THE INVESTIGATION OF K-COMPLEX

The Investigation of K-Complex and Vertex Sharp Wave Activity in Response to Mid-Inspiratory Occlusions and Complete Obstructions to Breathing During NREM Sleep

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Study Objectives: To determine whether the cortical response to mid-inspiratory occlusions can be used as a model of the cortical response to obstructive events during sleep; and to determine whether the vertex sharp wave (VSW) and K-complex are exclusivecontributors to the N350 and N550 components respectively of the stage 2 sleep event-related potential.

Design: Two types of respiratory stimuli were used to elicit evoked potential responses during stage 2 NREM sleep. These were mid-inspiratory occlusions and complete breath obstructions. Trials were grouped according to the type of phasic response elicited; isolated K-complex (KC), VSW associated with a K-complex (VSW/KC), isolated VSW, and no evoked response (other). Evoked responses were averaged separately within these categories.

Setting: Data were collected in the University of Melbourne Sleep Laboratory.

Participants: Six young healthy male adults.

Interventions: N/A

Measurements and Results: Data were recorded from 29 scalp sites referenced to linked ears. Mask pressure (Pm) and airflow were also recorded. Intra-thoracic pressure, as indicated by Pm, reached a more negative level following complete obstructions than brief occlusions. However, both types of respiratory stimuli elicited the two late latency components. Although latency varied across the two respiratory conditions in a manner consistent with the intra-thoracic pressure rise time differences, the elicitation characteristics and topographic distribution of these components did not vary across the two types of stimuli. In addition, an N350 was only present in the average for those categories that included VSWs, while an N550 was only present in those categories that contained K-complexes.

Conclusions: Mid-inspiratory occlusions can be used as a model of obstructive events. VSWs contribute exclusively to the N350 component, while K-complexes contribute exclusively to the N550 component.

Key words: K-complex; vertex sharp wave; N350; N550; sleep; evoked potentials

INTRODUCTION

RECENT STUDIES HAVE INVESTIGATED CORTICAL RESPONSES TO EXPERIMENTALLY INDUCED INSPIRATORY OCCLUSIONS. These responses, when averaged, produce a pattern of activation known as the respiratory-related evoked potential (RREP). During NREM sleep, the RREP consists of a series of early and late components. The two early components1,2 are thought to reflect activation of primary somatosensory and supplementary motor cortices.3 The dominant features of the RREP during NREM sleep are two late negative components, the N350 and N550.2,4,6

To date, RREP studies performed during sleep have used occlusion2,4,6 or negative pressure7 stimuli, introduced as interruptions to inspiration. This differs from clinical conditions in which obstructions (apnea) or increases in upper airway resistance (hypopnea and upper airway resistance syndrome) are experienced from the commencement of each breath.

The only study to investigate respiratory obstructions presented prior to the onset of inspiration has done so during wakefulness.8 These authors compared Evoked Potentials (EPs) elicited by mid-inspiratory occlusions to those elicited by obstructions presented prior to the onset of inspiration. They reported that the early positive peak of the RREP (P1) was longer in latency and smaller in amplitude when elicited by obstructions presented prior to the commencement of inspiration. This finding was interpreted as being due to differences in both the rise time and intensity of the pressure profiles produced in each condition. Mid-inspiratory occlusions demonstrated a faster rise time and greater peak intra-thoracic pressure response compared to onset obstructions. This result has not however been extended to other components or to responses seen during sleep.

Auditory studies have investigated the effect of stimulus rise time on the EP response during sleep. Bastien and Campbell9 reported that K-complexes were easier to elicit using high-intensity fast rise-and-fall time stimuli than low-intensity slow rise-and-fall time stimuli. However, these authors observed no amplitude or latency difference on the N550 component between these two conditions. These data are important in the context of the respiratory occlusion stimulus paradigm, as RREP studies conducted during sleep have used an occlusion stimulus with a rapid
onset. This is different to what would be expected when breathing against an already blocked airway.

The first aim of this study was to compare the EP response elicited by mid-inspiratory occlusions to the more ecologically valid method of presenting occlusions beginning prior to inspiration, and lasting the entire breath, in a group of subjects free from respiratory problems.

The N550 component of the auditory evoked potential (AEP) has been shown to be due to the inclusion of K-complex responses in the average. This relationship has also been shown for the N550 in the RREP. The AEP N350 has also been thought to be related to K-complexes, although a number of studies have reported an N350 in the average of trials not containing any K-complexes. It has been suggested that the N350 may represent a K-complex trigger, producing a K-complex once a certain N350 amplitude threshold has been reached. Furthermore, a suggestion by Harsh et al. that the N350 may relate to vertex sharp waves (VSW) has recently been confirmed for both AEP and RREP N350 components.

The N550 has a frontally maximum distribution whereas the N350 has a vertex maximum distribution. This difference in topographical distribution supports the view that different intracranial generators produce the N350 and N550 components. However, the possibility that the N350 is exclusively associated with VSWs and the N550 with K-complexes, has not been tested.

Thus, the second aim of this study was to determine whether K-complexes and VSWs are exclusive in their contribution to the N550 and N350 components respectively. It was also of interest to determine whether the N350 component does indeed represent a K-complex trigger.

METHOD

Subjects and Design

The subjects were six males (mean age 21.8±2.50 years). All were healthy non-smokers who were free from sleep-related respiratory disorders. Subjects were studied over two to four non-consecutive nights in the sleep laboratory. Two types of respiratory stimuli were applied during stage 2 NREM sleep. These were mid-inspiratory occlusions, lasting for one second, and complete obstructions to breathing, that were presented prior to inspiration and lasted an entire breath. For both types of respiratory stimuli, each individual trial was categorized according to one of four different conditions depending to the type of phasic response that was visually identified (see below). The study was approved by the University of Melbourne’s Human Ethics Committee, and subjects gave written informed consent prior to participation.

PROCEDURES

General Laboratory Procedures

Subjects were asked to refrain from consuming caffeine or alcohol on the day prior to each sleep session and were required to maintain a supine position during data collection. As K-complex and VSW activity are both present and easy to identify within stage 2 sleep, the experimental protocol was conducted exclusively during this stage.

Application of Respiratory Stimuli

Subjects wore a facemask (Hans Rudolph Series 7940) that was positioned so that subjects could comfortably respire with minimal facial muscle activity. A silicone rubber seal (Hans Rudolph Ultimate Seal) was placed between the mask and the face of the subject to eliminate the possibility of a mask leak. The mask was secured using a head strap and was attached to a two-way non-rebreathing valve (Hans Rudolph Series 2600). The mask and breathing valve had a dead space of approximately 120ml. Inspiratory airflow was measured using a heated Morgan pneumotachograph placed in the inspiratory line, and connected to a differential pressure transducer (Validyne DP45-14), with the output converted to a voltage signal using a carrier demodulator (Validyne CD15). The inspiratory port of the non-rebreathing valve was connected to approximately three meters of tubing, which was connected to a two-way stopcock in an adjacent room. The stopcock allowed either breathing of normal room air or the application of an occlusion or obstruction. Inspiration was interrupted by the experimenter turning the stopcock to the occlusion setting during either the early part of inspiration or during expiration (prior to an inspiratory effort), as determined from the flow signal. The use of the stopcock, as opposed to a pressure activated occlusion valve, meant that both occlusion and obstruction stimuli could be applied without an associated auditory component.

Mid-inspiratory occlusions lasted approximately one second, with complete obstructions lasting an entire breath. The two respiratory conditions were presented in a counterbalanced fashion for blocks of 20 trials each across the night. That is, 20 occlusion trials were performed, followed by 20 obstruction trials. On subsequent nights, the order of initial stimulus presentation was reversed. There was a minimum of three breaths between stimulus presentations. Mask pressure (Pm) was monitored from a tap on the inspiratory port of the breathing mask using a second differential pressure transducer (Validyne DP45-14). This provided an index of the pressure inside the mask relative to ambient pressure. Given a patent airway, this signal becomes more negative with inspiration on a normal breath, and shows a substantially greater negative pressure when the inspiratory line is blocked. This latter shift is due to contraction of inspiratory muscles and expansion of the thoracic cavity, with a fixed volume of air present. Despite the use of the silicone seal, the experimenter still monitored the airflow signal for the possible presence of mask leaks. If this occurred, a new silicone seal was attached between the face of the subject and the mask.

Measurement of EEG, Sleep State, and Digital Recording

EEG activity was recorded (using an ECI electrocap) from 29 tin electrodes referenced to linked ears. (FP1, FP2, Fz, F3, F4, F7, F8, FCz, FC3, FC4, FT7, FT8, Cz, C3, C4, T3, T4, CPz, CP3, CP4, TP7, TP8, Pz, P3, P4, T5, T6, O1 and O2), based on the International 10/20 system. A vertical EOG was recorded from electrodes placed at the supra and infra orbital ridges of the right eye. A horizontal EOG was recorded from electrodes placed at the outer canthus of each eye. An electrode placed midway between Fz and Cz served as the ground. Electrode impedances were tested and maintained below 5 KΩ.

A Neuroscan system continuously recorded all EEG sites, the
vertical EOG, an airflow signal and Pm at a sampling rate of 1000Hz. The bandpass filters for EEG and Pm were set to 0.1—100 Hz. The C3, Oz, EEG, EOG, EMG, airflow signal and Pm activity were also recorded continuously on a Compumedics system at a sampling rate of 500Hz. Bandpass filters were set at 0.1—40 Hz for the EEG; 0.1—40 Hz for the EOG and 3—40 Hz for the EMG. These signals were used to determine sleep state using standard criteria.18

Data Reduction

Individual trials were excluded from the analysis if they demonstrated an arousal response according to AASM criteria.19 Periods in which respiration was disturbed or affected by body movements (as exhibited by EEG, EOG, and EMG recordings) were also excluded from the analysis.

Phasic Event and RREP Component Definition

For both types of respiratory stimuli, K-complexes were visually identified in the raw EEG using Rechtschaffen and Kales’ criteria. That is, “a waveform having a well delineated negative sharp wave which is immediately followed by a positive component. The total duration of the complex should exceed 0.5 sec.”19(p. 6). An amplitude criterion was also implemented in that the negative wave had to have an amplitude of at least 75mV at the Fz electrode site. Vertex sharp waves were visually identified as a large negative sharp wave that had a duration of less than 500ms. The negative wave also had to be preceded and followed by a positive wave. The amplitude had to be at least 50mV at Cz and be maximal at the vertex. Examples of each response type are illustrated in Figure 1.

For both types of respiratory stimuli, each individual trial was categorized according to one of four different conditions according to the type of phasic response that was visually identified. To be included in the analysis, the phasic response had to appear within 1 second of stimulus onset for the occlusion stimulus and between 1.0—2.5 seconds following stimulus onset for the complete obstructive stimulus. These were 1) KC—where only a K-complex was identified; 2) VSW/KC—both a VSW and K-complex were identified; 3) VSW—only a VSW was identified; 4) “other”—no clear evoked response was identified.

Due to the substantial variation in latency in the average evoked response to the two types of respiratory stimuli (see RESULTS), the terms “N350” and “N550” were used to describe the appearance of the first and second late latency components in response to mid-inspiratory occlusions. The terms “N350-type” and “N550-type” were used to describe the appearance of the first and second late latency component in response to complete obstructions. The N350 and N550 were defined as the minimum voltage that appeared between 250—450ms and 500—800ms post-stimulus respectively. The N350-type and N550-type were defined as the minimum voltage that appeared between 1300—1600ms and 1600—1900ms post-stimulus respectively.

Epoching of trials

The continuous Neuroscan files were epoched between 500ms pre-stimulus and 1300ms post-stimulus for mid-inspiratory occlusion trials and between 500ms pre-stimulus and 3000ms post-stimulus for complete obstruction trials. Mid-inspiratory occlusion trials were epoched using the flow signal, which proved to be an accurate method of time locking the evoked response to the onset of the stimulus. These trials were epoched from the beginning of the reduction in flow (which corresponded to the beginning of the increase in Pm). Due to the lack of flow associated with complete obstructions, these trials were epoched using the Pm signal. A computer-based algorithm was applied to the Pm signal that enabled determination of the onset of rise in mask pressure that was associated with application of the complete obstruction. Trials were then epoched according to the beginning of the rise in Pm.

Statistical Analyses

In the following analyses, “stimulus type” refers to the two types of respiratory stimuli: mid-inspiratory occlusions and complete obstructions; “phasic response condition” refers to the four types of responses observed on trials, based on the presence or absence of VSWs and K-complexes: KC, VSW/KC, VSW and “other” phasic responses; and “scalp site” refers to the 29 mapped scalp sites. Post hoc tests, where reported, were conducted using single factor ANOVA.

Evaluation of differences in scalp topography

In an ANOVA model that includes scalp site as one of the factors, the method often used to determine whether the scalp distribution differs between levels of another factor is to evaluate the interaction term in the model. Unfortunately this creates difficulties when using EEG amplitudes, as the assumption of ANOVA is that experimental effects are additive. The biophysical properties of EEG are such that effects tend to be multiplicative. To overcome this breach of assumption, McCarthy and Wood20 devised a scaling technique that normalises the amplitude data, allowing topographic differences to be assessed using the ANOVA interaction term. Therefore, any analysis that was designed to investigate topographical differences between particular conditions was conducted on scaled data using the McCarthy

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Figure 1—Example waveforms illustrating the different types of phasic responses. Data are presented from Cz and Fz. “VSW” illustrates a vertex sharp wave; “VSW/KC” a vertex sharp wave immediately followed by a K-complex; “KC” an isolated K-complex, and “other” a case where no phasic responses were produced. All examples were drawn from the one subject.
and Wood normalisation procedure.\textsuperscript{20} In addition, all probability levels were Huynh-Feldt corrected.

**Mask Pressure:**

The first analysis was conducted to investigate Pm differences. A two (stimulus type) by four (phasic response condition) repeated measures ANOVA was conducted on peak Pm amplitude and latency data respectively.

**N550 and N550-type components:**

The second analysis was conducted to investigate the N550 and N550-type components. N550 and N550-type amplitude were analyzed using a three factor repeated measures ANOVA [two (stimulus type) by two (phasic response condition) by 29 (scalp site)]. In addition, a two factor repeated measures ANOVA [two (stimulus type) by two (phasic response condition)] was also conducted on both amplitude and latency data at Fz. For these analyses, “phasic response condition” refers only to the phasic responses that demonstrated an N550 or N550-type component (KC and VSW/KC).

Differences between N550 (occlusions) and N550-type (obstructions) scalp topography were assessed by evaluating the stimulus type (2) by scalp site (29) interaction term in an ANOVA model conducted separately on the KC and VSW/KC phasic response conditions. Differences in N550 and N550-type scalp topography between the KC and VSW/KC conditions were assessed by evaluating the phasic response condition (2) by scalp site (29) interaction term in an ANOVA model conducted separately on mid-inspiratory occlusion and complete obstruction data. These analyses were conducted separately because the three-factor design did not allow us to compare the stimulus type by scalp site and phasic response condition by scalp site interactions independent of each other.

**N350 and N350-type component:**

As no clear N350-type component could be observed in the VSW or VSW/KC averaged response to complete obstructions, the third analysis investigated the N350 component in response to mid-inspiratory occlusion stimuli only. N350 amplitude was analysed using a two factor repeated measures ANOVA [two (phasic response condition) by 29 (scalp site)]. In addition a one-factor ANOVA with two levels (phasic response condition) was conducted on N350 amplitude and latency data at Cz. For these analyses, “phasic response condition” refers only to the phasic responses that demonstrated an N350 component (VSW and VSW/KC). In addition a matched pairs t-test was conducted on N350 amplitude and latency data at Cz in order to compare the N350 component between the VSW and VSW/KC phasic response conditions. Furthermore, exploratory analyses were conducted to examine the absence of an N350 component in the obstruction condition.

Differences in N350 scalp topography between the VSW and VSW/KC conditions were assessed by evaluating the phasic response condition by scalp site interaction term in an ANOVA model conducted on normalized mid-inspiratory occlusion data.

**RESULTS**

**Percentage of Occurrence**

The number of trials that resulted in an arousal type response, scored according to AASM criteria, was only slightly higher for complete obstructions (16.9%) than for mid-inspiratory occlusions.

**Table 1**—The means (and standard deviations) of the percentage of phasic responses to mid-inspiratory occlusions and complete obstructions. Percentages are based on the total number artifact-free responses.

<table>
<thead>
<tr>
<th>Component Type</th>
<th>KC % (VSD)</th>
<th>VSW/KC % (VSD)</th>
<th>VSW % (VSD)</th>
<th>Other % (VSD)</th>
<th>Arousal % (VSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occlusion</td>
<td>13.0 (5.0)</td>
<td>5.1 (2.5)</td>
<td>4.4 (2.2)</td>
<td>62.7 (10.1)</td>
<td>14.8 (4.1)</td>
</tr>
<tr>
<td>Obstruction</td>
<td>11.1 (4.8)</td>
<td>6.1 (5.1)</td>
<td>4.4 (2.4)</td>
<td>61.5 (12.5)</td>
<td>16.9 (7.1)</td>
</tr>
</tbody>
</table>

**Table 2**—Mean (and standard deviation) of peak mask pressure change for mid-inspiratory occlusions and complete obstructions

<table>
<thead>
<tr>
<th>Phasic response condition</th>
<th>Occlusion (Pm)</th>
<th>Obstruction (Pm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude (cmH\textsubscript{2}O)</td>
<td>Latency (ms)</td>
</tr>
<tr>
<td>KC</td>
<td>3.1 (1.1)</td>
<td>682 (98)</td>
</tr>
<tr>
<td>VSW/KC</td>
<td>3.4 (1.8)</td>
<td>682 (87)</td>
</tr>
<tr>
<td>VSW</td>
<td>3.5 (1.5)</td>
<td>717 (95)</td>
</tr>
<tr>
<td>Other</td>
<td>4.6 (1.8)</td>
<td>716 (82)</td>
</tr>
</tbody>
</table>
sions (14.8%). The number of trials rejected due to EEG or movement artifact was less than 3%. On average there were 207±54 mid-inspiratory occlusions and 192±62 complete obstructions not associated with AASM arousals, and thus available for phasic event classification. Table 1 displays the mean percentages of all trials that were elicited within each phasic response condition in response to either type of respiratory stimulus. This table reveals that neither a VSW nor a K-complex was elicited on slightly over half the trials (other category). A K-complex occurred alone on 13.0% of trials for mid-inspiratory occlusions and 11.1% of trials for complete obstructions. VSWs occurred alone on 4.4% of both mid-occlusion and obstruction trials. K-complexes appeared in association with a VSW on 5.1% of occlusion trials and 6.1% of obstruction trials.

**Mask Pressure**

Figure 2 illustrates the averaged Pm signal for the VSW/KC, KC, VSW and “other” phasic response conditions in response to mid-inspiratory occlusions (Figure 2a) and complete obstructions (Figure 2b). It reveals a large difference in average Pm morphology between the two types of respiratory stimuli. Complete obstructions demonstrated a significantly larger negative intrathoracic pressure peak [F(1,5)=31.10, p<.01] and a slower rise time to reach the peak [F(1,5)=133.8, p<.001] when compared to mid-inspiratory occlusions (Table 2).

A main effect of phasic response condition was observed for Pm amplitude with statistical analysis conducted across both types of respiratory stimuli [F(3,15)=6.25, p<.01]. Figure 2 illustrates that although the “other” phasic response condition demonstrated the largest Pm response to mid-inspiratory occlusions (Figure 2a), both the “other” and VSW phasic response conditions showed large Pm responses to complete obstructions (Figure 2b). Despite this apparent difference between the two types of respiratory stimuli, the interaction between stimulus type and phasic response condition was not significant [F(3,15)=1.10, p>.05]. Pm latency was not significantly affected by phasic response condition [F(3,15)=1.20, p>.05].

**N550 and N550-type component**

The three factor repeated measures ANOVA performed on 29 scalp sites revealed a main effect of site [F(28,140)=83.64, p<.001], but no main effect of stimulus type [F(1,5)=0.06, p>.05] for N550 and N550-type amplitude. However, a stimulus type by scalp site interaction was observed [F(28,140)=1.8, p<.05]. Analysis of the data at Fz using the two factor ANOVA revealed a significant main effect of stimulus type at this site [F(1,5)=10.14, p<.05]. Figures 3a and 4a illustrate the respective mid-inspiratory and complete obstruction grand mean waveforms for each phasic response condition at Fz. The N550 in response to mid-inspiratory occlusions was larger than the N550-type component in response to complete obstructions. A significant main effect of stimulus type was also observed for N550 and N550-type latency [F(1,5)=236.0, p<.001]. The N550 in response to mid-inspiratory occlusions appeared earlier than the...
N550-type component in response to complete obstructions.

There was no main effect of phasic response condition with the three factor ANOVA conducted on all 29 sites \(F(3,15) = 0.86, p>.05\). However, analysis of the data at Fz using the two factor ANOVA, revealed that the N550 and N550-type components were larger in the VSW/KC compared to KC condition \(F(3,15)=27.65, p<.01\) (Figures 3a and 4a). The main effect of phasic response condition for N550 and N550-type latency was not significant. \(F(1,5) = 3.76, p>.05\).

Figure 5 illustrates the topographical distribution of the N550 in response to occlusions (Figure 5a) and N550-type in response to obstructions (Figure 5b) for all phasic response conditions. These figures illustrate that the both the N550 and N550-type components displayed prominent frontal distributions in the KC and VSW/KC conditions. This was confirmed statistically using the site by stimulus type interaction term in a two way ANOVA conducted on normalized data for both the KC \(F(28,140)=0.502, p>.05, e=0.404\) and VSW/KC \(F(28,140)=0.114, p>.05, e=0.198\) conditions.

In addition, a phasic response condition by scalp site interaction comparing the N550 in the VSW/KC and KC conditions in response to mid-inspiratory occlusions was non-significant \(F(28,140)=2.79, p=.08, e=0.114\). This same interaction was also non-significant for the N550-type component in response to complete obstructions \(F(28,140)=2.31, p=.08, e=0.189\). The fact that both of these F-values approached significance could be indicative of a trend toward a difference in scalp topography between these conditions. However, inspection of Figures 4a and b suggest that it was more likely that this statistic reflects amplitude differences that were not completely corrected for when using the McCarthy and Wood (1985) normalization procedure.

### N350 and N350-type components

It should be noted that as no clear N350-type component could be observed in the VSW and VSW/KC conditions in response to mid-inspiratory occlusions. There was a significant main effect of site \(F(28,140)=18.43, p<.001\) but no main effect of phasic response condition for N350 amplitude when the analysis was conducted on all 29 sites \(F(3,15)=1.51, p>.05\). In addition, a matched pairs t-test conducted at Cz revealed there was no difference on N350 amplitude between the VSW and VSW/KC phasic conditions (Fig 3b and Table 3).

<table>
<thead>
<tr>
<th>Phasic response condition</th>
<th>N550 (Fz) Occlusion Amplitude(µV)</th>
<th>Latency(ms)</th>
<th>N550-type (Fz) Obstruction Amplitude(µV)</th>
<th>Latency(ms)</th>
<th>N350 (Cz) Occlusion Amplitude(µV)</th>
<th>Latency(ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC</td>
<td>-103.33 (33.12)</td>
<td>730 (62)</td>
<td>-93.12 (18.22)</td>
<td>1750 (168)</td>
<td>-0.72 (5.30)</td>
<td>373 (29)</td>
</tr>
<tr>
<td>VSW/KC</td>
<td>-118.00 (23.33)</td>
<td>662 (58)</td>
<td>-106.20 (20.52)</td>
<td>1790 (182)</td>
<td>-38.87 (11.27)</td>
<td>363 (36)</td>
</tr>
<tr>
<td>VSW</td>
<td>-7.00 (4.73)</td>
<td>665 (69)</td>
<td>-4.04 (4.75)</td>
<td>1750 (162)</td>
<td>-35.55 (4.09)</td>
<td>363 (35)</td>
</tr>
<tr>
<td>Other</td>
<td>-5.43 (1.83)</td>
<td>667 (63)</td>
<td>-2.04 (4.75)</td>
<td>1730 (176)</td>
<td>1.95 (3.74)</td>
<td>345 (27)</td>
</tr>
</tbody>
</table>

Figure 5 illustrates the topographical distribution of the N550 and N350 component in all phasic response conditions. Panel d displays the topographic map of the N350-type response from the VSW condition. It also displays the N350-type response (*) taken from the averaged waveform using the negative peak of the K-complex as the common time reference in the VSW/KC condition. Voltage (mV) values are taken from grand mean evoked potential averages. Each map represents a top down view of the head. Voltage scales differ across respiratory stimulus types in order to illustrate the prominent topographical focus for each component.
A repeated measures ANOVA conducted on N350 latency at Cz revealed a significant main effect of phasic response condition \([F(3,15)=4.97, p<.05]\). However, a post hoc comparison between the VSW/KC and VSW condition revealed no significant N350 latency difference between these two phasic response conditions (Figure 3b).

Figure 5c illustrates the topographical distribution of the N350 component in all conditions in response to mid-inspiratory occlusions. This figure reveals that the N350 had a clear vertex distribution in both the VSW and VSW/KC phasic response conditions that did not differ statistically \([F(28,140)=2.10, p>.05, e=0.188]\).

**DISCUSSION**

The data indicate that despite the obvious difference in latency, the cortical response seen to complete obstructions is very similar to that seen to mid-inspiratory occlusions. In addition, it appears that K-complexes contribute exclusively to the N550 component of the RREP, while VSWs contribute exclusively to the N350 component of the RREP. Lastly, there was no evidence to suggest that the N350 component represented a K-complex trigger.

**N550 and N550-type components**

A prominent N550 in response to mid-inspiratory occlusions and a prominent N550-type component in response to complete obstructions were seen only when KCs (with a minimum amplitude of 75 V) were included in the phasic response condition averages. The frontal maximum of these components was similar to that reported for other RREP\(^2,4,6\) and AEP\(^2,4,6,14,16\) studies.

The complete obstruction N550-type component at Fz was smaller in amplitude, longer in latency, and substantially broader than the mid-inspiratory occlusion N550 component. One explanation for this difference in morphology is that the response to
the complete obstruction was more variable in latency than the response to the mid-inspiratory occlusion. In support of this, integration of the area under the KC phasic response grand mean waveform for both occlusions and obstructions resulted in larger values for the obstruction N550-type component, despite its smaller amplitude. Also, RMS averaging of the waveforms produced similar results for both respiratory stimulus types (60.7mV for obstruction waveform and 58.9mV for the occlusion waveform).

The increased response latency variation to the obstructive stimulus was best explained by increased variation in the timing of the eliciting stimulus relative to valve closure. The RREP eliciting event is believed to be the rapid increase in negative intra-thoracic pressure that is associated with the occlusion stimulus. Mid-inspiratory occlusions produce a rapid rise in intra-thoracic pressure (as measured by mask pressure) that can be easily time-locked to the occlusion stimulus. However, the Pm response to complete obstructions showed a much slower and more gradual increase in intra-thoracic pressure. Although the EP elicited by complete obstructions was time-locked from the onset of the increase in mask pressure, the cortical response was more likely to be triggered by a critical intra-thoracic pressure that occurred at some point along the Pm continuum. As this pressure could not be determined, the latency of the evoked response to complete obstructions had to be referenced to the onset of mask pressure.

The N550 and N550-type components were both larger when preceded by an N350 (VSW/KC condition) than when they were not (KC condition). These results, in addition to the topographical data, suggest that the presence of an N350 and N350-type component in the VSW/KC condition added a degree of source strength to the N550 and N550-type components respectively.

N350 and N350-type components

A prominent N350 was seen only when VSWs (with a minimum amplitude of 50V) were included in the mid-inspiratory occlusion phasic response condition averages. The N350 in response to mid-inspiratory occlusions had the same amplitude in both the VSW and VSW/KC condition. The prominent vertex distribution of the N350 is consistent with other RREP and AEP studies. The present data therefore indicate a clear and exclusive relationship between vertex sharp waves and the N350 component.

A small, but broad N350-type component was identified in the VSW condition in response to complete obstructions. Topographical analysis revealed that the N350-type (obstruction) and N350 (occlusion) had very similar distributions at the scalp. These data suggest that the N350-type component is present in response to complete obstructions, but that it gets “washed out” due to latency jitter when averaged from the beginning of the change in Pm. That is, the pressure change causing the afferent discharge though to the cortex, occurred somewhere toward the end of the long rise time in intra-thoracic pressure, and was highly variable depending on the rate of rise (slope) in intrathoracic pressure on individual trials.

In contrast, there was no prominent N350-type component in the VSW/KC condition. However, this component became apparent when the VSW/KC data were re-epoched using the peak of the K-complex as the common reference point. This method is similar to the one used by Niiyama et al. These authors used the peak of the N350 as a reference point to average both evoked and spontaneous K-complexes. The N350-type component in the VSW/KC condition in the present study appeared 240ms prior to the peak of the averaged K-complex, which is earlier, but still comparable to the distance between the N350 and N550 component peaks in the VSW/KC condition in response to mid-inspiratory occlusions (299ms). We would argue that had a similar procedure been possible on the VSW condition, a more prominent N350 component would be present in this condition also.

Frequency of elicited responses

The data on the elicitation of phasic responses between the two types of respiratory stimuli were remarkably similar. The proportion of “other” responses was very similar between occlusions and obstructions. In addition, there was only a small amount of variation between occlusions and obstructions across the KC, VSW/KC, and VSW phasic response condition types.

Relationship between the N350 component and K-complexes

Previous AEP research has argued that the N350 is part of the K-complex and that it may represent a K-complex trigger once a certain N350 amplitude threshold has been reached. This argument was based on reports that the N350 was larger following the averaging of trials containing K-complexes than following the averaging of trials not containing K-complexes. However, the present study determined that N350 amplitude was the same in the VSW and VSW/KC conditions, which clearly argues against the N350 trigger hypothesis.

There are methodological issues that explain the different N350 amplitude results observed in the present study when compared to previous research. The smaller N350 in the K-complex absent compared to K-complex present condition in previous research may have been an artefact of the averaging process. That is, the proportion of trials containing a VSW would have been greater in the K-complex present compared to K-complex absent condition, which would thus contribute to the smaller N350 seen in the latter condition.

In addition, Colrain et al. recently added to the phasic response condition grouping by dividing trials into KC, VSW, and “other” categories. These authors observed that the N350 was largest in the VSW average, and was smaller, but still present in the KC average. With the exception of the VSW condition in the Colrain et al. study, N350 amplitude would have also been reduced in the phasic response averages in previous research due to the inclusion of both VSW and no VSW trials in the average. Thus, with the addition of a fourth phasic response grouping (VSW associated with a K-complex), the present study identified an N350 in only the VSW and VSW/KC conditions with no N350 discernible in the KC and “other” conditions.

The identification of K-complex trials that were not preceded by a VSW would suggest that the N350 is not exclusively related to K-complex elicitation. Extrapolation of the proportion data presented in Table 1 indicates that for both types of respiratory stimuli, when a K-complex was elicited, the probability of it appearing in isolation (not with a VSW) was 60.0% for mid-inspiratory occlusions and 51.4% for complete obstructions. These data suggest that if a K-complex was elicited, the probability of it being preceded by a VSW was slightly less than...
chance. These data are consistent with a proposition by Bastien and Campbell and Ujszaszi and Halasz. These authors argue that the N350 and N550 reflect different neural events that do not involve each other functionally, but are possibly indicative of parallel processes. Both groups have reported the N350 to increase in latency with increased depth of sleep, whereas N550 latency shortened with sleep depth. The N350 component has also been shown to appear during periods of stage 1 theta activity, prior to both stage 2 sleep and K-complex elicitation. Finally, as indicated in the present study and previously, the N350 and N550 have different scalp topographies (vertex for N350 and fronto-central for N550). These data argue that the N350 should therefore be viewed as a component independent of the N550 component of the averaged K-complex.

A significant point of interest in the present study was that with the removal of VSWs and K-complexes from the averaged evoked response (“other” condition), the evoked potential waveform was essentially a flat line. That is, the late positive component (P2 and P450) that have been reported during NREM sleep in other RREP studies were no longer present. This would suggest that at last in young adults, the makeup of the evoked potential waveform during sleep is predominantly due to the presence of the two large negative phasic responses (VSWs and K-complexes).

**Summary**

Despite differences in the physiological nature of the two types of respiratory stimuli, the types of components elicited appear to be remarkably similar. An N550 and N550-type component were observed in both the KC and VSW/KC conditions that had almost identical elicitation characteristics and topographical features for both types of respiratory stimuli. In addition, N550, and N550-type amplitude and latency differences between mid-inspiratory occlusions and complete obstructions can be accounted for by differences in the time-course of changes in intra-thoracic pressure.

An N350-type component was observed in the VSW/KC condition in response to complete obstructions that had a similar topographical distribution to the N350 in response to mid-inspiratory occlusions. The difficulty in seeing the N350-type component using normal averaging techniques can again be explained by the event-related time locking problem. As such, we would argue that mid-inspiratory occlusions can be used as a useful model of obstructive events in normal subjects to indicate EEG responses to respiratory stimuli during NREM sleep.

### REFERENCES

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