

Time of night and first night effects on arousal response in healthy adults

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Abstract

Objective: Several factors, such as homeostatic and circadian influences, may affect the density of cortical and subcortical arousals (AR). The purpose of this study was to examine the time-of-night and the first night effect on AR response.

Methods: AR were classified into microarousals (MA), phases of transitory activation (PAT), delta (D-burst) and K-complex burst (K-burst). The AR density and duration was analyzed during two consecutive nights with the analysis of sleep stage and sleep cycle in thirty-six healthy subjects.

Results: D- and K-burst showed a trend toward progressive decline across sleep cycles ($p < 0.0001$). While MA rate was unaffected throughout sleep cycles, PAT index increased across the night ($p = 0.002$). The density and duration of each group of AR exhibited reproducibility without significant differences between nights. An individual inter-night variability in AR density was found independently of night and sleep structure.

Conclusions: While homeostatic and circadian influences affect nighttime subcortical and MA responses, a wakefulness drive modulates the occurrence of AR with movements. Although the pattern of AR responses was highly reliable from the first to second night, the substantial inter-individual variability suggests the existence of an individual susceptibility.

Significance: The first night effect on arousal response is affected by individual susceptibility and circadian and homeostatic influences.

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Keywords: Arousals; Subcortical arousals; First night effect; Sleep stage; Sleep cycle

1. Introduction

Arousal (AR) from sleep, even though it does not cause a full awakening (Akerstedt et al., 2002), induced a sleep fragmentation contributing to daytime sleepiness and cognitive and psychomotor dysfunctions (Martin et al., 1996; Chugh et al., 1996). Although AR by definition means *cortical activation* and includes the different types of transient events such as microarousals (MA) (American Sleep Disor-

ders Association, 1992) and the phases of transitory activation (PAT) (Schieber et al., 1971; Collard et al., 1996), endogenous or an exogenous stimuli may result in overt autonomic activation without clear-cut EEG changes (Carley et al., 1997; Pitson and Stradling, 1998; Pillar et al., 2002) or with the appearance of EEG synchronization (Sforza et al., 2000; Halasz, 2005), called, respectively, “autonomic” and “subcortical” AR (Halasz et al., 2004). These AR induce fluctuations in cardiovascular (Pillar et al., 2002; Catcheside et al., 2002) and respiratory parameters (Rees et al., 1995) similar to those produced by cortical arousals and contribute to fatigue and sleepiness (Guilleminault et al., 2007). Thus, the concept of “arousal” includes a range of physiological responses related to different levels of central nervous system activation (Halasz

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et al., 2004) and determining the different patterns of electroencephalographic (EEG) and autonomic activation (Catcheside et al., 2002; Trinder et al., 2003).

During sleep, several mechanisms monitor external and internal stimuli to assure sleep continuity as well as to protect organisms against threatening stimuli. If so, changes in arousability might occur during the night in order to adapt humans to survival response and preserve sleep continuity. Arousal associated with movements are more frequent in stage 1 of non-rapid-eye-movement (NREM) sleep and in rapid-eye-movement (REM) sleep (Halasz et al., 2004), and they increase linearly through the night, the last sleep cycles containing more movements than the first ones (Collard et al., 1996). When we consider MA, they are more frequent during the ascending slopes of sleep cycles (Halasz et al., 1979) and in the last part of the night (Terzano et al., 2000), the tendency to arouse increasing across the night (Franco et al., 2001) as a function of accumulated sleep time. Sleep propensity also affects the occurrence of cortical arousals as suggested by reduction in MA density after total or partial sleep deprivation (De Gennaro et al., 2001). Therefore, we can say that as a consequence of integration of homeostatic and circadian mechanisms (Halasz, 1993) acting on arousal systems, sleep stage (Bonnet and Arand, 1997), sleep pressure (Parrino et al., 1993; De Gennaro et al., 2001), type (Kato et al., 2004) and intensity of sensory stimuli (Halasz, 1993; Williams et al., 1964) affect arousal responsiveness and arousal threshold. Moreover, since polysomnography itself represents an external stimulus, a “first night effect” may be present in experimental studies inducing not only reduced total sleep time and lower sleep efficiency but also rise in AR density (Agnew et al., 1966; Le Bon et al., 2001).

Much of our knowledge on the factors influencing arousal responsiveness comes from the studies on classical microarousals in normal subjects and in some patients with sleep disorders, (Sforza et al., 1999; Sforza et al., 2002) few data in the literature assessing factors affecting frequency and temporal evolution of subcortical arousals (Terzano et al., 2000; Terzano et al., 2002). The aim of the current retrospective study was thus to assess sleep cycle and time of the night effect on arousal responses by considering cortical and subcortical arousals separately. We further compared these effects on arousal density during two consecutive nights in order to characterize the first night effect on arousal responses. By doing so, we intended to obtain a general overview of the main factors influencing arousal response and their respective impacts on cortical and subcortical activation.

2. Methods

2.1. Subjects

The subject sample consisted of thirty-six subjects, 30 males and six females, aged 24.6 ± 6.9 yr (range 18–44) recruited among reservists of the Canadian Forces, and

participating as volunteers in a sleep-deprivation study (Pigeau et al., 1995) including two baseline nights, 64 h of sleep deprivation under continuous EEG recording and two recovery nights. Prior to the study, all subjects were screened for any current or past medical, neurological or psychiatric history, and they were drug-free at the time of the study. All the participants were without the history of excessive daytime sleepiness or sleep complaints and all had regular life habits and sleep–wake schedule. The Toronto Defense Research and Development Canada Ethics Committee and the Department of Health and Welfare Canada approved the experiment and all the subjects gave a written consent.

Sleep data of the two baseline nights were used in the analysis, the first night allowing exclusion of sleep disorders such as insomnia, sleep apnea syndrome (<5 apnea–hypopnea index) and periodic limb movement disorder (<5 periodic leg movements during sleep).

2.2. Nocturnal sleep recording

The subjects were fitted with electrophysiological recording equipment (Oxford Medilog 9200 ambulatory recorder) to measure four electroencephalograms (EEG) (C_3 , C_4 , P_3 , P_4 referenced to linked ears), an electro-oculogram (EOG), an electromyogram (EMG) and an electrocardiogram (ECG). During the first baseline night, respiration was monitored with thermistors and thoracic movements, and a tibialis electromyographic (EMG) activity was recorded using surface electrodes placed on the right and left legs. Sleep stages were visually scored by the same observer (ES) according to the standard criteria (Rechtschaffen and Kales, 1968) using 20-s epochs, the investigator being kept blind to subject and experimental condition. NREM–REM sleep cycles were defined according to the criteria of Feinberg and Floyd (Feinberg and Floyd, 1997). For each experimental night, standard sleep parameters were computed over the complete sleep time-period. All the recordings were analyzed for sleep staging and arousal scoring using the PRANA[®] software package (PhiTools[®], Strasbourg, France).

2.3. Data analysis

2.3.1. Visual EEG scoring

Arousals were categorized into four groups according to the previously published criteria (Sforza et al., 2000). We classified (Fig. 1): (1) Delta bursts (D-bursts) as a sequence of delta waves, lasting at least 2-s, exceeding by at least 1/3 the amplitude of background activity and detectable on at least three EEG derivations. (2) K-bursts as a sequence of two or more K-complexes immediately following each other without alpha activity, detectable on at least three EEG derivations. (3) Microarousals (MA) were defined according to ASDA criteria (American Sleep Disorders Association, 1992) as a return to alpha, theta or fast frequency, well differentiated from the background EEG activity. The duration

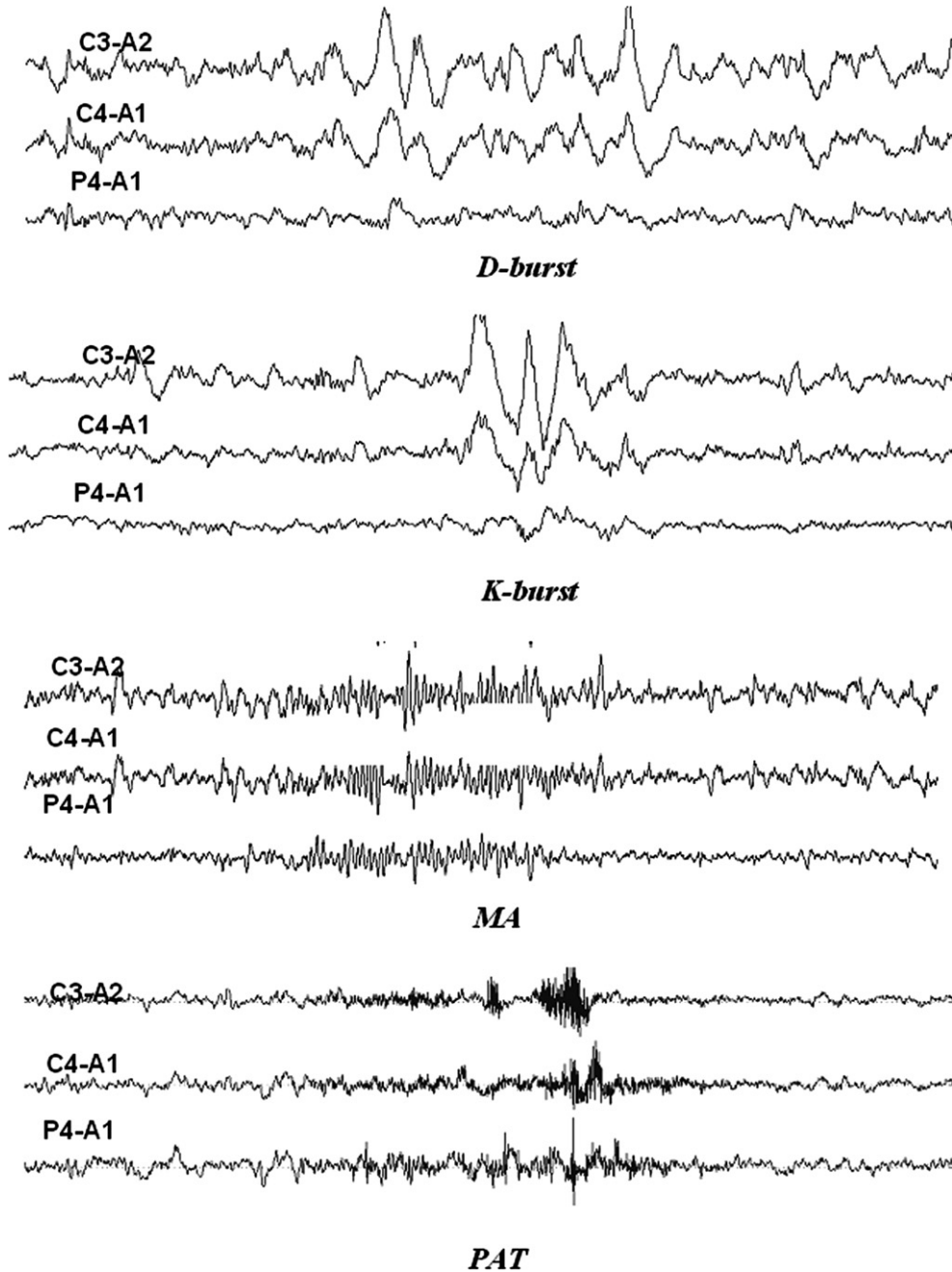


Fig. 1. EEG sample of each arousal type.

was, however, extended to include MA lasting between >1.5 -s and <3 -s. 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) The phases of spontaneous transitory activation (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, the appearance of muscular artifacts, the acceleration of heart rate and, during REM sleep, transitory disappearance of rapid-eye movements, according to the previously described criteria (Schieber et al., 1971; Collard et al., 1996). The duration of PAT

ranged from >3 -s up to 30-s. 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-s indicating return to sleep, and the termination of D- and K-bursts as a return to pre-arousal background activity.

For each type of arousals, the number, the index (number/hour of sleep) and the mean duration were computed for total sleep as well as for each sleep stage and each sleep cycle. For D- and K-bursts, duration and index were calculated considering sleep stage 2 and slow wave sleep (SWS)

(stages 3 and 4 of NREM sleep) only. For a quantitative description of the temporal variation over the course of the night, the analyses were performed for the first four sleep cycles recorded in both nights. Intra-subject variability was analyzed considering differences in absolute values between nights for each arousal type as well as for the total arousal density.

2.3.2. Statistical analyses

The differences between nights for sleep and arousal characteristics during the total sleep period and for each sleep stage and sleep cycle were determined with a two-tailed paired Student's *t* test.

The analysis of variance for repeated measures was done to assess the effect of sleep cycle on each AR index and duration. A trend analysis was performed to evaluate if changes across sleep cycles fit a linear or exponential slope. The arousal index and duration were submitted to two-way analysis of variance (ANOVA) with “night” (first and second night) \times “sleep cycle”, in order to assess their density and duration across sleep cycles as a function of the experimental night. For MA and PAT, comparisons of arousal index and duration between sleep stages were carried out by use of two-way ANOVA for repeated measures with “night” and “sleep stage” as the factors. Whenever significant main factor or interaction effects were present, *post hoc* Student–Newman–Keuls tests were used to assess significant differences. Bivariate correlation analysis using Pearson correlation coefficient was performed to determine whether variations in the amount of sleep stages were correlated with changes in the arousal indices between nights.

All statistical analyses were performed with the SPSS statistical software package (SPSS for Windows, 11, SPSS Inc, Chicago). The level of significance was set at $p < 0.01$ corrected for the number of comparisons. Data are reported as means \pm SEM.

3. Results

3.1. Polygraphic sleep parameters

The sleep parameters of the two nights are shown in Table 1. As expected, all the subjects slept well in both nights with higher sleep efficiency, lower sleep latency and a normal amount of each sleep stage in NREM sleep. REM sleep amount was greater in the second night ($p < 0.01$) without the differences in REM latency. No significant differences in sleep cycle duration were found between nights.

3.2. Effect of the night on arousal index

The effects of the experimental night on the arousal index and duration during the total sleep time are shown for each arousal type in Table 2. The statistical analysis for total arousal ($t = 1.6$, $p = 0.12$), D-burst ($t = 1.15$, $p = 0.26$), K-burst ($t = 0.64$, $p = 0.52$), MA ($t = 1.18$, $p = 0.248$) and PAT ($t = -0.82$, $p = 0.42$) revealed that

Table 1
Sleep parameters (mean \pm SEM) during the two nights

	First night	Second night
Total sleep time (min)	457.7(4.98)	454.8(3.32)
Sleep latency (min)	9.67(1.20)	8.44(0.87)
Sleep efficiency (%)	96.2(0.42)	96.7(0.22)
WASO (%)	6.23(0.57)	6.36(0.83)
Stage 1 (%)	8.41(0.59)	8.50(0.54)
Stage 2 (%)	53.9(0.83)	51.1(1.62)
SWS (%)	17.6(0.92)	16.3(0.86)
Stage REM (%)	20.0(0.59)	22.3(0.51) ^a
1st sleep cycle duration (min)	100.7(3.9)	94.3(4.23)
2nd sleep cycle duration (min)	106.8(3.87)	101.0(3.49)
3rd sleep cycle duration (min)	108.5(3.81)	105.2(4.20)
4th sleep cycle duration (min)	98.8(3.79)	97.4(3.90)

Abbreviations: WASO: wake after sleep onset; SWS: slow wave sleep.

^a Two-tailed Student paired test differences between nights (* $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$.)

Table 2

Polygraphic characteristics (mean \pm SEM) of scored arousals in the two consecutive nights

	First night	Second night
D-burst index (n/h)	6.32(0.64)	5.82(0.60)
K-burst index (n/h)	5.76(0.57)	5.45(0.63)
MA index (n/h)	13.5(0.90)	12.9(0.90)
PAT index (n/h)	3.62(0.31)	3.85(0.27)
D-burst duration (s)	6.32(0.11)	6.01(0.12)
K-burst duration (s)	5.32(0.67)	5.10(0.96)
MA duration (s)	8.22(0.13)	7.90(0.13)
PAT duration (s)	13.41(0.33)	13.02(0.35)

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

the indices and the duration of each category did not show significant differences between nights, these measures being consistently similar in the first and second nights. The indices were highly reproducible from one night to the other, with a between nights correlation coefficient, of 0.76 ($p < 0.0001$) for D-burst, 0.69 ($p < 0.0001$) for K-burst, 0.84 ($p < 0.0001$) for MA and 0.54 ($p = 0.001$) (Fig. 2). However, there was an individual variability in indices when comparing the two nights, with an average change in the absolute density of -1.18 ± 0.4 (range: -15.1 – 11.3) for total arousal index, -0.49 ± 0.3 (range: -8.5 – 4.9) for D-burst, -0.31 ± 0.3 (range: -7.1 – 4.9) for K-burst, -0.61 ± 0.3 (range: -7.5 – 4.4) for MA index, 0.23 ± 0.2 (range: -4.8 – 3.3) for PAT (Fig. 3). Pearson correlation test showed no relation between the changes in each AR index between nights and the changes in sleep parameters.

3.3. Effect of sleep–wake cycle

The results of the nocturnal variation in density and duration for each arousal type during the first four sleep cycles are illustrated in Figs. 4 and 5. The analysis of variance for repeated measures showed a significant sleep cycle

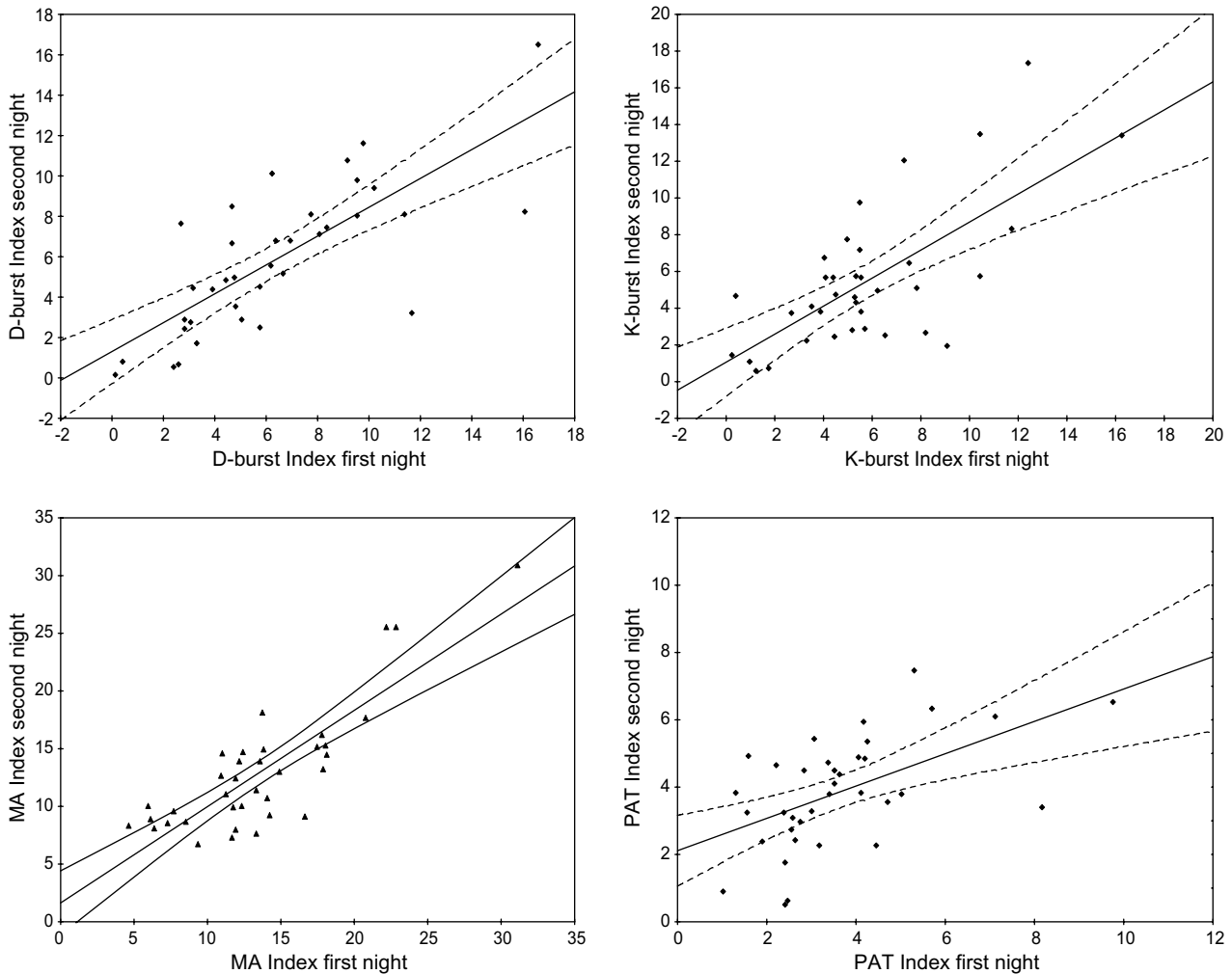


Fig. 2. Scatter-plots showing the reproducibility (correlation coefficient and 95% confidence interval) of the index of each arousal type in the two nights.

effect for D-burst ($F(3, 21) = 42.1, p < 0.0001$) and K-burst indices ($F(3, 21) = 10.8, p < 0.0001$) with a linear time trend to be higher at the beginning of the night, followed by a progressive decline over consecutive sleep cycles. As illustrated in Fig. 4, the D-burst density peaked sharply during the first sleep cycle and then linearly declined across sleep cycles ($F = 99.3, p < 0.0001$). For K-bursts, the density was higher and stable during the first two sleep cycles, followed by a linear decline in the following sleep cycles ($F = 25.5, p < 0.0001$). The ANOVA with cycle and night as factors revealed a significant main effect of cycle without any interaction for K-burst, the index being similar in the first and second nights ($F(3, 21) = 0.27, p = 0.84$). For D-burst, there was a tendency to decreased density in the second night, but this effect did not reach statistical significance ($F(3, 21) = 2.32, p = 0.08$). The decrease in the subcortical arousal density across the night was not associated with significant changes in D-burst ($F(3, 27) = 0.09, p = 0.96$) and K-burst ($F(3, 27) = 0.08, p = 0.93$) duration, this being similar in both the nights (Fig. 5).

The lower panels of Fig. 4 show the temporal occurrence of cortical arousals during the four sleep cycles in the two

nights. For MA, neither the index ($F(3, 21) = 2.35, p = 0.07$) nor the duration ($F(3, 21) = 2.35, p = 0.07$) showed a main effect of sleep cycle and non significant interaction MA index ($F(3, 21) = 1.28, p = 0.28$), and MA duration ($F(3, 21) = 0.93, p = 0.43$), showing considerable uniformity throughout the first and second nights. The ANOVA for repeated measures revealed a significant sleep cycle effect ($F(3, 21) = 14.6, p < 0.0001$) for PAT index, the average index linearly increasing ($F = 37.0, p < 0.0001$) in the third and four sleep cycles. Moreover, a significant interaction was present for PAT index, the average index being greater in the second night ($F(3, 21) = 4.52, p = 0.004$). Comparison for PAT duration did not show significant cycle ($F(3, 17) = 1.05, p = 0.37$) nor cycle \times night ($F = 0.48, p = 0.69$) effect, duration being similar in both the nights (Fig. 5).

3.4. Effect of sleep stage on MA and PAT indices and duration

The group means for MA and PAT index and duration in each sleep stage are shown in Table 3. The two-way

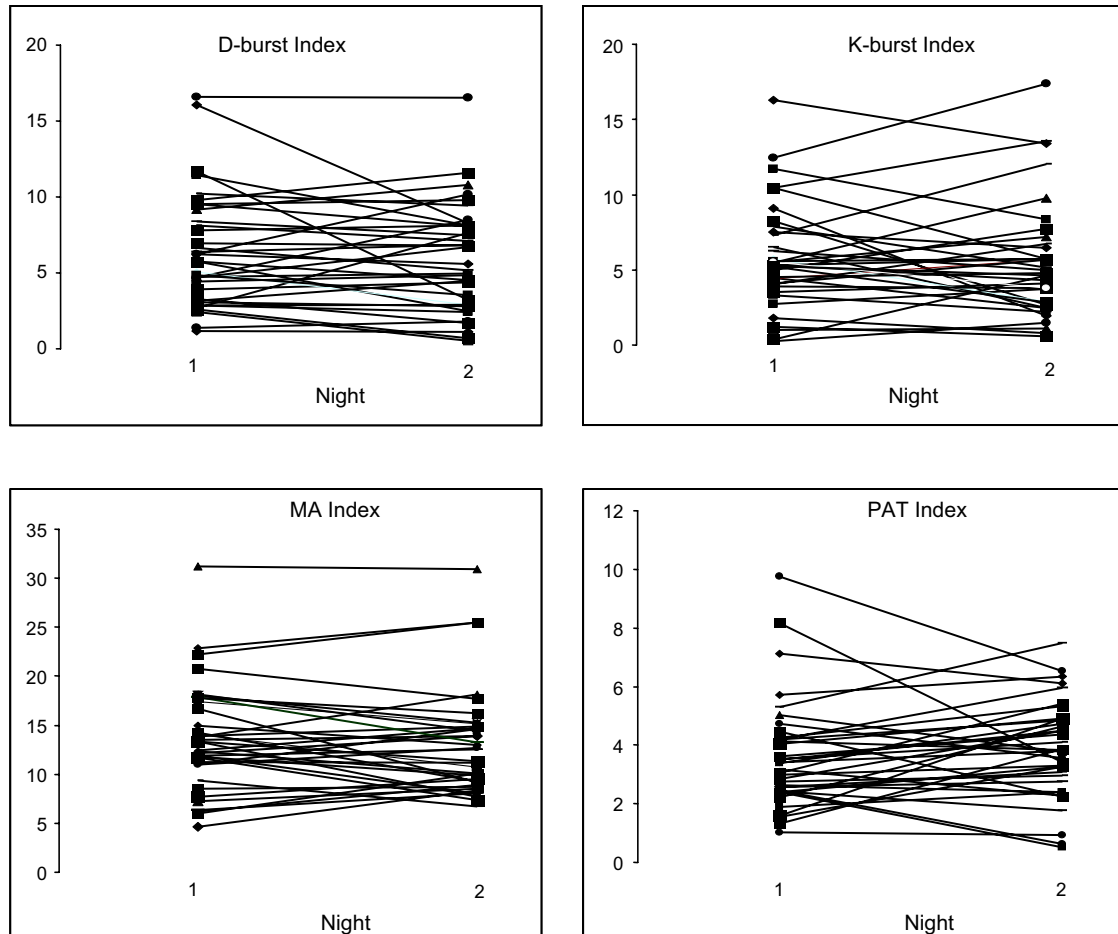


Fig. 3. Individual values of each arousal index during the two nights. Individual variability is more evident for K-burst and PAT.

repeated measures ANOVA revealed for MA index ($F = 184.3$, $p < 0.0001$) and duration ($F = 22.3$, $p < 0.0001$) a significant main effect of sleep stage, duration being greater in SWS and index in stage 1 of NREM sleep and REM sleep. No night effect was found either for MA index and MA duration, except during REM sleep. A similar pattern of variation was found for PAT index ($F = 35.9$, $p < 0.0001$) and PAT duration ($F = 15.7$, $p < 0.0001$), the index being greater in stage 1 and the duration peaking during slow wave sleep, but again without interaction, the trend in density and duration being similar in the first and second nights.

4. Discussion

The present study aimed to analyze factors affecting arousal responsiveness in adults considering cortical and subcortical arousals. The first finding of our study is that cortical and subcortical arousals showed a different time-of-night effect on their occurrence without any effect on their duration. While D- and K-burst showed a tendency to progressive and linear decline across sleep cycles, MA occurred in each sleep cycle with a density similar across the night. In contrast, PAT, increased linearly from the first

to the last sleep cycle. Moreover, there was no systematic night-to-night variation in arousal density, arousal duration and temporal occurrence, suggesting the absence of a “first night effect” on sleep microstructure. However, an individual variance in arousal occurrence was noted, not accounted for changes in sleep time or sleep efficiency. Overall, these data suggest that sleep and wakefulness drive influenced differently the appearance of arousals and PAT, the latter translating into a progressive rise of the wakefulness drive rather than a change in arousal threshold and sleep propensity. Moreover, the first night effect on AR types density suggests that individual brain responsiveness to arousing stimuli and individual predisposing factors more than environmental ones may influence variability in sleep microstructure.

The main finding of the current study is that the time of the night occurrence of the four arousal types indicates the interference of different systems both arousing and awakening subjects that are responsible for differences in the time course of cortical and subcortical arousals density. Overall, while subcortical arousal rate shows a trend to progressive decline across sleep cycles, PAT have a tendency to rise at the end of the night, MA showing a similar density throughout the night. The over-time nocturnal

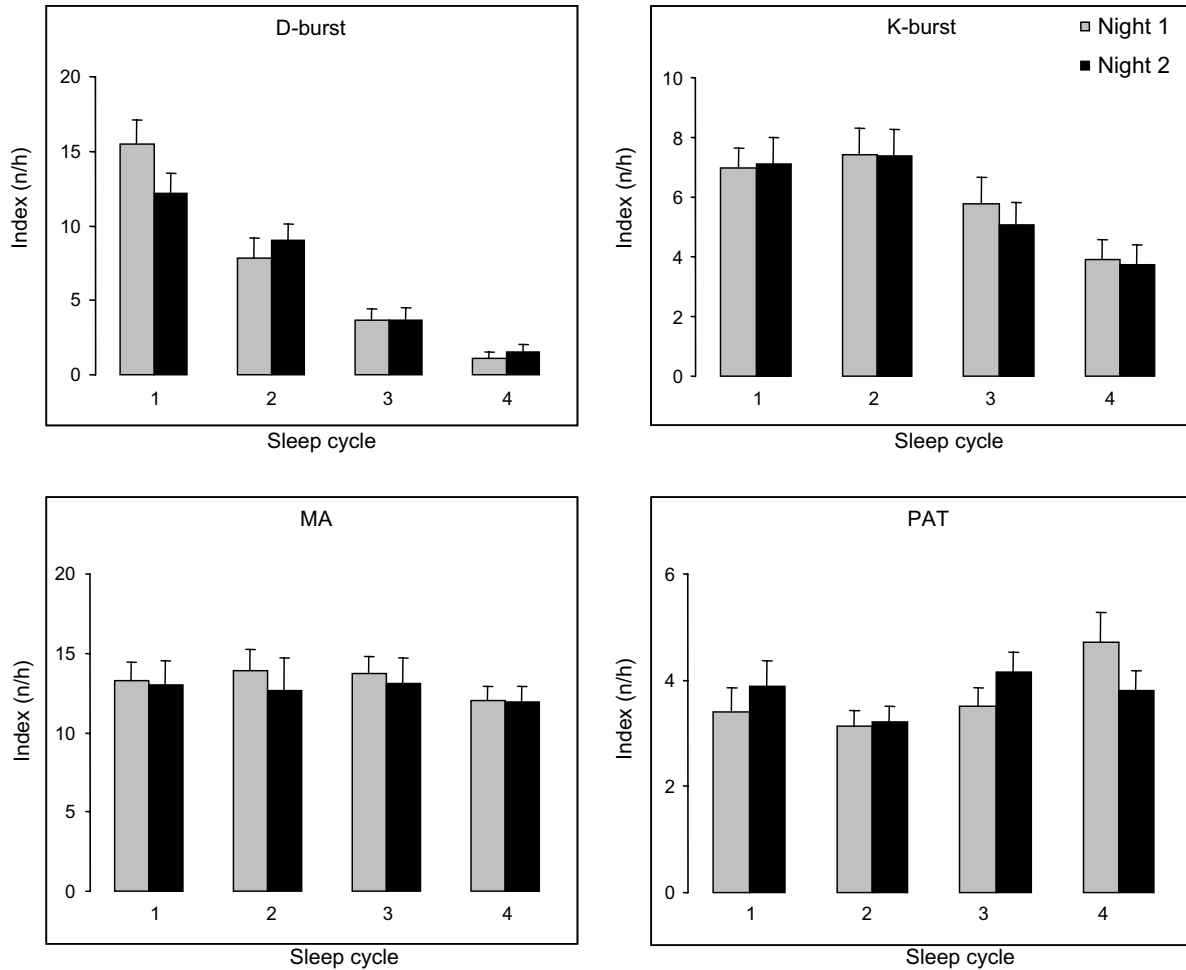


Fig. 4. Average index of the four arousal types in the first four sleep cycles during the first and the second nights. A main effect of sleep cycle was found for D-burst and K-burst showing a progressive and linear decline across the night, more marked for D-burst. MA rate remains stable across sleep cycles, while PAT index linearly rises in the third and four sleep cycle. The over-time evolution in the first and second nights was similar for all types of arousals.

evolution of the indices of the arousal response was not associated with variations in their duration suggesting a constant and similar response of arousing stimuli across the night. A critical question unanswered by our results is why the time of the night arousal responsiveness varied only for subcortical arousals and PAT and whether this trend is related to the differences in circadian and homeostatic influences or to different mechanisms generating the arousal response.

According to classical theories, circadian and homeostatic influences (Halasz, 1993; Achermann et al., 1993) and intrinsic oscillatory mechanisms, i.e., cyclic alternating pattern (CAP) (Terzano et al., 2002), regulate sleep and arousal threshold and as a consequence arousal occurrence. The homeostatic process dissipates over NREM sleep cycles and it is reflected by the time course of slow wave activity (SWA) and slow wave sleep (Aeschbach and Borbély, 1993). In contrast, the circadian process reflects the circadian timing of REM sleep and the drive for wakefulness (Achermann et al., 1993). If so, when sleep propensity and sleep pressure are high, as is the case at the beginning of the night, the arousal

threshold will be higher and sensory inputs may modify sleep in a lesser extent, generating D- and K-bursts (De Gennaro et al., 2000) or phases A1 of CAP (Parrino et al., 2001). Therefore, the nocturnal over-time decay in sleep propensity would explain the peaking of D- and K-bursts at the beginning of the night and the gradual falling throughout the sleep episodes. In the last part of the night, when the arousal threshold and the sleep propensity is lower, sensory inputs may modify the sleep structure shaping the individual course of sleep and inducing the rise in cortical arousals. However, in contrast to this hypothesis, we found that subcortical arousals showed a progressive decline across successive sleep cycles, MA did not change significantly across sleep cycles and PAT increased at the end of the night. This is also the case when we consider the nocturnal evolution of CAP (Terzano et al., 2000; Terzano et al., 2002), phases A1 following an exponential decline across sleep cycles, and phases A2 and A3, similar to MA and PAT (Parrino et al., 2001), showing a regular amount throughout the night (Terzano et al., 2000) or an increase in the last part of the night (Terzano et al., 2002). The constant amount

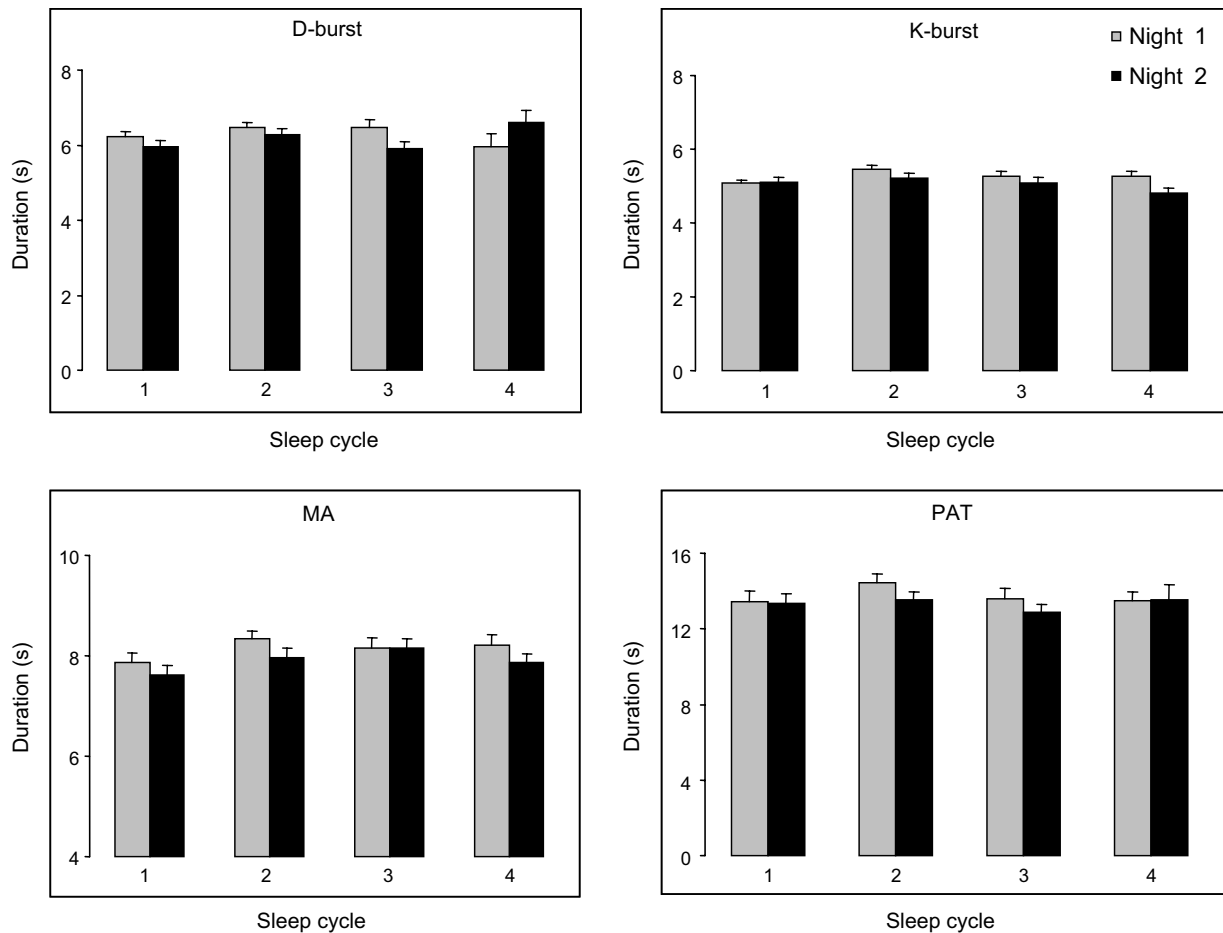


Fig. 5. Histograms of the duration of the four arousal types during each sleep cycle. No differences in duration were found throughout sleep cycles for all type of arousals both in the first and second nights.

Table 3
Polygraphic characteristics (mean \pm SEM) of the MA and PAT according to sleep stage in the two consecutive nights

	First night	Second night	<i>p</i>
MA index stage 1 (n/h)	35.1(2.48)	33.4(2.21)	Ns
MA index stage 2 (n/h)	12.5(1.07)	12.5(1.36)	Ns
MA index stages 3–4 (n/h)	4.1(1.25)	2.1(0.36)	Ns
MA index stage REM (n/h)	17.7(1.35)	15.6(1.19)	0.04
MA duration stage 1 (s)	8.38(0.17)	7.90(0.19)	0.03
MA duration stage 2 (s)	8.23(0.16)	7.92(0.18)	Ns
MA duration stages 3–4 (s)	10.7(0.61)	9.49(0.63)	Ns
MA duration stage REM (s)	8.09(0.18)	7.72(0.16)	0.07
PAT index stage 1 (n/h)	10.8(1.90)	9.47(1.25)	Ns
PAT index stage 2 (n/h)	2.79(0.38)	2.88(0.27)	Ns
PAT index stages 3–4 (n/h)	3.33(0.49)	2.72(0.26)	Ns
PAT index stage REM (n/h)	4.82(0.39)	4.91(0.49)	Ns
PAT duration stage 1 (s)	12.2(0.53)	11.7(0.61)	Ns
PAT duration stage 2 (s)	14.0(0.48)	13.9(0.49)	Ns
PAT duration stages 3–4 (s)	15.9(0.63)	15.6(0.89)	Ns
PAT duration stage REM (s)	12.8(0.40)	12.2(0.52)	Ns

See Table 2, *p*: Two-tailed Student paired test differences between nights. Ns, not significant.

of MA across the night and the more consistent rise of PAT at the end of the night suggest that MA and

PAT reflect two different aspects of the waking intrusion during sleep. The MA indicates a “transient” cortical activation followed by a return to sleep (Horner et al., 1997), expression of a lowering of arousal threshold and sleep pressure during sleep. In contrast, PAT corresponds to “awakening”, indicating a clear discontinuity of sleep episode and a stronger “waking drive” that reestablish full alertness (Bonnet, 2000). Such a hypothesis would predict the different effect of sleep loss on cortical arousals, sleep deprivation significantly reduces MA (De Gennaro et al., 2001) without effect on PAT density (Sforza et al., 2004).

The second aim of our study was to examine the first night effect on AR density and duration for cortical and subcortical arousals. Since we know that the “first night effect” (Agnew et al., 1966; Le Bon et al., 2001) elicits changes in sleep propensity and sleep stability, we hypothesized that the stressing effect imposed by the first night of recording would have an increasing effect on arousal frequency and that more indices of sleep fragmentation would be present in the first night when sleep instability would be greater. Contrary to expectation, as shown in Figs. 3 and 4, neither the index nor the duration of all AR types showed

significant changes across nights suggesting that polysomnography did not have a systematic adverse effect on sleep microstructure. Moreover, the temporal occurrence of all AR types was similar between nights, supporting the hypothesis that the time-of-night effect on arousal responsiveness follows an intrinsic and endogenous circadian rhythm persistent in following nights and similar in each subject. Finally, the intraclass correlation coefficients of 0.76, 0.69, 0.84 and 0.54 for the D-burst, K-burst, MA and PAT index, respectively, indicate a relatively good agreement, suggesting that the indices of sleep fragmentation were not underestimated on the first nocturnal recording. As previously published (Curcio et al., 2004), a systematic and strong first night effect was not observed in our subjects suggesting that a single night of recording may be an adequate measure of sleep microstructure in young and middle-aged healthy subjects (Bonnet and Arand, 2006). However, we observed an intra-individual variability between nights with a decrease in all types of arousal in 16 subjects, an increase in 12 and a stable value in eight. Subject factors that have been evaluated as sources of night-to-night variability include sleep architecture, age and values during the first night (Le Bon et al., 2001). In our subjects, no statistically significant differences were observed between nights for sleep efficiency, slow wave sleep or percent of each sleep stage. Moreover, neither sex nor AR index in the first night influenced the observed changes suggesting that these variables do not account alone for the AR density variation in our group. In addition, MA lasting $>1.5 < 3$ were few in our sample (1.9% of the total sample) suggesting that inclusion of these AR does not affect MA density and its variation across nights. Finally, we can suggest that the lack of inter-night differences in our subjects was related to a sleep deprivation condition, as suggested by the shorter sleep latency and the good sleep quality. However, as previously described (Pigeau et al., 1995), all subjects followed for at least one week a regular sleep schedule confirmed by sleep log and clinical interview excluding the effect of a sleep deprivation. Therefore, further studies, involving larger samples and subjects of different ages, are needed to define the mechanisms and factors underlying this variability, a biological individual trait-like predisposition (Van Dongen et al., 2005) probably responsible not only for sleep/wake parameters variability but also for differences in arousal responsiveness.

In summary, the quantification of arousal frequency in terms of EEG-arousals and subcortical arousals appears to be an important marker to assess sleep fragmentation in adults. The dissociated nighttime course of cortical and subcortical arousals indicates that arousal response is not a homogenous phenomenon taking place during sleep, homeostatic, circadian influences and wakefulness drive being integrated in the modulation of arousal responsiveness. The assessment of arousal density and duration between two nights failed to demonstrate a significant inter-night variability for duration, density and

over-time occurrence, suggesting the role of an individual susceptibility on the first night effect on sleep fragmentation.

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