decreased <sup>(23)</sup>. In goats, swallows during expiration also had a similar effect on  $T_E$ , and  $T_{Tot}$ . However, in contrast to data in humans <sup>(23)</sup>, the ensuing  $T_I$ ,  $V_T$ , and  $DIA_{Peak}$  were significantly reduced, suggesting that an inhibitory

effect of swallowing persisted in the following inspiratory timing and output. The increase in  $T_I$  with swallows during inspiration in goats was similar to that found in rabbits<sup>(20)</sup>, but is in contrast to the findings in humans<sup>(23)</sup>. Similarly,  $V_T$  was slightly increased, which is opposite of that found in humans<sup>(23)</sup>, and the findings of  $DIA_{Peak}$  in goats study further support the idea that total respiratory output is unaltered while respiratory timing increases with swallows during inspiration.

Although the information in the literature on the effect of swallows on the present and subsequent breath reveals general trends in the interaction between respiratory and swallowing centers, it does not investigate or eliminate a within- and/or multi-breath interaction. Most studies<sup>(20, 23)</sup> utilized the breath(s) before the swallows as controls. This latter point assumes that stimulated and spontaneous swallows only have an effect on breathing during and after the swallows. While our results support these findings, they also show that both timing and the output to the diaphragm are altered before a swallow. The neural substrate for this interaction is unknown. However, it is presumed that a build-up of secretions in the upper airway activates receptors that travel to the nucleus tractus solitarius via the superior laryngeal nerve<sup>(12)</sup>. Jean<sup>(14, 15, 16)</sup> reported that some neurons in the nucleus tractus solitarius exhibit a “pre-swallowing activity” that may act as trigger neurons for swallowing whose activity is increased with stimulation of the superior laryngeal nerve. Dick et al.<sup>(7)</sup> also observed that stimulation of the superior laryngeal nerve below the threshold for eliciting a swallow resulted in a prolongation of  $T_E$ . Together, these results suggest a peripheral feedback mechanism to the medullary neurons that may elicit changes in prior to a swallow and function to prepare the animal for a swallow.

The within-breath analysis of the effects of swallowing on the pattern of breathing provides an insight into the moment-to-moment relationship between the swallowing and respiratory centers. In infants, there is a linear relationship between the duration of inspiration and the  $T_I$  at the onset of airway closure due to a swallow<sup>(30)</sup>. The earlier the swallow occurred (within the first half of a breath), the shorter the inspiratory time. Thereafter, swallows produced a longer period of inspiration. Swallows during expiration also had the greatest effect in increasing  $T_{Tot}$ . This relationship produced a negative correlation between  $T_{Tot}$  and the occurrence of swallow during expiration. This finding is contrast to the observation in anesthetized and unanaesthetized adult humans, where spontaneous swallows occurred

primarily during expiration, producing a positive relationship between  $T_E$  and the occurrence of swallows during expiration, thereby increasing  $T_{Tot}$  <sup>(23, 24)</sup>. However, in anesthetized adult humans, swallows during inspiration abruptly interrupted inspiration and were followed by a short expiratory period, thereby producing a significant positive correlation between  $T_I$  and the onset time of the swallows <sup>(24)</sup>.

Similarly, there is a positive correlation between  $V_T$  and the onset of swallows during inspiration in anesthetized and unanaesthetized adults <sup>(23, 24)</sup>. In contrast, an abrupt cessation of inspiration followed by expiration was not observed in goats. Instead, swallows progressively lengthened  $T_I$  the later they occurred in inspiration (Table 2, Figure 5B). In addition, while swallows at the very beginning of inspiration were related to a reduced  $V_T$ , thereafter, a positive correlation (above control) was observed for  $V_T$  and the onset of the swallow during inspiration. In contrast, no change in  $DIA_{Peak}$  was observed. These observations suggest that not only did swallowing insert a pause into the inspiratory period while not widening the swallow complex and increasing the interruption in flow, it also had a mild progressive inhibition on ventilation (decreasing) while not altering total activity of the diaphragm.

During expiration in goats the relationships between the onset of a swallow and the values of  $T_E$ ,  $T_I$  and  $V_T$  are considerably different from previously reported data. In contrast to humans, our data show that the earlier the onset of a swallow during expiration, the shorter  $T_E$  observed, until approximately 75%  $\gamma_E$ , at which point swallowing increased  $T_E$  above control. This temporal effect of swallowing during expiration is also seen in the negative slope of  $n-1$   $T_I$  data, while a generalized overall inhibitory effect of swallowing was found in the relationship of the  $T_I$  vs.  $\gamma_E$  for the  $n$  breaths. Also consistent with this latter finding was the significant effect of expiratory swallows on the subsequent  $V_T$ , while  $DIA_{Peak}$  was not altered. These findings suggest that output to the diaphragm is not reduced during inspiration after a swallow; only the timing and the subsequent  $V_T$  are reduced.

#### *Phase Response Analysis in the Interaction of Breathing and Swallowing*

A major advantage of the phase response analysis is the capability to characterize the relationship between pattern generators without knowing a priori the specific neuroanatomical basis of the interaction <sup>(29, 31)</sup>. The phase response of stimulated and spontaneous swallows has only been investigated in humans

by Paydarfar et al. <sup>(27)</sup>, who found that the later a swallow occurred during inspiration, the later the onset of the subsequent breath. The peak of this response was near IE transition, after which no effect on the onset of the next cycle was seen until the swallow occurred at the very end of the respiratory cycle. This non-linear effect of swallowing on breathing is very similar to the results of Nashino et al. <sup>(23, 24)</sup>. In addition, Paydarfar et al. <sup>(27)</sup> demonstrated that swallows produced a ‘true’ resetting of the respiratory rhythm. In other words, instead of an immediate, short-term shift in the bulbospinal output produced by swallowing, a parallel shift in the subsequent breaths occurred. In goats, resetting of the respiratory rhythm generator also occurs after swallows at different onsets within the respiratory cycle, with a parallel shift in the starting of subsequent breaths. The continuity of the calculated co-phases ( $\theta$ ) further supports the concept of a continual oscillation of the respiratory pattern generator and true phase resetting.

However, the results from the phase response analysis in our study differ from those found in humans by Paydarfar et al. <sup>(27)</sup>. The basis for this difference lies in the within-breath effects of swallows on breathing. The type of resetting observed by Paydarfar et al. <sup>(27)</sup> was a type 0, in which the net change of the co-phase was zero over a cycle of  $\phi$ . Except for the phase delay and advance observed in the  $\theta_{n1}$  and  $\theta_{n2}$  curves, the net slope of the  $\theta_{n1}$ ,  $\theta_{n2}$ , and  $\theta_{n3}$  curves in our study approximated a negative slope of -1, demonstrating a type 1 (weak) resetting effect of swallowing on breathing in goats <sup>(29, 31)</sup>. This is also in contrast to a type 0 resetting observed in the breathing of anesthetized cats by different levels of superior laryngeal nerve stimulation <sup>(26)</sup>.

#### *EI and IE Transitions and Swallows*

The transition from EI or IE has been suggested as a critical period during the respiratory cycle <sup>(7, 23, 24, 26, 27)</sup>. Dick et al. <sup>(7)</sup> observed in decerebrate, unanaesthetized cats that superior laryngeal nerve stimulation during EI and IE transitions, consistently produced more swallows during EI transition and the least during IE transition which is consistent with our observations of spontaneous swallows. They interpreted the occurrence of the swallows during phase transitions as points of interactions between the respiratory and swallowing pattern generators in which medullary post-inspiratory neurons provide a possible neural substrate for this interaction. The definition of the IE transition period in our study

extended from the observed occurrence of the thyroarytenoid activity in goats <sup>(10)</sup>, which begins immediately following peak inspiratory activity. During eupnea, classifying swallows within the IE period (Figure 1A) coincides with the abrupt depolarization of the medullary post-inspiratory neurons. Too few swallows were seen during the IE transitions to interpret their effect, except to infer that the natural probability of swallows during this period is greatly reduced. The lack of swallows during the early phase of expiration also coincides with the repolarization of these medullary neurons in phase I of expiration. The observed values for timing and output for swallows during the EI transition were halfway between the observed values for swallows during expiration and inspiration. Immediately following swallows during EI transition,  $T_I$  and  $V_T$  were reduced, while there was no effect on  $DIA_{Peak}$ . Similar to the findings with swallows during inspiration,  $V_T$  in the subsequent breaths was increased while  $T_I$  and  $DIA_{Peak}$  were not changed. Therefore, these results support the concept that EI and IE transitions are critical periods of interaction between the respiratory and swallowing centers for timing but not respiratory output in goats. These results also suggest, that the underlying neural substrate integrating superior laryngeal stimulation and swallowing and the respiratory pattern generator in goats compared to humans and cats, is functionally different. However, to address these findings more conclusively, we believe that a combination of within- and multi-breath analyses of swallowing and breathing are essential to provide an in-depth view of the nature and type of relationship between the two generators under various physiological conditions and using a wide range of whole and reduced animals preparations.

### Conclusions

Our study shows that swallowing produces a phase-dependent resetting of the respiratory rhythm generator in goats. Furthermore, the data suggest that the interaction between swallowing and breathing occurs before and after a swallow. We suggest that the interrelationship between the pattern generators is functionally diverse in different species and may involve not just the pattern generators directly by also a diversity of peripheral feedback mechanisms and their integration centrally.

### **Acknowledgments**

The authors thank Nancy Schlick, Julie Wenninger and Alex Serra for their technical assistance, and Donna Dale for help in preparation of the manuscript.

This study was supported by National Heart, Lung, and Blood Institute Grant 25739 and Veterans Administration.

**References**

1. Amri M., A. Car, A. Jean. Medullary control of the pontine swallowing neurones in sheep. *Exp. Brain Res.* 55(1):105-10, 1984.
2. Anderson C. A., T. E. Dick, J. Orem. Swallowing in sleep and wakefulness in adult cats. *Sleep.* 18(5):325-9, 1995.
3. Bartlett, D. Upper airway motor systems. In: Cherniack, N.S., Widdicombe, J.G. (Eds.) *The Handbook of Physiology, Section 3: The Respiratory System. Vol II: Control of Breathing, part 1.* American Physiological Society, Bethesda, MD, pp. 233-245, 1986.
4. Bianchi, A. L., M. Denavit-Saubié, and J. Champagnat. Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. *Physiol. Rev.* 75; 1-26, 1995.
5. Clark, G. A. Deglutition apnoea. *J. Physiol. (Lond.)* 54: 59, 1920.
6. Crelin, E. S. *Functional anatomy of the Newborn.* New Haven CT: Yale University Press, 1973.
7. Dick T. E., Y. Oku, J. R. Romaniuk, N. S. Cherniack. Interaction between central pattern generators for breathing and swallowing in the cat. *J. Physiol. (Lond.)* 465:715-30, 1993.
8. Doty, R. W. Neural organization of deglutition. In: *Handbook of Physiology. Alimentary Canal, Motility.* Washington, DC: Am. Physiol. Soc., 1988, sect. 6 vol. IV, p. 1861-1902.
9. Doty, R. W., and J. F. Bosma. An electromyographic analysis of reflex deglutition. *J. Neurophysiol.* 19:44-60, 1956.
10. Feroah, T.R., H.V. Forster, L.G. Pan, T. Rice. Reciprocal activation of hypopharyngeal muscles and their effect on upper airway area. *J. Appl. Physiol.* 88:611-626, 2000.
11. Harding, R., D. A. Titchen. Oesophageal and diaphragmatic activity during sucking in lambs. *J. Physiol. (Lond.)* 321:317-29, 1981.
12. Jean, A., and A. Car. Inputs to the swallowing medullary neurons from the peripheral afferent fibers and the swallowing cortical area. *Brain Res.*, 178:567-573, 1979.

13. Jean, A., A. Car, and J. P. Kessler. Brainstem organization of swallowing and its interaction with respiration. In *'Neural Control of the Respiratory Muscles'*, eds. CRC Press. 1997.
14. Jean A. Control of the central swallowing program by inputs from the peripheral receptors: A review. *J. Autonom. Nerv Sys.* 10(3-4):225-33, 1984.
15. Jean A. Localisation et activité des neurones déglutiteurs bulbaires. *J Physiol. (Paris)*. 64(3): 227-68, 1972.
16. Jean, A. Brainstem organization of the swallowing network. *Brain Behav. Evol.* 25(2-3):109-16, 1984.
17. Issa, F. G., S. Porostocky. Effect of continuous swallowing on respiration. *Resp. Physiol.* 95(2):181-93, 1994.
18. Kawasaki, M., J. H. Ogura, S. Takenouchi. Neurophysiologic observations of normal deglutition. I. Its relationship to the respiratory cycle. *Laryngoscope.* 74; 1747-1765, 1964.
19. Lewis, J., M. Bachoo, C. Polosa, and L. Glass. The effects of superior laryngeal nerve stimulation on the respiratory rhythm: phase-resetting and after effects. *Brain Res.* 517:44-50, 1990.
20. McFarland D. H., J. P. Lund. An investigation of the coupling between respiration, mastication, and swallowing in the awake rabbit. *J. Neurophysiol.* 69(1):95-108, 1993.
21. Miller, F. R., and C. S. Sherrington. Some observations on the buccopharyngeal stage of reflex deglutition in the cat. *Q. J. Exp. Physiol.* 9; 147, 1916.
22. Negus, V. E. The mechanism of swallowing. *J. Laryngol. Otol.* 58:46-59, 1943.
23. Nishino T. Yonezawa T. Honda Y. Effects of swallowing on the pattern of continuous respiration in human adults. *Am. Rev Respir. Dis.* 132(6):1219-22, 1985.
24. Nishino T., K. Hiraga. Coordination of swallowing and respiration in unconscious subjects. *J. Appl. Physiol.* 70(3):988-93, 1991.
25. Oku Y. Dick TE. Cherniack NS. Phase-dependent dynamic responses of respiratory motor activities following perturbation of the cycle in the cat. *J. Physiol. (Lond)*. 461:321-37, 1993.

26. Paydarfar, D., F. L. Eldridge, and J. P. Kiley. Resetting of mammalian respiratory rhythm: existence of phase singularity. *Am. J. Physiol.* 250(19): R721-R727, 1986.
27. Paydarfar, D., R. J. Gilbert. C. S. Poppel, P. F. Nassab. Respiratory phase resetting and airflow changes induced by swallowing in humans. *J. Physiol. (Lond)*. 483(1):273-88, 1995.
28. Peiper, A. *Cerebral Function in Infancy and Childhood*. New York: Consultants Bureau, 1963.
29. Tass, P. A. *Phase Resetting in Medicine and Biology: stochastic modeling and data analysis*. Springer-Verlag, New York. 1999.
30. Wilson SL. Thach BT. Brouillette RT. Abu-Osba YK. Coordination of breathing and swallowing in human infants. *J. Appl. Physiol.* 50(4):851-8, 1981
31. Winfree A. T. *The Geometry of Biological Time*. Springer-Verlag, New York. 1990.

Table 1. Intact vs. Tracheostomy goats and general swallowing data.

Goat	Tracheostomy	Number of swallows	Swallows * min <sup>-1</sup> (mean ± Std)
1	Yes	42	1.87 (± 0.42)
2	Yes	72	2.28 (± 0.93)
3	Yes	47	1.58 (± 1.01)
4	Yes	58	1.70 (± 1.30) *
5	No	123	3.51 (± 1.23) *
6	No	108	3.09 (± 1.02)
7	No	190	2.11 (± 1.66) *
8	No	76	2.17 (± 1.13)
9	No	186	4.97 (± 1.56)
10	No	174	5.14 (± 1.91)
11	No	109	2.88 (± 1.34) *
12	No	282	2.02 (± 1.11)
13	No	150	4.12 (± 1.97) *

\* swallowing data for this animal was not normally distributed as determined by Kolmogorov-Smirnov test.

Table 2. Inspiratory Gamma Analysis

	<i>n</i>	n-1			N		
		Slope	Intercept	R <sup>2</sup>	Slope	Intercept	R <sup>2</sup>
<b>T<sub>E</sub></b>	240	0.02 (± 0.03)	99.4 (± 1.84)	0.002	-0.03 (± 0.03)	98.6 (± 1.56)	0.005
<b>T<sub>I</sub></b>	240	0.02 <sup>†</sup> (± 0.02)	100 (± 1.23)	0.004	0.37* <sup>†</sup> (± 0.03)	110 <sup>‡</sup> (± 1.82)	0.368
<b>V<sub>T</sub></b>	240	0.03 <sup>†</sup> (± 0.03)	97.1 (± 1.39)	0.008	0.23* <sup>†</sup> (± 0.03)	88.8 <sup>‡</sup> (± 1.65)	0.21
<b>DIA<sub>Peak</sub></b>	121	0.05 (± 0.07)	96.5 (± 3.16)	0.006	0.04 (± 0.06)	94.2 <sup>‡</sup> (± 2.86)	0.003

All results are presented as average (± standard deviation)

\* Slope is significantly different from zero (p < 0.01)

<sup>†</sup> Slopes (n-1 vs. n) are significantly different from each other. (p < 0.01)

<sup>‡</sup> Y intercepts are significantly different from control (100%). (p < 0.02)

Table 3. Expiratory Gamma Analysis

	<i>n</i>	n-1			N		
		Slope	Intercept	R <sup>2</sup>	Slope	Intercept	R <sup>2</sup>
<b>T<sub>E</sub></b>	259	0.08* <sup>†</sup> (± 0.02)	94.49 (± 2.16)	0.046	0.42* <sup>†</sup> (± 0.05)	71.42 <sup>‡</sup> (± 4.2)	0.238
<b>T<sub>I</sub></b>	259	-0.10 * <sup>†</sup> (± 0.02)	109.8 <sup>‡</sup> (± 2.1)	0.068	-0.001 <sup>†</sup> (± 0.03)	93.82 <sup>‡</sup> (± 2.74)	0.001
<b>V<sub>T</sub></b>	259	-0.02 (± 0.03)	98.96 (± 2.34)	0.003	-0.001 (± 0.04)	88.26 <sup>‡</sup> (± 3.45)	0.000
<b>DIA<sub>Peak</sub></b>	194	0.01 (± 0.05)	97.6 (± 4.46)	0.001	-0.03 (± 0.05)	98.5 (± 4.88)	0.002

All results are presented as average (± standard deviation)

\* Slope is significantly different from zero (p < 0.01)

<sup>†</sup> Slopes are significantly different from each other. (p < 0.01)

<sup>‡</sup> Y intercepts are significantly different from control (100%). (p < 0.05)

Table 4. Non-Linear regression analysis of phase response curves

$\theta$	a	b	c	d	Sy.x	R <sup>2</sup>
<b>n-1</b>	0.03	-1.27	0.51	-0.28	0.07	0.97
<b>n1</b>	0.95	0.30	-2.90	1.71	0.09	0.95
<b>n2</b>	1.90	0.82	-4.12	2.42	0.134	0.90
<b>n3</b>	2.87	0.97	-4.36	2.53	0.158	0.87

### Figure Legends

Figure 1: Categorization and Examples of Swallows. (A) solitary swallows with no swallows in the prior or following breaths were classified into one of four categories based on when they occurred during the breath:  $SW_E$ , from the end of the previous inspiration to 0.1 sec before the start of the next inspiration;  $SW_{EI}$ , from 0.1 sec prior to and 0.15 sec following the start of inspiration;  $SW_I$ , from 0.15 sec following the start of inspiration to 0.1 sec prior to the end of inspiration;  $SW_{IE}$ , from 0.1 sec prior to the end of inspiration. The percent of control respiratory values evaluated in this study were compared for  $SW_E$ ,  $SW_{EI}$ ,  $SW_I$ , and  $SW_{IE}$  on the breath prior (n-1), during (n), and after (n+1) the swallows (see Methods). (B) Examples of solitary  $SW_E$  and  $SW_I$ : BP, blood pressure; V, inspiratory flow; TP, thyropharyngeus;  $TP_{MTA}$ , moving time average of TP; SP, stylopharyngeus;  $SP_{MTA}$ , moving time average of SP; DIA, diaphragm;  $DIA_{MTA}$ , moving time average of DIA. Dotted lines (a and c) mark the beginning of the swallows. Notice the electrical activity in DIA during expiration just prior to and after the start of the swallow. This activity in the DIA is often associated with a brief inspiratory flow and is termed *Schluckatmung* or a “swallow breath”. Arrows indicate a short absence of DIA activity either during a *Schluckatmung* (b) or an inspiration (d). (C) Examples of solitary  $SW_{EI}$  and  $SW_{IE}$ . Labels same as Panel B. Dotted lines indicate the beginning of the swallows.

Figure 2: Illustration of inspiratory and expiratory Gamma Analysis. (A) Inspiratory Gamma Analysis.  $\gamma_I$  was calculated as the period of time from the beginning of inspiration to the start of the swallow during inspiration. (B) Expiratory Gamma Analysis.  $\gamma_E$  was calculated as the period of time from the beginning of expiration to the start of a swallow during expiration.  $T_I$ ,  $T_E$ ,  $V_T$ , and  $DIA_{Peak}$  for the breath prior to and during the swallow were calculated. Control values were calculated from the two prior to and one following the breaths used in this analysis.

Figure 3: Phase response analysis. In this analysis, a set of six consecutive breaths (without movements and augmented breaths) was evaluated, with a solitary swallow in the fourth breath. (A) The old phase ( $\phi$ ) was calculated from the start of inspiration to the start of the swallow. The co-phases

( $\theta$ ) were calculated from the start of the swallow to the start of inspiration of the previous breath ( $\theta_{n-1}$ ), and the start of the three subsequent breaths ( $\theta_{n1}$ ,  $\theta_{n2}$ ,  $\theta_{n3}$ , respectively). In a hypothetical model in which swallowing had no effect on respiration rhythm, the normalized measures of  $\phi$  and  $\theta$  (expressed as a fraction of control) would be equal to one ( $\phi + \theta = 1$ ). (B) A plot of subsequent  $\theta_n$  vs.  $\phi$  in which a family of swallows that occurred during respiration had no effect on respiratory rhythm. In this hypothetical example, the slope of  $\theta_{n1}$  vs.  $\phi$  would be  $-1$ , and the effect on any subsequent breaths ( $n$ ) would also be anticipated by the equation:  $\theta_n = -\phi + n$ . As a result, the slope of the relationship between the onset of swallowing and respiration rhythm would be represented by series of linear parallel lines with a slope of  $-1$ .

Figure 4: Categorical analysis of respiratory data for different phases of respiration for the breath prior to ( $n-1$ ), during ( $n$ ), and after ( $n+1$ ) the swallow. The phase of respiration in which the swallows occurred is defined in the Methods and Figure 1A. The number of swallows analyzed for each category:  $SW_E = 317$ ,  $SW_{EI} = 296$ ,  $SW_I = 507$ , and  $SW_{IE} = 8$ . (A)  $T_I$ , (B)  $T_E$ , (C)  $T_{Tot}$ , (D)  $V_T$ , (E)  $T_I/T_{Tot}$ , (F)  $V_T/T_I$ , (G)  $DIA_{Peak}$ . All values are expressed as a percent of control and presented as means with standard deviation bars. The control values for each animal were estimated from breaths without artifacts or swallows as defined in the Methods, and used to normalize each animal's individual observation. \* Means are significantly different from control ( $P < 0.01$ ). † Means are significantly different from  $n$  breath ( $P < 0.01$ ).

Figure 5: Gamma Inspiratory ( $\gamma_I$ ) Analysis of respiratory data. (A)  $T_E$ , (B)  $T_I$ , (C)  $V_T$ , (D)  $DIA_{Peak}$ . 240 swallows qualified as defined in the Methods and Figure 2A. Fewer events with an adequate  $DIA_{Peak}$  data were observed and presented ( $n = 121$ ). Each value is presented as a percent of control of the individual animal control value.

Figure 6: Gamma Expiratory ( $\gamma_E$ ) Analysis of respiratory data. (A)  $T_E$ , (B)  $T_I$ , (C)  $V_T$ , (D)  $DIA_{Peak}$ . The number of swallows as defined for this analysis in the Methods and Figure 2B in the graphs for  $T_E$ ,  $T_I$ , and  $V_T$  were 259. The number of observations for  $DIA_{Peak}$  was 194. Each value is presented as a percent of control of the individual animal's control value.

Figure 7: The distribution of swallows throughout normalized respiration and the phase response analysis of the effect of the onset of swallowing respiratory timing. A total of 427 swallows meet the criteria for this analysis as presented in the Methods. (A) Swallows as a percent total swallows is presented relative to their onset during a normalized respiratory cycle ( $\phi$ ). The average transition from inspiration to expiration (IE) was approximately  $0.47 \pm 0.006$  of  $\phi$ . (B) The individual values for the phase response analysis of the co-phase for the breath prior to ( $\theta_{n-1}$ ), during ( $\theta_{n1}$ ), and two ( $\theta_{n2}$ ,  $\theta_{n3}$ ) after a swallow is presented. The solid line for  $\theta_{n-1}$ ,  $\theta_{n1}$ ,  $\theta_{n2}$ , and  $\theta_{n3}$  represents the best-fit curves for the non-linear regression analysis (Table 4). The formulas for the calculations of  $\phi$ ,  $\theta_{n-1}$ ,  $\theta_{n1}$ ,  $\theta_{n2}$ , and  $\theta_{n3}$  are presented in the Methods and Figure 3A. (C) The changes in  $T_I/T_{Tot}$  for swallows at various  $\phi$  and the changes in the breath prior to (n-1), during (n1), and two after (n2 and n3).  $T_I/T_{Tot}$  values are expressed as a fraction of control breaths as defined in the Methods. Because of the effect of swallowing to cause phase delay in the respiratory rhythm, values greater than 1.0 was observed for  $\phi$ .

Figure 8: Changes in measures of respiratory output for swallows at various  $\phi$  in the breath prior to (n-1), during (n1), and two after (n2 and n3). (A)  $V_T(n) * V_T(c)^{-1}$ . (B)  $DIA_{Peak}(n) * DIA_{Peak}(c)^{-1}$ . Values are expressed as a fraction of control breaths as defined in the Methods. Because of the effect of swallowing to cause phase delay in the respiratory rhythm, values greater than 1.0 were observed for  $\phi$ .

Figure 9: The differences in co-phases ( $\theta_{n-1}$ ,  $\theta_{n1}$ ,  $\theta_{n2}$ , and  $\theta_{n3}$ ) from the previous co-phase or a co-phase ( $\theta_{Ideal}$ ) in which swallowing would not have an effect on respiratory rhythm. All values are expressed as a fraction of percent change. As described in the Methods, the expected difference between  $\theta_{n-1}-\theta_{Ideal}$  is zero (0, dotted lines). However, the expected difference between the measured co-phases if swallowing had no effect on respiratory timing would be one (1; dotted lines). The individual differences were calculated and averaged over 0.05 periods of  $\phi$ . The means and standard deviation bars are presented for each 0.05 periods of  $\phi$  and compared to the expected. As mentioned in Figure 7 and 8, values greater than 1.0 were observed for  $\phi$  because of

the effect of swallowing to cause phase delay in the respiratory rhythm. \* Means are significantly different from control ( $P < 0.01$ ).

Figure 1

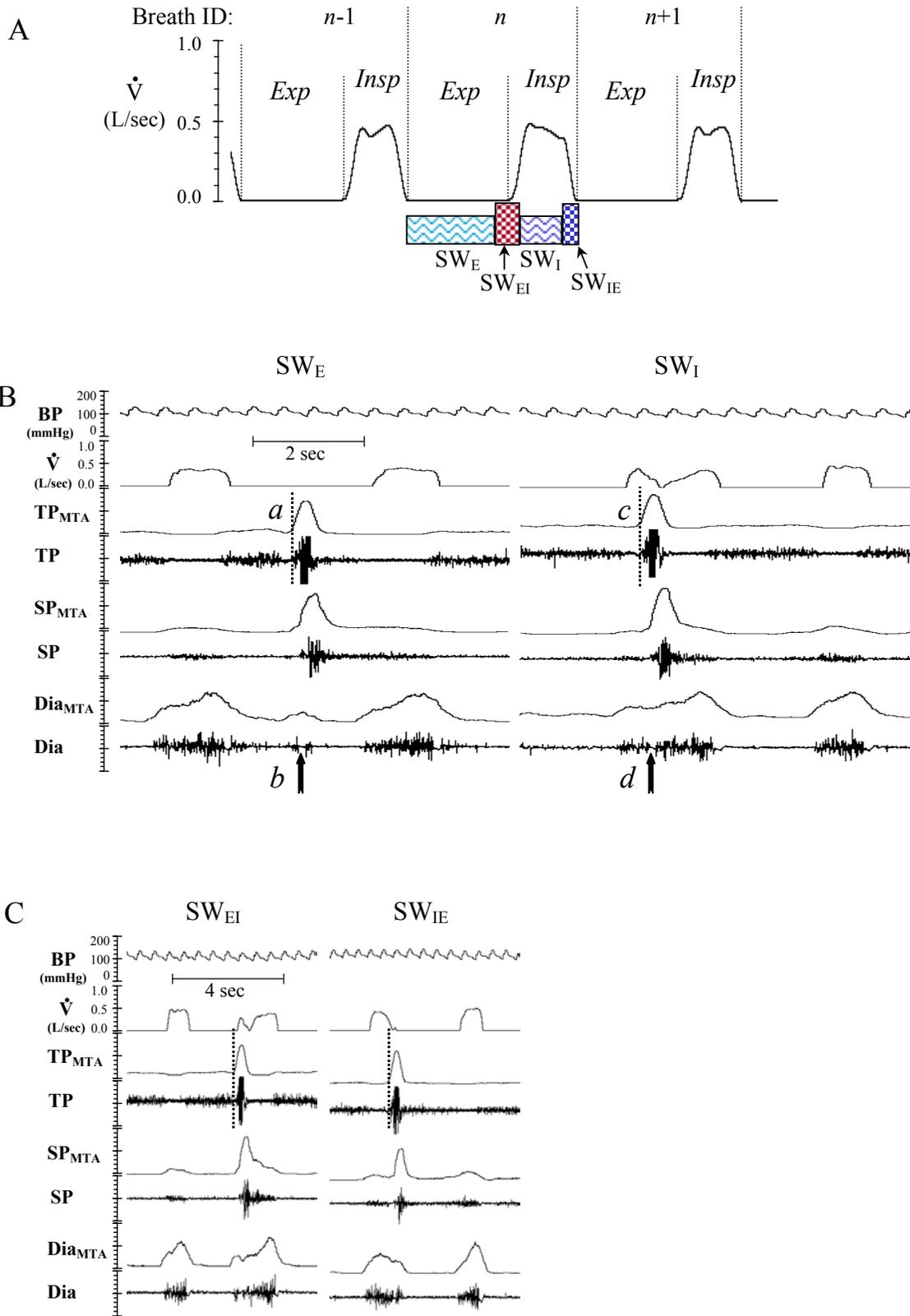


Figure 2

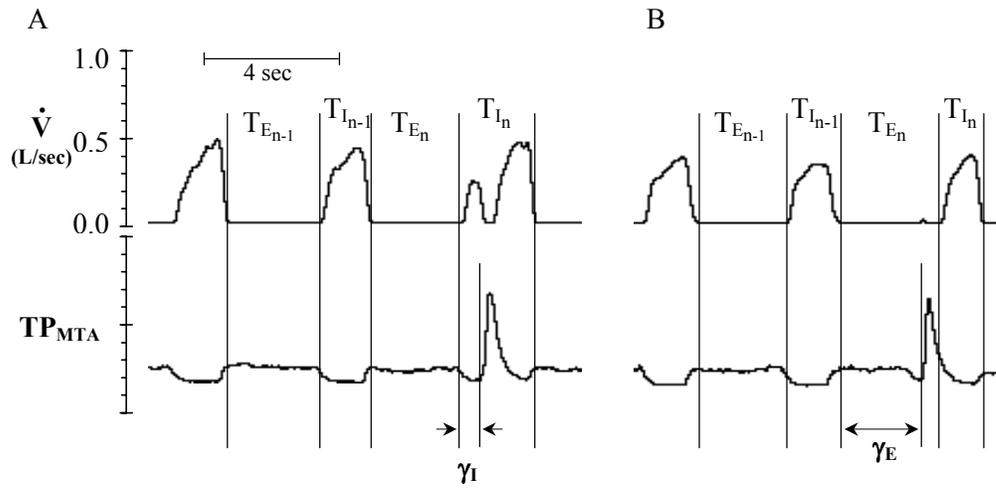


Figure 3

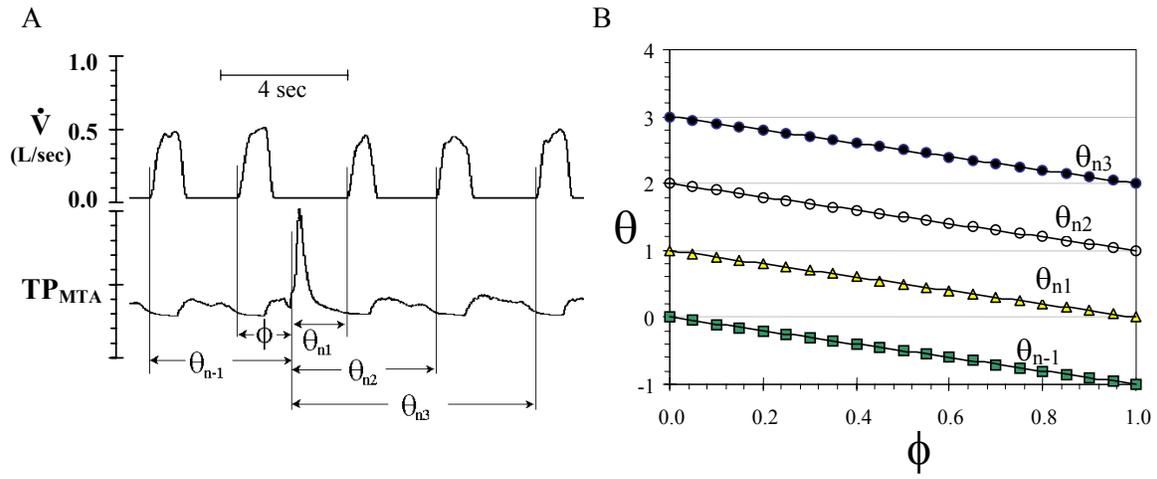


Figure 4

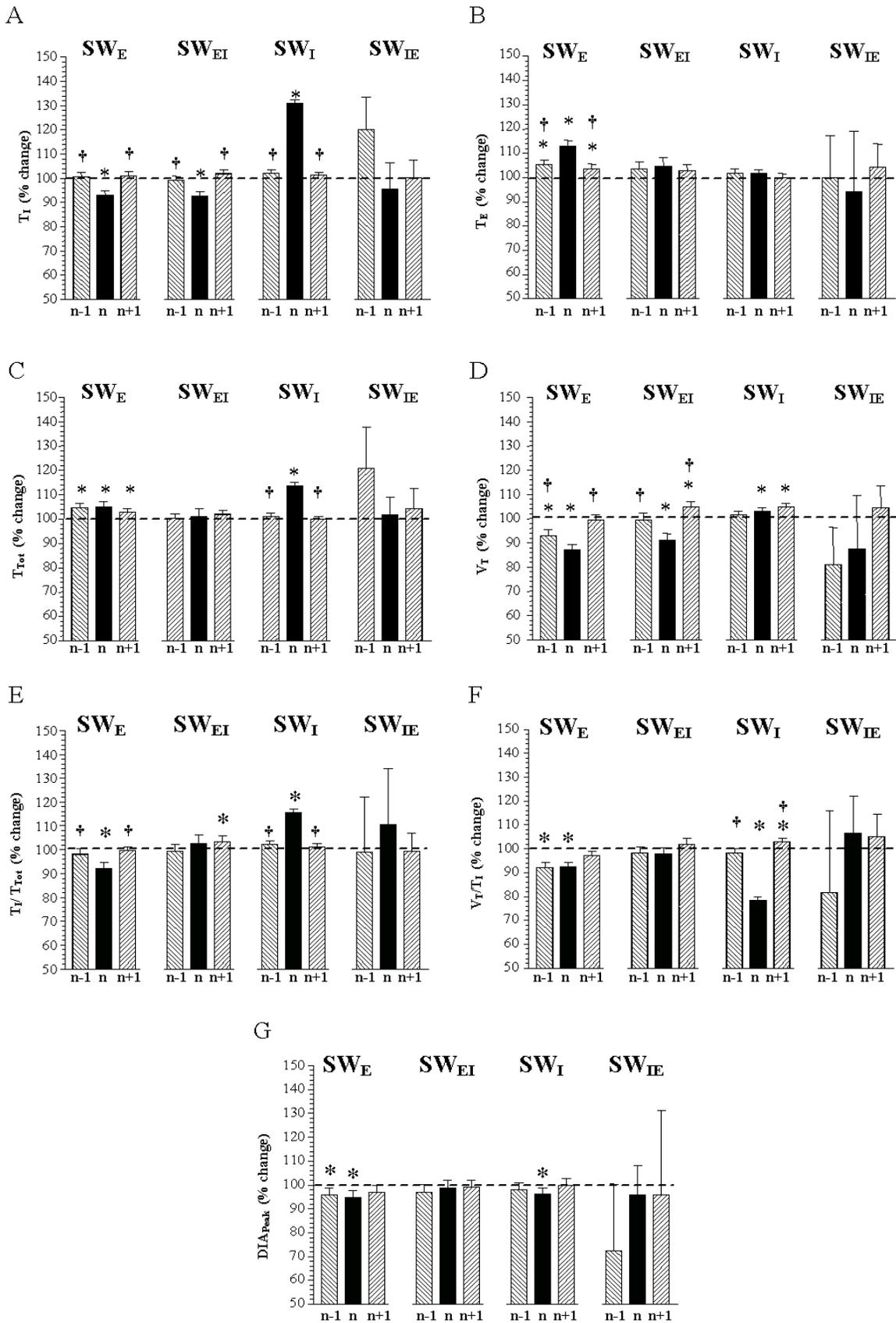
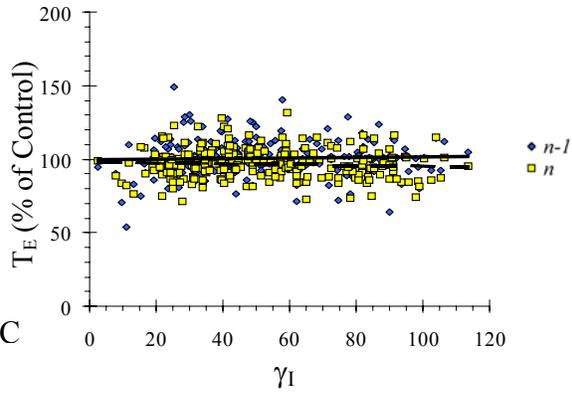
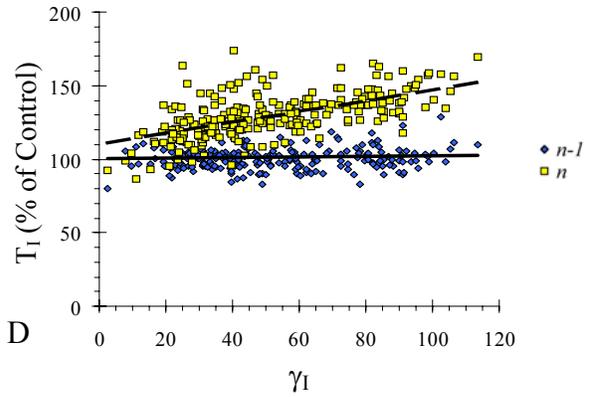


Figure 5

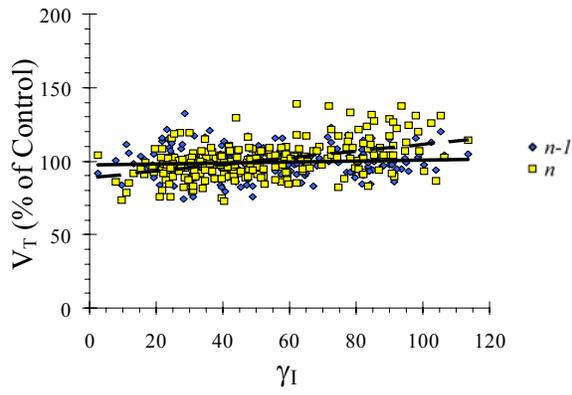
A



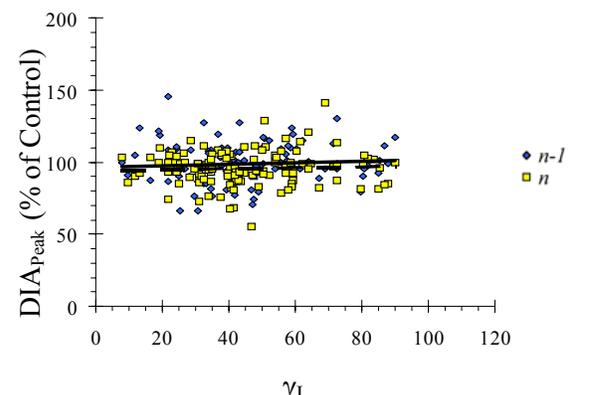
B



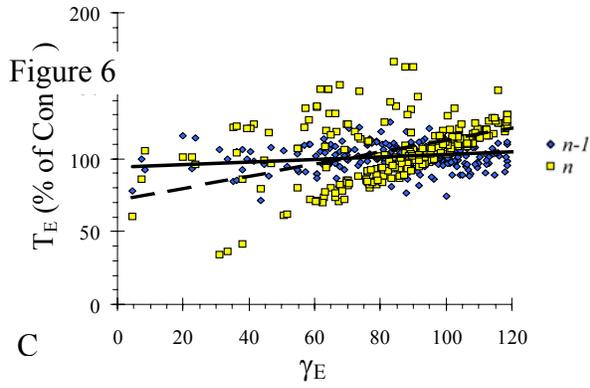
C



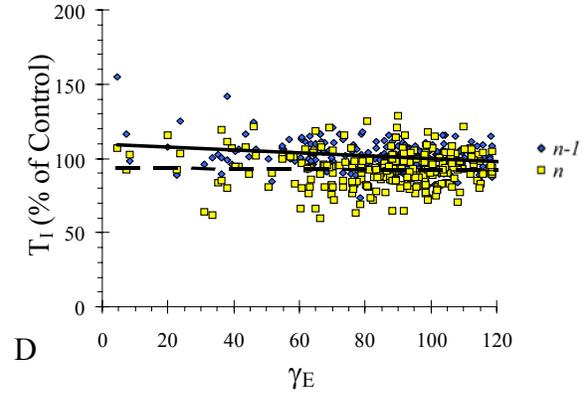
D



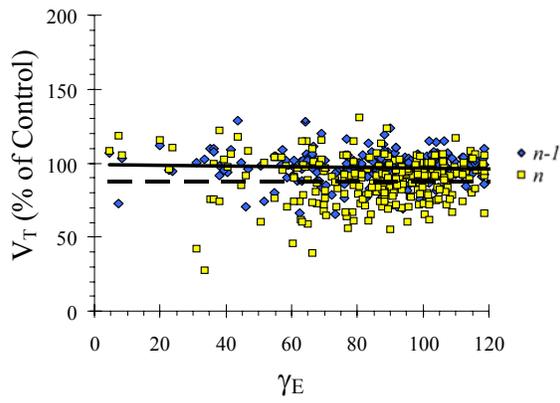
A



B



C



D

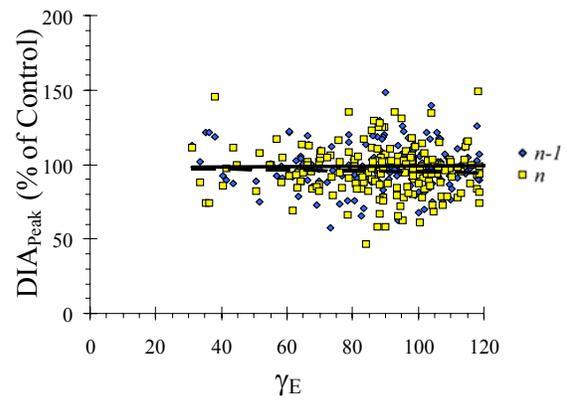


Figure 7

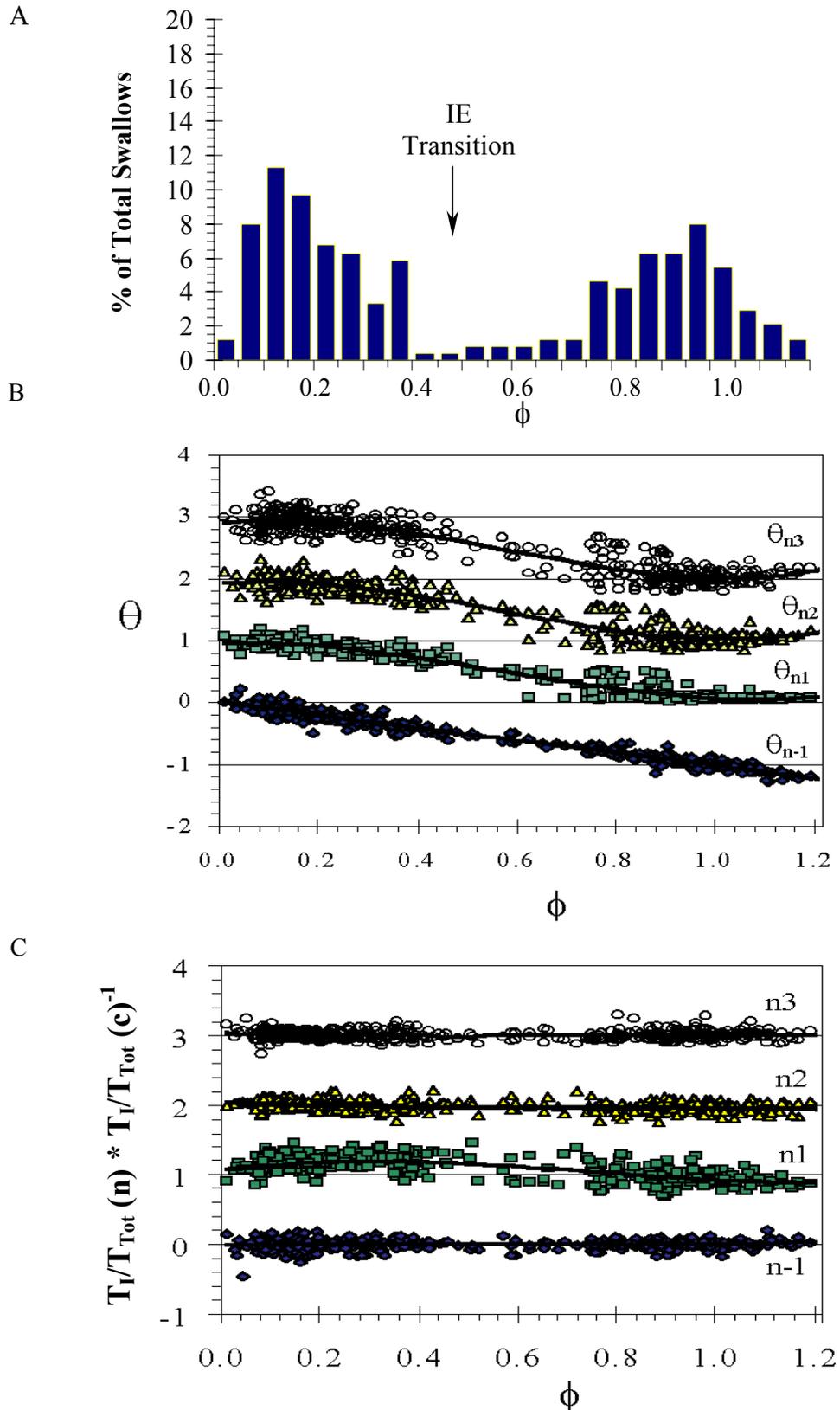


Figure 8

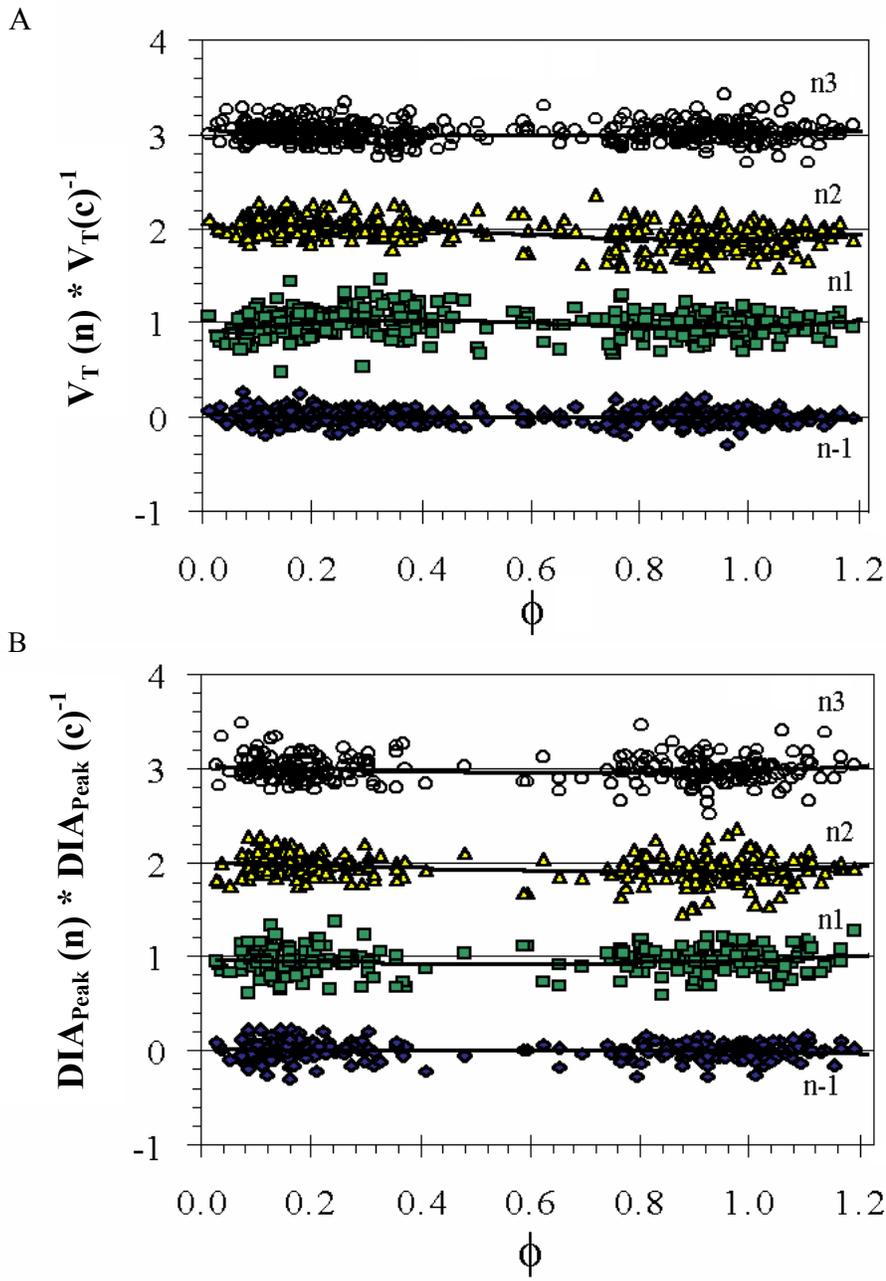


Figure 9

