

# Effects of Sleep Deprivation on Spontaneous Arousals in Humans

Emilia Sforza, MD, PhD<sup>1</sup>; Florian Chapotot, PhD<sup>2</sup>; Ross Pigeau, MD<sup>3</sup>; Paul Naitoh Paul, MD<sup>4</sup>; Alain Buguet, MD<sup>5</sup>

<sup>1</sup>*Sleep Laboratory, Department of Psychiatry, Geneva University Hospital, Geneva, Switzerland;* <sup>2</sup>*Unité de Neurophysiologie du Stress, Centre de Recherches du Service de Santé des Armées, La Tronche, France;* <sup>3</sup>*Human Factor Division, Defence Research and Development Canada-Toronto, North York, Ontario, Canada;* <sup>4</sup>*Applied Psychophysiology Group, Psychophysiological Research for Health, San Diego, USA;* <sup>5</sup>*Institut de Médecineropicale du Service de Santé des Armées, Le Pharo, Marseille, France*

**Study Objectives:** The hierarchical definition of arousals from sleep includes a range of physiologic responses including microarousals, delta and K-complex bursts, and variations in autonomic system. Whether patterns in slow-wave electroencephalographic activity and autonomic activation are primary forms of arousal response can be addressed by studying effects of total sleep deprivation. We therefore examined changes in arousal density during recovery sleep in healthy subjects.

**Design:** Participants spent 6 consecutive 24-hour periods in the laboratory. Nights 1 and 2 were baseline nights followed by 64-hour total sleep deprivation, then 2 consecutive recovery nights.

**Setting:** Sleep-deprivation protocol was conducted under laboratory conditions with continuous behavioral and electrophysiologic monitoring.

**Participants:** Twelve drug-free men aged  $27.4 \pm 7.9$  years were studied. None reported sleepiness or altered sleep-wake cycle, and none had neurologic, psychiatric or sleep disorders.

**Intervention:** N/A.

**Measurements and Results:** Arousals were classified into 4 levels: microarousals, phases of transitory activation, and delta and K-complex

bursts. Sleep deprivation induced changes in the density of considered arousals except phases of transitory activation, with a distinct pattern among the different types. The greatest change was found for microarousals, which showed a significant decrease in the first recovery night ( $P = .01$ ), with return to baseline thereafter. A fall in K-complex and delta-burst density was noted in the first recovery night, not, however, reaching statistical significance. The phases of transitory activation rate were virtually unaffected throughout the experimental nights.

**Conclusions:** We conclude that homeostatic sleep processes exert an inhibitory effect on arousal response from sleep with a significant effect only on the microarousal density. Decreased delta and K-complex burst rates, though not significant, support the hypothesis that they may be activating processes, probably modulated by factors independent from those implicated in cortical arousal.

**Key Words:** Arousals, subcortical arousals, sleep deprivation, sleep homeostatic processes

**Citation:** Sforza E; Chapotot F; Pigeau R et al. Effects of sleep deprivation on spontaneous arousals in humans. *SLEEP* 2004;27(6):1068-75.

## INTRODUCTION

AROUSALS FROM SLEEP PLAY A SIGNIFICANT ROLE IN NEUROCOGNITIVE FUNCTIONS AND DAYTIME SLEEPINESS. This finding has been supported mainly by studies using acoustic stimulation,<sup>1,2</sup> which reveal that, in normal subjects, the frequency of provoked arousals contributes to reduced alertness and increased sleepiness.<sup>2</sup> Moreover, the occurrence of arousals in patients with sleep-related breathing disorders<sup>3,4</sup> and periodic limb movements<sup>5</sup> contributes to subjective and objective sleepiness. Despite evidence that arousals from sleep disrupt sleep structure, the rate of recurrence of arousals alone is insufficient to account for daytime sleepiness, either in normal subjects or in patients with sleep-related disorders. This finding probably results from the definition of microarousals (MA)<sup>6</sup> scored when an abrupt shift in alpha or fast electroencephalogram (EEG) frequencies occurs lasting at least 3 seconds but neglecting changes of both autonomic and motor systems.

Although cortical activation is the gold standard for definition of arousal, several studies<sup>7-12</sup> show that the concept of “arousal”

includes a range of physiologic responses with differences not due to qualitative changes in arousal mechanisms but, rather, to different levels of central nervous system activation. At the lower range of arousal responses are those inducing reflex motor responses,<sup>13-15</sup> autonomic activation,<sup>16-18</sup> and appearance of slow-wave EEG activity, ie, delta bursts (D-bursts) and K-complex bursts (K-bursts),<sup>7,10</sup> all defined as “subcortical arousals.” At the upper range are arousal responses implying a cortical activation represented by MA<sup>6</sup> and phases of transitory activation (PAT),<sup>19</sup> therefore called “cortical arousals.”

The functional significance of subcortical arousals remains unknown, and there is considerable debate as to whether the appearance of EEG slow-wave activity and K-bursts is indicative of a partial arousal process that leaves the central nervous system more likely to be aroused, or reflects a sleep maintenance process that inhibits arousal and prevents sleep disruption. In favor of the first hypothesis are data from experimental<sup>8,11</sup> and clinical studies<sup>10,13-15</sup> showing increases in heart and respiratory rate accompanying bursts of delta activity or K-complexes in response to arousing stimuli. Other researchers have proposed a “sleep instability” hypothesis in that D-bursts or K-bursts reflect an underlying ability of the brain to generate synchronized EEG activity to stabilize sleep and to prevent sleep fragmentation<sup>20-24</sup> affected by homeostatic<sup>25,26</sup> and circadian<sup>27</sup> influences.

Whether D-bursts and K-bursts are signs of arousal response or markers of sleep maintenance processes can be addressed by studying the effects of a total sleep-deprivation protocol. Given the hypothesis that D-bursts and K-bursts may act as an arousing response sharing some functional properties with MA but generated by different neural systems, one would predict that after

## Disclosure Statement

This is not an industry-sponsored study. Drs. Sforza, Chapotot, Pigeau, and Buguet have indicated no financial conflicts of interest.

Submitted for publication November 2003

Accepted for publication April 2004

Address correspondence to: Emilia Sforza MD, PhD, Laboratoire de sommeil, Département de Psychiatrie, Hôpitaux Universitaires de Genève, 2 Chemin du Petit Bel Air, Ch 1225 Chêne Bourg, Geneva, Switzerland; Tel: 0041 22 3055329; Fax: 0041 22 3055343; E-mail: Emilia.Sforza@hcuge.ch

sleep deprivation a decline both in MA and D-bursts and K-bursts would occur, with differences related to neural pathways regulating their generation. Otherwise, if subcortical arousals were markers of sleep maintenance processes, we would expect a strong reduction in the density of D- and K-bursts as a consequence of greater sleep pressure and lesser arousal instability. To this end we examined variations in cortical and subcortical arousals in healthy subjects who were totally sleep deprived and for whom 2 consecutive recovery nights were available.

## METHODS

### Subjects

The subject sample consisted of 12 healthy men recruited among reservists of the Canadian Forces, participating as volunteers. The average age was  $27.4 \pm 7.9$  years, range 19 to 44 years. Prior to the study, all subjects were screened for any current or past medical, neurologic, or psychiatric history and were drug-free at the time of the study. All participants were without history of excessive daytime sleepiness or sleep complaints, and all were entrained to a regular sleep-wake schedule. The Defence and Civil Institute of Environmental Medicine ethics committee and the Department of Health and Welfare Canada approved the experiment, and all subjects gave written consent.

Complete details of the protocol and methods have been published previously.<sup>28</sup> The subjects participated in a 6-day study, with 2 baseline days and nights, followed by continuous mental work for 64 hours starting at 6:00 AM on the third day and ending with 2 recovery days and nights. The subjects were allowed to sleep a maximum of 13 hours during the first recovery night, whereas time in bed was scheduled between 10:00 PM and 6:00 AM during the 2 consecutive baseline recordings and the second recovery night. During the 64 hours of sustained wakefulness, the subjects worked continuously in 105-minute work sessions with 15-minute breaks according to experimental and individual needs. Work sessions were devoted to cognitive tasks and subjective questionnaires and were repeated until completion of the sleep-deprivation procedure. Continuous EEG recording was used to assess the sleep-deprivation condition during the 64-hour period.

Sleep data of the second baseline night and the first and second recovery nights were used in the analysis.

### Nocturnal Sleep Recording

The subjects were fitted with electrophysiologic recording equipment (Oxford Medilog 9000, Oxford, UK) to measure 4 EEG leads (C3, C4, P3, P4 referenced to linked ears), an electrooculogram, an electromyogram (EMG), and an electrocardiogram. During the first baseline night, respiration was monitored with thermistors and thoracic movements, and tibialis electromyographic activity was recorded using surface electrodes placed on the right and left legs. None of the participants showed an index of respiratory events (apnea+hypopnea index) greater than 10 per hour of sleep or a periodic leg movement index greater than 5.

Sleep stages were visually scored according to standard criteria<sup>29</sup> using 20-second epochs, with the investigator blind to subject and experimental condition. For each experimental night, standard sleep parameters were computed over the complete

sleep time period, and all recordings were analyzed for sleep staging and arousal scoring with the ERA© software package (Prana, Phitools®, Strasbourg, France).

## Data Analysis

### Visual EEG Scoring

Arousals were categorized into 4 groups according to previously published criteria<sup>10</sup> (Figure 1). Briefly, we distinguish (1) D-bursts as a sequence of delta waves, exceeding by at least one-third the amplitude of background activity in stages 3 and 4, and detectable on at least 3 EEG derivations.<sup>30</sup> (2) K-bursts as a sequence of 2 or more K-complexes without alpha activity, detectable on at least 3 EEG derivations. The K-complex was defined as a negative deflection, followed by a positive component with a minimum duration of 0.5 seconds and a minimum peak-to-peak amplitude of  $75\mu\text{V}$ .<sup>31</sup> (3) Microarousals (MA) were defined according to American Sleep Disorders Association criteria<sup>6</sup> as a return to alpha, theta, or fast frequency, well differentiated from the background EEG activity. The duration was extended, however, to include MA lasting between 1.5-seconds and 3 seconds.<sup>18</sup> MA detection criteria for rapid-eye-movement (REM) sleep included an increase in submental EMG amplitude, in addition to a shift in EEG activity. (4) PAT were defined as an acceleration of the background EEG activity with decreasing amplitude and appearance of alpha and beta activity associated with a concomitant increase in EMG, appearance of muscular artifacts, acceleration of heart rate, and, during REM sleep, transitory disappearance of REMs, according to previously described criteria.<sup>19</sup> The point of onset of each arousal was defined as the first occurrence of alpha or fast EEG activity or delta and K-complex. The termination of MA and PAT was defined as the onset of theta activity for at least 10 seconds, indicating return to sleep, and the termination of D-bursts and K-bursts as a return to prearousal background activity.

For the baseline night and the 2 consecutive recovery nights, the duration and the index (number per hour of sleep) of arousal, obtained by dividing the amount of each arousal type by total sleep time and by each sleep stage duration, were calculated. For D-bursts and K-bursts, duration and index were calculated for the 3 nights considering Stage 2 sleep and slow-wave sleep (SWS) (Stages 3 and 4 of non-REM [NREM] sleep) only. The same parameters were also measured considering homogenous sleep duration of about 7 hours during the 3 considered nights. In order to evaluate the effect of the sleep cycle on arousal density, the numbers of all arousal types were also analyzed throughout the first 5 sleep cycles recorded in all experimental nights.

### Statistical Analyses

For all considered arousals, comparisons of arousal index and duration between nights were carried out by use of 1-way analysis of variance (ANOVA) for repeated measures with Night as the factor. MA and PAT index and duration were also submitted to 2-way ANOVA, Night (baseline, first recovery, second recovery)  $\times$  Sleep Stage in order to assess their density across sleep stages as a function of the experimental night. Whenever significant main factor or interaction effects were present, posthoc Student-Newman-Keuls tests were used to assess significant differences. Bivariate correlation analysis using Pearson correlation coefficient

cient was used to determine whether or not variations in the amount of sleep stages were correlated with changes in the arousal indexes. In all statistical analyses, differences were considered to be significant when the  $P$  value was below .05. Data are reported as means  $\pm$  SEM. All statistical analyses were performed with the SPSS statistical software package (SPSS for Windows, 10, SPSS, Inc., Chicago, Ill).

## RESULTS

### Polygraphic Sleep Parameters

Details of sleep parameters during the baseline night and the 2 recovery nights are given in Table 1. As expected, the first recovery night was characterized by important modifications of sleep structure. The major changes were the increase in total sleep time ( $P = .001$ ), the rise in SWS ( $P = .001$ ) and REM sleep ( $P = .001$ ), and a significant reduction in stage 2 ( $P = .001$ ). Sleep latency was also markedly reduced ( $P = .001$ ). In contrast, the wake after sleep onset (WASO) and the number of awakenings did not differ significantly. Comparison of the second recovery night versus baseline showed a decrease in total sleep time and Stage 2 and an increase in SWS, with differences reaching significance only for total sleep time ( $P = .007$ ).

### Effect of the Night on Arousal Index

The effects of the experimental night on the arousal index and duration during total sleep time are shown for each arousal type

in Table 2. For any type of arousal, no significant differences in duration were found throughout the 3 experimental conditions.

A significant night effect ( $F = 4.95, P = .013$ ) in arousal index was found for MA, showing a marked decrease in the first recovery night and a return to prerecovery value in the second recovery night. One-way ANOVA for D-bursts ( $F = 2.7, P = .08$ ), K-

**Table 1**—Sleep Parameters During the Baseline and the 2 Recovery Nights

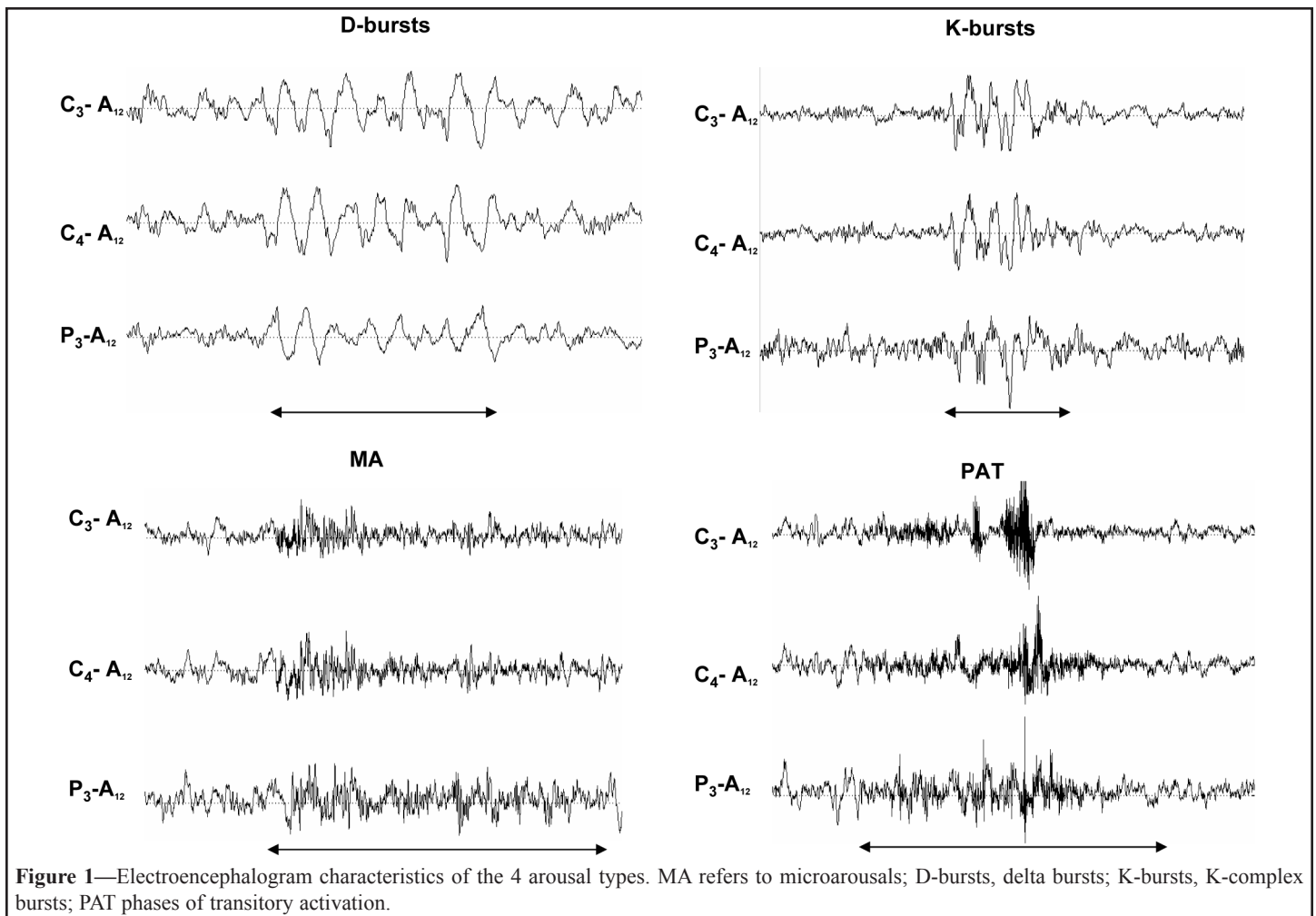
Parameter	Baseline	Night	
		First Recovery	Second Recovery
Total sleep time, min	454.2 $\pm$ 4.2	721.9 $\pm$ 13.0* $\dagger$	400.3 $\pm$ 15.1*
Sleep latency, min	21.4 $\pm$ 6.9	9.1 $\pm$ 2.3* $\dagger$	33.2 $\pm$ 6.7
Sleep efficiency, %	96.0 $\pm$ 0.5	97.1 $\pm$ 0.6	92.6 $\pm$ 3.2
WASO, min	23.4 $\pm$ 3.5	25.5 $\pm$ 5.2	45.1 $\pm$ 15.2
Awakenings, no.	1.5 $\pm$ 0.3	1.7 $\pm$ 0.6	1.5 $\pm$ 0.4
Sleep stage, %			
1	7.8 $\pm$ 3.2	5.7 $\pm$ 6.2	8.1 $\pm$ 3.8
2	52.1 $\pm$ 10.8	45.7 $\pm$ 10.0* $\dagger$	50.1 $\pm$ 13.7
SWS	16.8 $\pm$ 9.9	21.4 $\pm$ 14.3*	19.2 $\pm$ 9.9
REM	23.4 $\pm$ 3.9	27.27 $\pm$ 9.2* $\dagger$	22.6 $\pm$ 4.9

Data are presented as mean  $\pm$  SEM.

WASO refers to wake after sleep onset; SWS, slow-wave sleep; REM, rapid eye movement.

\*Significant difference from baseline

$\dagger$ Significant difference from second recovery night



**Figure 1**—Electroencephalogram characteristics of the 4 arousal types. MA refers to microarousals; D-bursts, delta bursts; K-bursts, K-complex bursts; PAT phases of transitory activation.

bursts ( $F = 1.92, P = .16$ ), and PAT ( $F = 1.39, P = .26$ ) indexes did not show any significant difference between baseline and recovery nights. For K-bursts, the index fell from the baseline value of  $3.3 \pm 0.7$  to a value of  $1.6 \pm 0.3$  during the first recovery night. A similar trend was found for D-bursts for which the index decreased from the baseline value of  $3.1 \pm 0.8$  to a final value of  $1.7 \pm 0.3$  in the first recovery night. No significant change in the PAT index was noted for the 3 nights under study.

In order to check whether the lack of changes in the D-bursts, K-bursts, and PAT index was related to prolonged sleep time in the first recovery night, the same ANOVA design was also performed, considering for each of the 3 nights a similar sleep duration of about 7 hours. As depicted in Figure 2, the results of this ANOVA completely paralleled those previously described. The K-bursts ( $F_{2,27} = 1.11, P = .34$ ), the D-bursts ( $F_{2,27} = 1.19, P = .32$ ), and the PAT ( $F_{2,27} = 0.34, P = .72$ ) index tended to decrease in the first recovery night, but the effect was not significant. Again a significant night effect ( $F = 3.7, P = .03$ ) was found for MA, with the MA index falling from the initial value of  $16.2 \pm 2.1$  to an average value of  $8.5 \pm 1.7$  in the first recovery night.

In the baseline and second recovery nights, no significant correlations were found between the total index of MA and PAT and total sleep time, sleep efficiency, and the amount of SWS. The only significant correlation was between the index of MA ( $r = 0.70, P = .01$ ) and PAT ( $r = 0.84, P = .001$ ) and the amount of stage 1 sleep.

### Effect of Sleep Cycle on Arousal Density

Figure 3 illustrates the average number of the 4 arousal types across the first 5 sleep cycles in the 3 experimental nights. ANOVA for repeated measures shows a significant sleep-cycle effect for D-bursts ( $F_{4,13} = 15.3, P < .0001$ ), K-bursts ( $F_{4,13} = 7.36, P = .0002$ ), and MA ( $F_{4,13} = 3.88, P = .005$ ). The significant differences were between the lower levels during the fourth and fifth sleep cycle ( $P < .0005$ ) and the highest value in the first 3 sleep cycles. Despite a main effect of sleep cycle, no significant interaction effect was present when the 3 experimental nights

were considered (D-bursts ( $F_{8,13} = 1.93, P = .06$ ), K-bursts ( $F_{8,13} = 1.82, P = .07$ ), and MA  $F_{8,13} = 1.59, P = .13$ ), their average value showing considerable uniformity in baseline and recovery nights. The ANOVA for repeated measures revealed no significant sleep-cycle effect ( $F_{4,13} = 2.40, P = .06$ ) for PAT, but a significant interaction ( $F_{8,13} = 3.04, P = .03$ ), their average value being greater in the 2 recovery nights and in the last 2 sleep cycles.

### Effect of Sleep Stage on MA and PAT Index

The group means for MA and PAT index in each sleep stage are shown in Figure 4. The 2-way repeated measures ANOVA revealed for MA a significant main effect of sleep stage ( $F = 162.6, P < .0001$ ) and night ( $F = 6.7, P = .004$ ) and a significant interaction ( $F = 3.5, P = .004$ ). During the first recovery night, as compared to baseline, there was a significant fall in MA index affecting stage 1 ( $P = .002$ ) and stage 2 ( $P = .05$ ) sleep, with no effect during SWS. A tendency to a decrease in MA index was found in REM sleep, but the differences were not significant. Comparison of the second recovery night versus baseline revealed in all sleep stages a trend toward reaching baseline levels, the second recovery night remaining lower as compared with baseline conditions but without significant difference. The 2-way ANOVA did not show a significant effect for MA duration, neither across the different nights nor across sleep stages.

A distinct pattern of variation in the PAT index during the 2 recovery nights was evident from the 2-way ANOVA, revealing a significant main effect of sleep stage ( $F = 26.5, P < .001$ ) and night ( $F = 11.8, P < .001$ ) and a significant interaction ( $F = 7.5, P < .001$ ). During the first recovery night, there was an increase in the PAT index during stage 1 sleep and SWS, with differences reaching significance for SWS ( $P < .001$ ). This tendency persisted in the second recovery night but, again, with significance only

**Table 2**—Polygraphic Characteristics of Scored Arousals in the Baseline and Recovery Nights

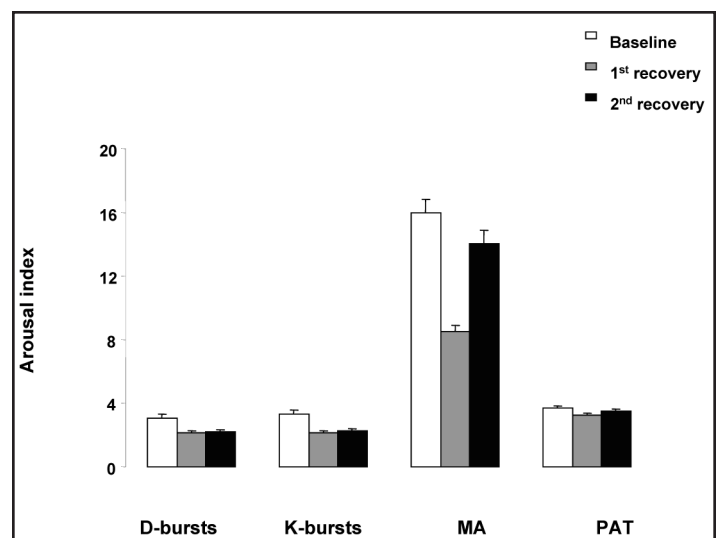
Parameter	Night		
	Baseline	First Recovery	Second Recovery
Index, no./h			
D-bursts	$3.1 \pm 0.8$	$1.7 \pm 0.3$	$2.5 \pm 0.4$
K-bursts	$3.3 \pm 0.7$	$1.6 \pm 0.3$	$2.3 \pm 0.4$
MA	$16.2 \pm 2.1$	$8.3 \pm 1.0^{*†}$	$14.8 \pm 2.3$
PAT	$3.5 \pm 0.4$	$4.0 \pm 0.4$	$3.7 \pm 0.4$
Duration, s			
D-bursts	$5.2 \pm 0.2$	$5.2 \pm 0.2$	$5.4 \pm 0.2$
K-bursts	$4.5 \pm 0.1$	$4.3 \pm 0.2$	$4.6 \pm 0.1$
MA	$7.4 \pm 0.2$	$7.3 \pm 0.2$	$7.4 \pm 0.2$
PAT	$14.4 \pm 1.3$	$13.5 \pm 0.8$	$12.8 \pm 0.7$

Data are presented as mean  $\pm$  SEM.

Abbreviations: D-bursts: bursts of delta waves; K-bursts: bursts of K-complexes; MA: microarousals; PAT: phases of transitory activation.

\*Significant differences from baseline.

†Significant differences from 2nd recovery night.



**Figure 2**—Index of the 4 arousal types during the baseline and 2 recovery nights. The index was calculated from the first 7 hours of sleep in each of the 3 experimental nights, to obtain a homogenous sleep duration. A fall in all arousal-type indexes was present in the 2 recovery nights, with significant variation, however, only for the microarousal index in the first recovery night. MA refers to microarousals; D-bursts, delta bursts; K-bursts, K-complex bursts; PAT phases of transitory activation.

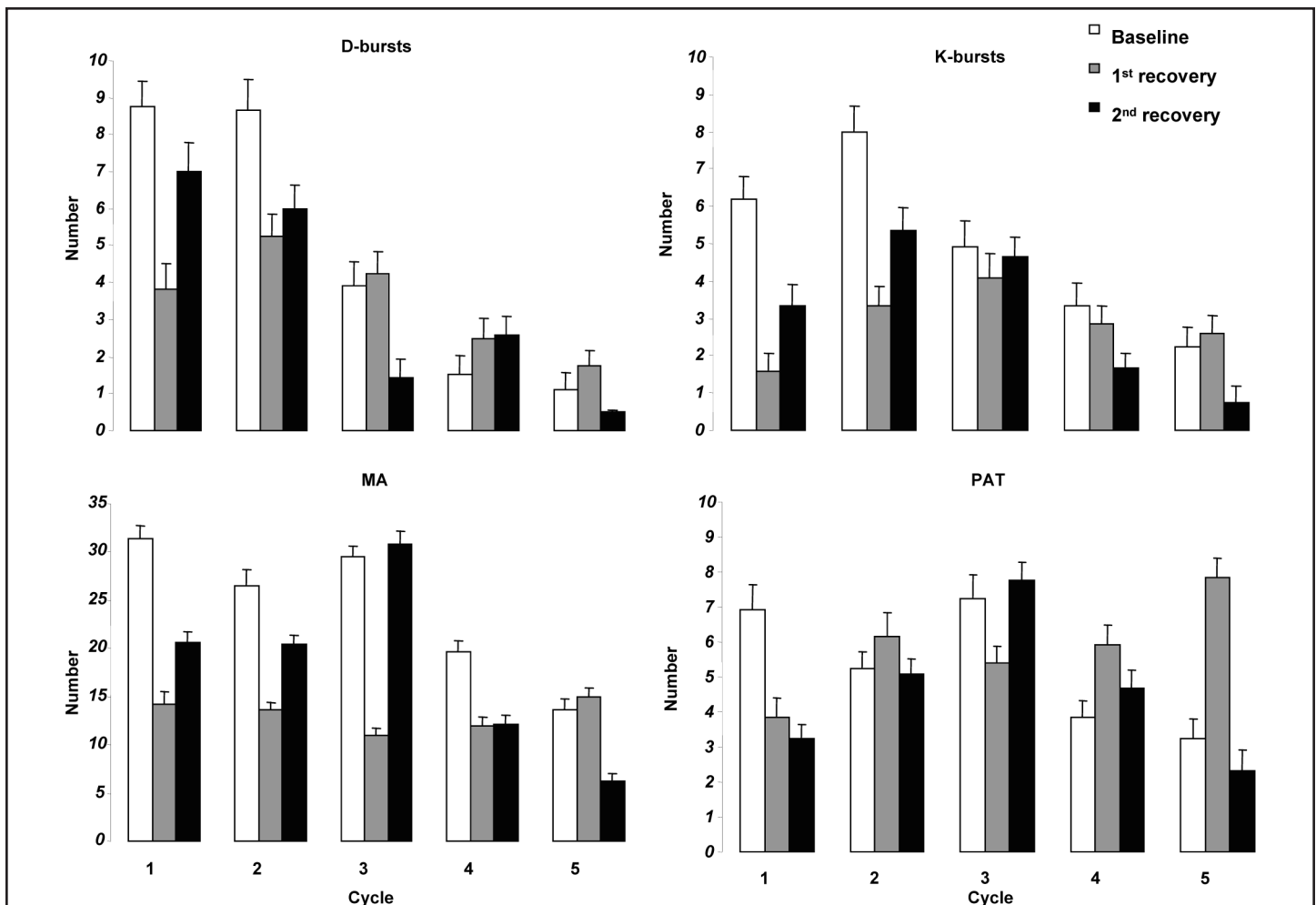
for SWS ( $P < .001$ ). No differences in the PAT duration across the nights were present for any sleep stage.

## DISCUSSION

The present study was undertaken to define the effect of sleep deprivation on spontaneous arousal occurrence during recovery sleep. The first interesting finding of our study was that the strong increase in sleep drive following sleep deprivation was associated with a change in the arousal indexes with a distinct pattern among the different types of arousal response. As illustrated in Figure 2, the MA index was significantly reduced in the first recovery night while the rate of PAT was virtually unaffected throughout the nights. Second, a decrease in the rate of subcortical arousals, as reflected by the D-bursts and K-bursts, was present, although not reaching statistical significance. Although the latter result is not consistent with the idea that K-bursts and D-bursts reflect either an aroused or a sleep-protective brain state, the decrease in their indexes across the recovery nights, following the MA fall, gives some indirect support to the hypothesis that slow EEG events (K-bursts and D-bursts) and conventional arousals share some functional properties as activating processes.

Consistent with previous reports,<sup>25,26</sup> the first finding of our study is that the increase in sleep pressure following sleep deprivation significantly affects the arousal response from sleep with a significant inhibitory effect only on MA. Furthermore, despite the fact that sleep deprivation induces a recuperative increase of SWS, there were no significant correlations or even trends toward a relationship between the amount of SWS and the decrement in MA density. With the caution of inferences based on correlation analysis carried out on a small sample of subjects, the current finding points to an independence of the mechanisms generating MA and those subserving NREM sleep. Moreover, if we consider MA and PAT as markers of cortical responsiveness to arousing stimuli, we note that sleep deprivation does not induce a comparable decrease in MA and PAT, a dramatic and significant arousal density reduction being observed only for MA, and more apparent in light sleep. A critical question not answered by our results is whether the term *cortical* arousal may be misleading, with MA and PAT reflecting 2 different aspects of the waking intrusion during sleep. While MA indicates a transient cortical activation followed by a return to sleep,<sup>32</sup> reflecting brain response to stimuli, PAT may correspond to *awakening*, indicating a clear discontinuity of sleep episode and sometimes its end.<sup>33</sup> In adults, spontaneous awakening depends on the combined effect of circadian and homeostatic influences<sup>33</sup> and on the phase of the ultradian NREM-REM cycle.<sup>34</sup> Therefore we can suggest that, as a consequence of a higher sleep pressure related to sleep deprivation, reduced cortical responsiveness to arousing stimuli

vation significantly affects the arousal response from sleep with a significant inhibitory effect only on MA. Furthermore, despite the fact that sleep deprivation induces a recuperative increase of SWS, there were no significant correlations or even trends toward a relationship between the amount of SWS and the decrement in MA density. With the caution of inferences based on correlation analysis carried out on a small sample of subjects, the current finding points to an independence of the mechanisms generating MA and those subserving NREM sleep. Moreover, if we consider MA and PAT as markers of cortical responsiveness to arousing stimuli, we note that sleep deprivation does not induce a comparable decrease in MA and PAT, a dramatic and significant arousal density reduction being observed only for MA, and more apparent in light sleep. A critical question not answered by our results is whether the term *cortical* arousal may be misleading, with MA and PAT reflecting 2 different aspects of the waking intrusion during sleep. While MA indicates a transient cortical activation followed by a return to sleep,<sup>32</sup> reflecting brain response to stimuli, PAT may correspond to *awakening*, indicating a clear discontinuity of sleep episode and sometimes its end.<sup>33</sup> In adults, spontaneous awakening depends on the combined effect of circadian and homeostatic influences<sup>33</sup> and on the phase of the ultradian NREM-REM cycle.<sup>34</sup> Therefore we can suggest that, as a consequence of a higher sleep pressure related to sleep deprivation, reduced cortical responsiveness to arousing stimuli



**Figure 3**—Average number of the 4 arousal types in the first 5 sleep cycles during the baseline and 2 recovery nights. A main effect of sleep cycle was found for delta bursts (d-bursts), K-complex bursts (K-bursts) and microarousals (MA), showing a progressive decline across the night without significant differences between baseline and recovery nights. PAT refers phases of transitory activation.

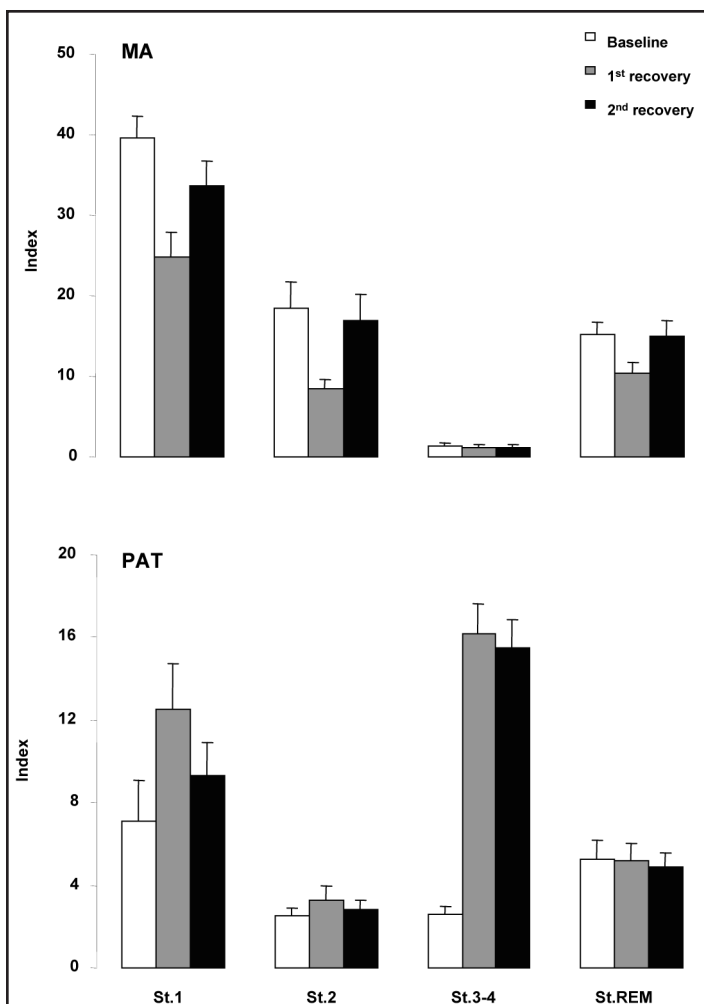
can be present during light NREM sleep inducing the fall in MA. As sleep proceeds, the homeostatic and circadian wake pressure would increase, inducing a short awakening, ie, a PAT, to restart the NREM-REM cycle.

The second finding of our study is that a trend to reduced K-burst and D-burst density was noted in the recovery nights without, however, reaching statistical significance. This observation opens discussion on the meaning of these transitory slow EEG activities arising from sleep and on the mechanisms underlying their generation. It is generally accepted that the term “arousal” refers to abrupt frequency shifts toward fast EEG rhythms, which interrupt the continuity of sleep. However, we have to consider that the usual definition of “arousal” includes also a range of responses affecting cardiac, respiratory, and motor systems, without always producing overt EEG activation.<sup>35</sup> Thus, the functional significance of the arousal responses without EEG activation is still controversial, and 2 hypotheses have recently received most attention. The first assumes that K-bursts and D-bursts are independent from arousal from sleep and that they simply translate a “sleep instability” allowing both sleep maintenance and sleep defense. This hypothesis has its theoretical basis on the cyclic alternating pattern (CAP), consisting of phases of higher arousal, eg, phase A, and phases with lower arousal, eg, phase B. Within

phase A, 3 subtypes have been identified<sup>30</sup>—the A1 consisting of synchronized EEG phasic events such as K-bursts and D-bursts, the A2 in which desynchronized EEG activity is mixed with slow EEG activities, and the A3 in which desynchronized EEG patterns are associated with motor and autonomic activation. Comparison of the CAP components with the hierarchical arousal organization shows some analogies, MA and PAT corresponding to phases A2 and A3 of the CAP, with subcortical arousal being considered as equivalent to phase A1 of the CAP.<sup>36,37</sup> Such a hypothesis would predict a negative correlation between subcortical arousal density and sleep recuperation, inducing, as consequence of greater sleep pressure in the recovery nights,<sup>25-27</sup> a strong decrease in their rate. In contrast, in our subjects D-burst and K-burst density showed a tendency to decrease in the recovery nights without, however, a strong decline. Thus, slower EEG patterns with autonomic activation can have different meaning and may be sustained by factors different from those implicated for MA.

According to the “arousal” hypothesis<sup>8,10-12,35</sup> D-bursts and K-bursts, as well as rises in heart and respiratory rate,<sup>8,12</sup> are the primary types of arousal response, leaving the central nervous system more likely to be aroused in response to stimulation but still preserving continuity of sleep. If the arousal process continues and the magnitude of the stimulus is greater,<sup>14,15</sup> a progression to cortical activation may occur inducing MA. Accordingly, the 2 types of arousals are not randomly scattered but appear structurally distributed within sleep with an over-time evolution across sleep cycles similar for MA and D-bursts and K-bursts (Figure 3). Moreover, since the autonomic component of the arousal response precedes the EEG component,<sup>8-10</sup> both types of arousal may reflect the function of a common arousal-promoting system acting during sleep, involving separate physiologic substrates and operating in temporal succession,<sup>37</sup> namely “the brainstem system” and the “cortical system.” The first system includes caudal brainstem neurons<sup>38-40</sup> directly contributing to the overall changes of slow EEG activity and autonomic activation and considered as the primary level generator of the arousal response.<sup>7,10,14,15</sup> The second system includes forebrain and upper brainstem neurons that project rostrally to thalamic areas and finally to the cortex,<sup>37,39,41</sup> inducing, when activated, the EEG desynchronization and the concomitant activation of sensory and motor systems that defined MA. On the basis of these different neurophysiologic sources, slow EEG events and MA may undergo different modulatory influence.<sup>37</sup> If so, it can be anticipated that the fall in cortical arousals after manipulation of homeostatic sleep pressure would be proportionally greater than the decrease in subcortical arousals. In line with this hypothesis, we found a decrease in the K-bursts and D-bursts during recovery sleep that, even though not significant, followed the trend of MA. Thus, even though speculative, sleep deprivation increasing the cortical arousal threshold may modulate the decline of MA through a direct effect on cortical excitability or responsiveness, with slighter influence on brainstem neural generators.

The lack of significant difference in the density of subcortical arousal may be explained by several factors, including some methodologic limitations of our study. First, it could be questioned whether some of the findings resulted from time differences across the night. This does not appear to be the case, since a separate analysis (Figure 2) considering homogenous sleep duration at baseline and first recovery night confirmed the same



**Figure 4**—Histograms of the indexes of microarousals (MA) and of phases of transitory activation (PAT) during each sleep stage. While the MA index tended to fall significantly during light sleep, the PAT rate rose during stage (St.) 1 of non-rapid eye movement (NREM) sleep and during slow-wave sleep.

pattern of variation in D-burst and K-burst density. Second, the well-known variation in the power of delta activity during the first recovery night could have contributed to a bias in the visual detection of subcortical arousals. However, the high power of delta activity during the first recovery night would be more likely to induce an overestimation rather than an underestimation of the subcortical arousals.

In summary, it can be concluded that the strong increase in sleep drive following total sleep deprivation was associated with a declining rate of arousal responsiveness in the first recovery night. However, despite the fact that sleep deprivation blunts the arousal threshold, the significant reduction in MA density was associated with an insignificant fall in the rate of D-bursts and K-bursts. While the mechanisms of this dissociation in the arousal response cannot be completely ascertained from our data, this finding might corroborate the hypothesis of the existence of 2 separate neural systems<sup>10,37,42</sup> integrated in the arousal network and undergoing different modulatory influences. How this functions and how it is integrated by sleep processes remains poorly understood and needs further research.

## ACKNOWLEDGEMENTS

We are grateful to Andrea Hawton, Angela Ricci, Alain Roux, John Bowen, Tim Pointing, and Patty Odell for valuable technical assistance. We are indebted to Captain Kirk Evoy, who coordinated and arranged the selection of Canadian Forces volunteers, and to Dr. David Salisbury for providing medical assistance.

## REFERENCES

- Williams HL, Hammack JT, Daly RL, Dement WC, Lubin A. Responses to auditory stimulation, sleep loss and the EEG stages of sleep. *Electroencephalogr Clin Neurophysiol* 1964;16:269-79.
- Philip P, Stoohs R, Guillemainault C. Sleep fragmentation in normals: a model for sleepiness associated with upper airway resistance syndrome. *Sleep* 1994;17:242-7.
- Douglas NJ, Martin SE. Arousals and the sleep apnea/hypopnea syndrome. *Sleep* 1996;19:S196-7.
- Chugh DK, Weaver TE, Dinges DF. Neurobehavioral consequences of arousals. *Sleep* 1996;19:S198-201.
- Rosenthal L, Roehrs T, Sicklesteel J, Zorick F, Wittig R, Roth T. Periodic movements during sleep, sleep fragmentation and sleep-wake complaints. *Sleep* 1984; 7:326-30.
- American Sleep Disorders Association. EEG arousal: scoring rules and examples. *Sleep* 1992;15:174-84.
- Halasz P. Hierarchy of micro-arousals and the microstructure of sleep. *Neurophysiol Clin* 1998;28:461-75.
- Quattrocchi JJ, Shapiro J, Verrier RL, Hobson JA. Transient cardiorespiratory events during NREM sleep: a feline model for human microarousals. *J Sleep Res* 2000;9:185-91.
- Black JE, Guillemainault C, Colrain IM, Carrillo O. Upper airway resistance syndrome. Central electroencephalographic power and changes in breathing effort. *Am J Respir Crit Care Med* 2000;162:406-11.
- Sforza E, Jouny C, Ibanez V. Cardiac activation during arousals in humans: further evidence for hierarchy in the arousal response. *Clin Neurophysiol* 2000;111:1611-9.
- BuSha B, Leiter JC, Curran AK, Li A, Nattie EE, Damall RA. Spontaneous arousals during quiet sleep in piglets: a visual and wavelet-based analysis. *Sleep* 2001;24:499-513.
- Sforza E, Jouny C, Ibanez V. Time-dependent variation in cerebral and autonomic activity during periodic leg movements in sleep: implications for arousal mechanisms. *Clin Neurophysiol* 2002;113:883-91.
- Lijowska AS, Reed NW, Mertins Chiodini BA, Thach BT. Sequential arousal and airway defensive behavior of infants in asphyxial environments. *J Applied Physiol* 1997;83:218-8.
- McNamara F, Wulbrand H, Thach BT. Characteristics of the infant arousal response. *J Applied Physiol* 1998;85:2314-21.
- McNamara F, Lijowska AS, Thach BT. Spontaneous arousal activity in infants during NREM and REM sleep. *J Physiol* 2002;538:263-9.
- Pitson D, Chhina N, Knijn S, Van Herwaaden M, Stradling J. Changes in pulse transit time and pulse rate as markers of arousal from sleep in normal subjects. *Clin Science* 1994;87:269-73.
- Carley DW, Applebaum R, Basner RC, Onal E, Lopata M. Respiratory and arousal response to acoustic stimulation. *Chest* 1997;112:1567-71.
- Martin SE, Engleman HM, Kingshott RN, Douglas NJ. Microarousals in patients with sleep apnoea/hypopnoea syndrome. *J Sleep Res* 1997;6:276-80.
- Schieber JP, Muzet A, Feriere PJR. Les phases d'activation transitoire spontanée au cours du sommeil normal chez l'homme. *Arch Sci Physiol* 1971;25:443-65.
- Terzano MG, Mancina D, Salati MR, Costani G, Decembrino A, Parrino L. The cyclic alternating pattern as a physiological component of normal NREM sleep. *Sleep* 1985;8:137-45.
- Wauquier A. K-complexes: are they signs of arousal or sleep protective? *J Sleep Res* 1995;4:138-43.
- Terzano MG, Parrino L. Origin and significance of the cyclic alternating pattern (CAP). *Sleep Medecine Rev* 2000;4:101-23.
- De Gennaro L, Ferrara M, Bertini M. The spontaneous K-complex during stage 2 sleep: is it the "forerunner" of delta waves? *Neurosci Lett* 2000;291:41-3.
- Crowley K, Trinder J, Kim Y, Carrington M, Colrain IM. The effects of normal aging on sleep spindle and K-complex production. *Clin Neurophysiol* 2002;113:1615-22.
- De Gennaro L, Ferrara M, Bertini M. EEG arousals in normal sleep: variations by total and selective sleep deprivation. *Sleep* 2001;24:673-9.
- De Gennaro L, Ferrara M, Spadini V, Curcio G, Cristiani R, Bertini M. The cyclic alternating pattern decreases as a consequence of total sleep deprivation and correlates with EEG arousals. *Neuropsychobiology* 2002;45:95-8.
- Parrino L, Spaggiari MC, Boselli M, Barusi R, Terzano, MG. Effects of prolonged wakefulness on cyclic alternating pattern (CAP) during sleep recovery at different circadian phases. *J Sleep Res* 1993;91-5.
- Pigeau R, Naitoh P, Buguet A, et al. Modafinil, d-amphetamine and placebo during 64 hours of sustained mental work. I. Effects on mood, fatigue, cognitive performance and body temperature. *J Sleep Res* 1995;4:212-28.
- Rechtschaffen A, Kales A. A Manual of Standardized Terminology, Technique and Scoring System for Sleep Stages of Human Sleep. Los Angeles: Brain Information Service. Brain Information Institute, UCLA ; 1968.
- Parrino L, Boselli M, Spaggiari MC, Smerieri A, Terzano MG. Cyclic alternating pattern (CAP) in normal sleep: polysomnographic parameters in different age groups. *Electroencephalogr Clin Neurophysiol* 1998;107:439-50.
- Paiva T, Rosa A. The K-complex variability in normal subjects. In: Terzano MG, Halasz P, Declerck AC, eds. Phasic Events and Dynamics of Sleep. New York, Raven Press; 1991:167-84.
- Horner RL, Sanford LD, Pack AI, Morrison AR. Activation of a distinct arousal state immediately after spontaneous awakenings from sleep. *Brain Res* 1997;778:127-34.
- Akerstedt T, Billiard M, Bonnet M, et al. Awakening from sleep. *Sleep Med Rev* 2002;6:267-86.

34. Barbato G, Wehr TA. Homeostatic regulation of REM sleep in humans during extended sleep. *Sleep* 1998;21:267-76.
35. Harper RM, Richard CA, Henderson LA, Macey PM, Macey KE. Structural mechanisms underlying autonomic reactions in pediatric arousals. *Sleep Med* 2002;3:S53-6.
36. Parrino L, Smerieri A, Rossi M, Terzano MG. Relationship of slow and rapid EEG components of CAP to ASDA arousals in normal sleep. *Sleep* 2001;24:881-5.
37. Terzano MG, Parrino L, Rosa A, Palomba V, Smerieri A. CAP and arousals in the structural development of sleep: an integrative perspective. *Sleep Med* 2002;3:221-9.
38. Moruzzi G. The physiological properties of the brain stem reticular system. In: Adrian ED, Bremer F, Jasper HH, eds. *Brain Mechanisms and Consciousness*. Oxford: Blackwell; 1954:21-48.
39. Langhorst P, Stroh-Werz M, Dittmar K, Camerer H. Facultative coupling of reticular neuronal activity with peripheral cardiovascular and central cortical rhythms. *Brain Res* 1975;87:407-18.
40. Berlucchi G. One or many arousal systems? Reflections on some Giuseppe Moruzzi foresights and insights about the intrinsic regulation of brain activity. *Arch Ital Biol* 1997;135:5-14.
41. Steriade M, McCarley RW. *Brainstem Control of Wakefulness and Sleep*. New York: Plenum Press; 1990:223.
42. Thach BT. Graded arousals responses in infants: advantages and disadvantages of a low threshold for arousal. *Sleep Med* 2002;3:S37-40.