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Rythmes circadiens et mécanismes homéostatiques de récupération chez des personnes de type matinal ou vespéral

par
Valérie Mongrain

Département de Sciences Neurologiques
Faculté de Médecine

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Rythmes circadiens et mécanismes homéostatiques de récupération chez des personnes de type matinal ou vespéral

présentée par :
Valérie Mongrain

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Roger Godbout, PhD, président-rapporteur
Marie Dumont, PhD, directrice de recherche
Jacques Montplaisir, MD, PhD, membre du jury
Diane Boivin, MD, PhD, examinatrice externe
Gilles Lavigne, DMD, PhD, représentant du doyen de la FES

Résumé

Le but premier de ce projet de recherche était d'étudier l'interaction entre les processus circadien et homéostatique de régulation du sommeil et de la vigilance. Pour ce faire, le modèle des chronotypes a été utilisé puisque les types matinaux et vespéraux (ayant un horaire de sommeil respectivement hâtif ou tardif) diffèrent dans leur relation temporelle entre la phase circadienne et l'épisode de sommeil (angle de phase). Le sommeil et la vigilance ont été comparés entre 12 types matinaux et 12 types vespéraux pour étudier les marqueurs du processus homéostatique, les effets d'une augmentation de pression homéostatique et la récupération homéostatique suite à une augmentation de la durée de l'éveil, chez des individus synchronisés avec différents angles de phase. Les premiers résultats ont révélé que les participants matinaux et vespéraux étaient synchronisés avec des angles de phase similaires. Cependant, ceci nous a permis de proposer que des différences dans le processus homéostatique puissent engendrer des chronotypes différents. Les analyses des marqueurs du processus homéostatique en sommeil et à l'éveil, en conditions normales et suite à une fragmentation du sommeil, ont montré que les types matinaux ont une dynamique homéostatique plus rapide comparativement aux types vespéraux. De plus, nos analyses ont révélé l'existence de 2 catégories de chronotypes : des individus avec des phases circadiennes très différentes et une régulation homéostatique similaire, chez lesquels la phase circadienne semble déterminante pour la préférence d'horaire de sommeil, et des individus avec des phases circadiennes semblables mais une régulation homéostatique différente, chez qui les différences homéostatiques semblent engendrer la préférence pour l'horaire de sommeil. Finalement, certaines analyses nous ont permis d'observer que les différences circadiennes et homéostatiques ne covarient pas chez les chronotypes mais qu'elles semblent être indépendantes. Les résultats de ce programme de recherche ont montré pour la première fois des différences dans la régulation homéostatique du sommeil chez des individus normaux et en santé. De plus, ce projet a permis de faire ressortir que des différences homéostatiques modifient la relation de phase stable entre le cycle éveil-sommeil et la phase circadienne.

Mots-clés : rythmes circadiens, régulation du sommeil, chronotypes, humain, mélatonine, température, analyse spectrale de l'électroencéphalogramme (EEG), activité à ondes lentes, vigilance, performance.

Abstract

The aim of this research projet was to study the interaction between the circadian and homeostatic processes of sleep regulation. To achieve this goal, chronotypes where used as a model because morning and evening types (having respectively early and late sleep schedules) differ in the timing of their sleep episode in relation to their circadian phase (phase angle). Sleep and alertness were compared between 12 morning chronotypes and 12 evening chronotypes to measure markers of the homeostatic process, to evaluate the effects of an increase in homeostatic sleep pressure, and to study the recovery following an increase in time awake, in individuals synchronized with different phase angle. Contrary to expectations, the first results of the project revealed that the morning and evening subjects were synchronized with a similar relationship between their sleep episode and circadian phase. This observation led us to propose that homeostatic differences could generate differences in chronotypes. Subsequent analyses confirmed, using markers of homeostatic sleep presure during sleep and wakefulness before and after sleep fragmentation, that morning individuals had faster dynamics of homeostatic sleep pressure than evening types. Moreover, our analyses revealed the existence of 2 kinds of chronotypes: individuals with different circadian phases and similar homeostatic dynamics in which the circadian phase appears to determine sleep schedule preference, and individuals with similar circadian phases but different dynamics of homeostatic sleep regulation in which variations in homeostatic sleep regulation seem to generate the sleep schedule preference. Finally, we observed that circadian and homeostatic differences did not covary but instead appeared to be independant in this population. The results of this research program are the first to show variations in homeostatic sleep regulation in healthy normal humans. Additionally, this project led to the discovery that variations in the dynamics of homeostatic sleep regulation modify the stable phase relationship between the sleep-wake cycle and circadian phase.

Keywords : circadian rhythms, sleep regulation, chronotypes, human, melatonin, temperature, electroencephalogram (EEG) spectral activity, slow-wave activity, vigilance, performance.

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Liste des abréviations

en français :

AOL : activité à ondes lentes

ASPS : syndrôme du sommeil en avance de phase

CRSNG : conseil de recherche en sciences naturelles et en génie du Canada

DSPS : syndrôme du sommeil en délai de phase

ECG : électrocardiogramme

EEG : électroencéphalogramme

EMG : électromyogramme

EOG : électrooculogramme

EVA : échelle visuelle analogique

FES : Faculté des études supérieure

FRSQ : Fonds de recherche en santé du Québec

HSCM : Hôpital du Sacré-Coeur de Montréal

Hz : Hertz (unité de fréquence : 1 Hz = 1 cycle par seconde)

J1 : premier 24 heures du projet de recherche, nuit et journée d'adaptation

J2 : second 24 heures du projet de recherche, nuit et journée de base

J3 : troisième 24 heures du projet de recherche, nuit et journée de fragmentation 1

J4 : quatrième 24 heures du projet de recherche, nuit et journée de fragmentation 2

J5 : dernier 24 heures du projet de recherche, nuit et journée de récupération

QMV : Questionnaire de matinalité-vespéralité

processus C : processus circadien de régulation du sommeil et de la vigilance

processus S : processus homéostatique de régulation du sommeil et de la vigilance

SLP : sommeil lent profond (stades 3 et 4 de sommeil)

TIDE : test itératif d'endormissement

Types-M : types matin

Types-S : types soir

en anglais :

AD : first 24 hours of the protocol, night and day of adaptation

ANOVA : analysis of variance

BL : second 24 hours of the protocol, night and day of reference

CIHR : Canadian Institute of Health Research

CSM : Composite scale for morningness

CTQ : Circadian type questionnaire

CTS : circadian timing system

CV : coefficient of variation

DLMO : Dim light melatonin onset

DLMOangle : phase angle of entrainment between DLMO and wake time

DSPS : delayed sleep phase syndrome

DTS : diurnal type scale

FFT : fast Fourier transform

FR1 : third 24 hours of the protocol, night and day of fragmentation 1

FR2 : fourth 24 hours of the protocol, night and day of fragmentation 2

E-types : evening types

EEG : electroencephalogram

EM : eye movements

EMG : electromyogram

EOG : electrooculogram

Hz : Hertz (frequency unit : 1 Hz = 1 cycle per second)

M-types : morning types

MD : Marie Dumont

MEQ : morningness-eveningness questionnaire

MSLT : multiple sleep latency test

NREM : non-rapid-eye movement

NSERC : Natural Sciences and Engineering Research Council of Canada

POMS : profile of mood state

PSG : polysomnographic recording

Process C : circadian process of sleep regulation

Process S : homeostatic process of sleep regulation

PVT : Psychomotor vigilance task

r : slope of the decay given by the nonlinear regression analysis

REC : last 24 hours of the protocol, night and day of recovery

REM : rapid-eye movement

RT : reaction time

SEM : standard error of the mean

SWA : slow-wave activity

SWA_t : slow-wave activity at time t used to compute nonlinear regression analysis

SWA_0 : parameter of initial slow-wave activity given by the nonlinear regression analysis

$SWA\infty$: horizontal assymptote for infinite time given by the nonlinear regression analysis

t : independant parameter time used to compute nonlinear regression analysis

T_{min} : temperature minimum

Tangle : phase angle of entrainment between T_{min} and wake time

VAS : visual analog scale

VM : Valérie Mongrain

wEEG : wakefulness electroencephalogram

à eux,

les XY les plus importants de ma vie...

Isaak, Niki, ...

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1. Introduction

1.1 Chronotypes

Dans la population humaine, l'existence de types circadiens différents est reconnue depuis 1939 (cité dans Kleitman, 1963). Certaines personnes sont matinales, se couchent et se lèvent tôt et préfèrent fonctionner le matin; tandis que d'autres sont vespérales, se couchent et se lèvent tard et favorisent un horaire plus tardif pour leurs activités. Ces différents types circadiens sont appelés chronotypes et sont facilement identifiables par questionnaire. Plusieurs questionnaires ont été développés pour faciliter l'identification des chronotypes; notamment le "Circadian Type Questionnaire" (CTQ) par Folkard et al. (1979), la "Diurnal Type Scale" (DTS) par Torsvall et Akerstedt (1980) et le "Composite Scale for Morningness" (CSM) par Smith et al. (1989). De plus, dans les dernières années, plusieurs nouveaux questionnaires de chronotype ont aussi été développés (Hidalgo et al., 2002; Natale et Cicogna, 2002; Roenneberg et al., 2003b). La majorité des questionnaires de chronotypes résulte en la classification des individus en type matin (Type-M), type intermédiaire ou type soir (Type-S).

Cependant, le questionnaire le plus largement utilisé demeure le questionnaire de Matinalité-Vespéralité (QMV) de Horne et Östberg (1976, "Morningness-Eveningness Questionnaire"), qui a été utilisé en plusieurs langues (Ishihara et al., 1984; Adan et Natale, 2002; Taillard et al., 2003). Les pointages au QMV se distribuent de façon quasi-normale dans la population générale (Posey et Ford, 1981) dont la majorité, environ 60-70 %, est constituée de personnes plutôt intermédiaires qui peuvent toutefois avoir tendance soit à la matinalité ou à la vespéralité (Natale et Cicogna, 2002). Donc, il semble approprié de parler de continuum de matinalité-vespéralité dans la population humaine en tant que caractéristique interindividuelle.

Les chronotypes se distinguent majoritairement par la position de leur horaire de sommeil; les Types-M choisissent de dormir environ 2 heures plus tôt que les Types-S (Mecacci et Zani, 1983; Kerkhof, 1991; Natale et Cicogna, 2002). Cependant, les chronotypes se distinguent également par la phase de nombreuses variables physiologiques et

psychologiques qui dépendent de l'oscillateur circadien. Plusieurs études ont démontré que non seulement l'horaire de sommeil mais qu'également le maximum de vigilance et de performance sont plus tôt chez les Types-M que les Types-S (Horne et al., 1980; Foret, 1982; Watts et al., 1983; Kerkhof, 1991; Lack et Bailey, 1994; Kerkhof and Van Dongen, 1996; Natale et Cicogna, 1996; Taillard et al., 2003). Certains travaux ont montré que le maximum de vigilance est aussi 2 heures plus tôt chez les Types-M que chez les Types-S (Åkerstedt et Froberg, 1976), tandis que d'autres travaux ont observé une différence beaucoup plus grande (Horne et al., 1980; Thayer et al., 1988; Lack et Bailey, 1994; Kerkhof et Van Dongen, 1996; Natale et Cicogna, 1996). Les rythmes de la température corporelle et de sécrétion de mélatonine, les deux marqueurs de la phase circadienne interne les plus souvent utilisés, apparaissent en moyenne également 2 heures plus tôt chez les Types-M comparativement aux Types-S (Ishihara et al., 1987; Corbera et Grau, 1993; Lack et Bailey, 1994; Kerkhof et Van Dongen, 1996; Duffy et al., 1999; Baehr et al., 2000; Bailey et Heitkemper, 2001; Griefahn, 2002). Le rythme cardiaque (Breithaup et al., 1981), l'excrétion d'épinéphrine (Fröberg, 1977) et la sécrétion salivaire de cortisol (Bailey et Heitkemper, 2001) ont aussi des rythmes plus avancés chez les Types-M que chez les Types-S.

Une façon de différencier les Types-M et S est de les étudier spécifiquement le matin et le soir. Une multitude d'études sur la typologie circadienne se sont servies de cette stratégie pour caractériser les chronotypes. De façon générale, les Types-M performent mieux, se sentent mieux, s'endorment moins rapidement au test d'endormissement et ont une température plus élevée que les Types-S le matin; tandis que l'inverse est vrai pour le soir (Pátkai, 1971a, b; Horne et al., 1980; Kerkhof et al., 1980, 1981; Clodoré et al., 1986, 1990; Petros et al., 1990; Caminada et De Bruijn, 1992; Volk et al., 1994; Natale et Cicogna, 1996, 2002). Dans le passé, cette façon de distinguer les chronotypes a même été proposée comme étant la seule approche valable pour leur identification (Åkerstedt et Fröberg, 1976).

De plus, les chronotypes apparaissent différents sur certaines autres caractéristiques. Concernant le sommeil, les Types-M présenteraient moins de variabilité intra-individuelle que les Types-S dans leurs durées de sommeil et leurs heures de lever et de coucher (Webb et Bonnet, 1978; Foret, 1982; Ishihara et al., 1987, 1988; Taillard et al., 1999; Monk et al.,

1994, 2004). Aussi, il semble que les Types-M aient plus de difficulté à dormir le jour (Breithaupt et al., 1978; Åkerstedt et Torsvall, 1981a; Foret et al., 1985), comme par exemple lors du travail de nuit. Ces caractéristiques ont été reliées à quelques reprises à une variable de flexibilité/rigidité du sommeil et des habitudes de vie. Les Types-M seraient des individus plus rigides (ainsi que plus stables et disciplinés) tandis que les Types-S seraient des personnes plus flexibles (Baehr et al., 2000). Cette particularité des Types-S semble leur conférer un certain avantage pour le travail de nuit (Östberg, 1973b; Folkard et al., 1979; Hildebrandt et Stratmann, 1979; Torsvall et Åkerstedt 1980; Åkerstedt et Torsvall, 1981a; Ishihara et al., 1987; Härmä, 1993; Vidacek et al., 1993).

Le chronotype a aussi beaucoup été étudié en relation avec plusieurs traits psychologiques. Plusieurs études ont tenté de trouver une association entre extraversion et vespéralité, certaines avec succès (Pátkai, 1971a; Folkard et al., 1979; Mecacci et al., 1986; Matthews, 1988 [femmes seulement]; Adan and Almirall, 1990; Wilson, 1990; Neubauer, 1992) d'autres sans succès (Webb et Bonnet, 1978; Torsvall et Åkerstedt 1980; Kerkhof et al., 1981; Moog, 1981; Monk et al., 1983; Petros et al., 1990; Anderson et al., 1991; Baehr et al., 2000). Pour raffiner cette branche d'étude, quelques auteurs ont ajouté que c'était plus spécifiquement la composante "impulsivité" de l'extraversion qui était associée à la vespéralité (Matthews, 1988 [femmes seulement]; Neubauer, 1992; Baehr et al., 2000; Caci et al., 2004, 2005a). Certains travaux ont trouvé un lien entre neuroticisme et vespéralité (Torsvall et Åkerstedt 1980; Kerkhof et al., 1981) mais cette association ne ressort pas dans tous les travaux (Moog, 1981; Anderson et al., 1991). Finalement, la "recherche de sensations" a aussi été associée à la vespéralité (Kerkhof et al., 1981; Caci et al., 2004; Chotai, 2005). Bien que la majorité des associations retrouvées ne soit pas toujours fortes ou n'atteignent pas le seuil de signification statistique, il ressort tout de même que les Types-S apparaissent comme des personnes qui se démarquent par leur tendance à être extraverties, impulsives, neurotiques, et avides de sensations. De plus, les Types-S ont un plus grand penchant pour la consommation de substances stimulantes comme la caféine (Ishihara et al., 1985; Wilson, 1990; Adan 1994; Tankova et al., 1994), et les fumeurs sont également des personnes plus vespérales (Ishihara et al., 1985; Wilson, 1990).

Le continuum de matinalité-vespéralité est influencé par le genre. En effet, les femmes semblent avoir des caractéristiques circadiennes plus matinales. Plusieurs études ont

rapporté un horaire de sommeil plus avancé (Vink et al., 2001; Hidalgo et al., 2002; Roenneberg et al., 2003b) ainsi qu'une phase circadienne plus avancée (Mecacci et al., 1991; Gibertini et al., 1999; Baehr et al., 2000) chez les femmes comparativement aux hommes. Les femmes montrent aussi une plus grande tendance à la matinalité par leur pointage à différents questionnaires de chronotype (Natale and Adan, 1999; Vink et al., 2001; Adan et Natale, 2002; Roenneberg et al., 2003b; Roenneberg et al., 2004; Caci et al., 2005a; Natale et al., 2005; Mongrain et al., 2006c), mais pas toujours (Adan et Guàrdia, 1993; Paine et al., 2006). De plus, lorsque des Types-M et des Types-S sont comparés chez les femmes, les différences de phase des marqueurs circadiens ne sont pas aussi fortes que chez les hommes (Griefahn, 2002). Les différences homme-femme dans la typologie circadienne pourraient être modulées par l'influence socio-culturelle ou par le bagage génétique.

Alors que l'horaire de sommeil est stable pendant l'enfance (Touchette et al., en préparation) et décale à une heure plus tardive au cours de la puberté (Carskadon et al., 2004), il montre une avance progressive dès la fin de l'adolescence (Roenneberg et al., 2004). Avec l'âge, il y a aussi un avancement de la position de la phase circadienne de température et de mélatonine (Czeisler et al., 1992; Duffy et al., 1998; Carrier et al., 1999, 2002; Dijk et al., 2000; Kawinska et al., 2005). Par le fait même, à l'aide de questionnaires de chronotype, l'âge a été associé à maintes reprises à une augmentation de la matinalité (Torsvall et Åkerstedt 1980; Moog, 1981; Ishihara et al., 1991, 1992; Carrier et al., 1997; Baehr et al., 2000; Rosenthal et al., 2001; Caci et al., 2005a; Paine et al., 2006).

1.2 Régulation du sommeil : modèle à 2 processus

Les modèles actuels de régulation du sommeil et de la vigilance découlent presque tous du modèle de régulation du sommeil proposé par Borbély en 1982, qui suggère que le sommeil est régi par 2 processus principaux (Daan et al., 1984; Borbély et al., 1989; Achermann et al., 1993; Beersma, 1998). Le processus circadien (processus C) est responsable de l'aspect rythmique du cycle éveil-sommeil. Il commande les moments de faible et de forte propension à l'éveil au cours de 24 heures. Le processus homéostatique (processus S) augmente exponentiellement au cours de l'éveil et diminue de la même façon durant le

sommeil : il reflète le besoin de dormir et l'intensité du sommeil (Daan et al., 1984). Le modèle définit l'intensité du sommeil comme étant la quantité de sommeil lent profond et d'activité à ondes lentes en sommeil. L'interaction entre ces deux processus permet de générer des épisodes de sommeil ininterrompus d'environ 8 heures et de maintenir l'éveil durant environ 16 heures, malgré l'accumulation de fatigue (Dijk et Czeisler, 1994, 1995). Effectivement, l'accumulation de la pression homéostatique pour le sommeil au cours de la période d'éveil est compensée par l'augmentation de la tendance circadienne à l'éveil qui atteint son maximum quelques heures avant l'heure habituelle du coucher. Inversement, au cours de la période de sommeil, la diminution de la pression homéostatique au sommeil est contrecarrée par la diminution graduelle de la tendance circadienne à l'éveil (voir représentation schématique à la figure 1 ci-après).

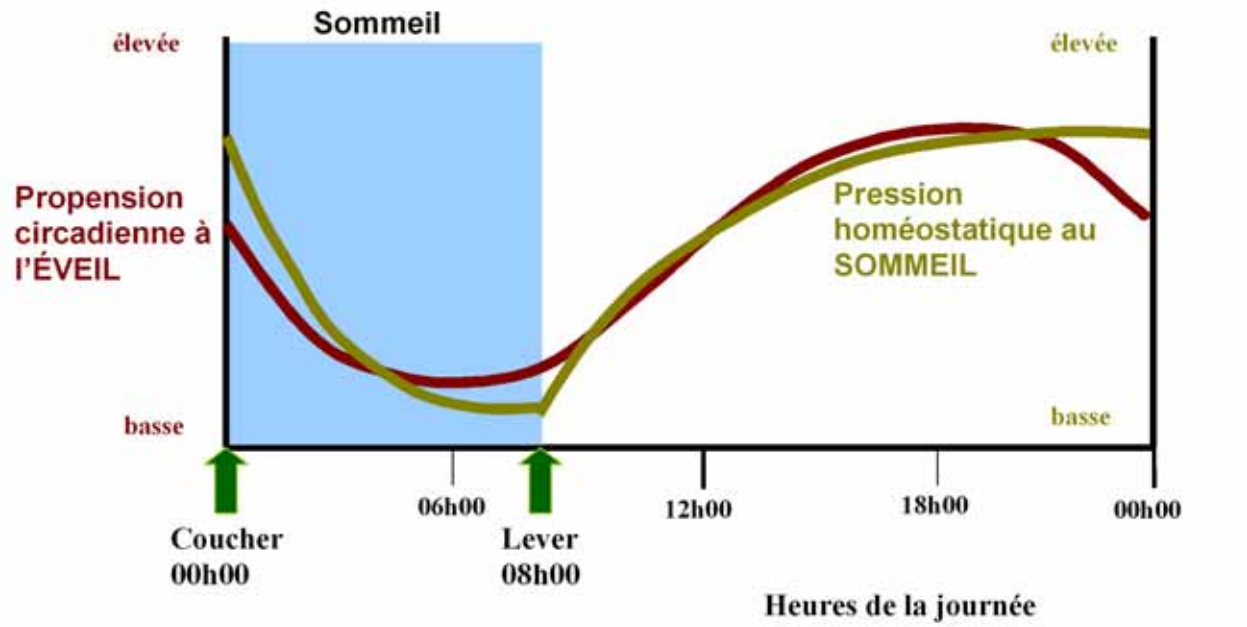

Figure 1 : Représentation schématique de l'interaction entre les processus circadien et homéostatique de régulation du sommeil. Le processus circadien contrôle un rythme de 24 heures de propension à l'éveil, dont l'échelle est à la gauche du graphique. Le processus homéostatique engendre une pression homéostatique pour le sommeil, dont l'échelle est indiquée à droite. L'exemple est montré pour un épisode de sommeil de minuit à 08h00.

Chez les mammifères, le processus C est généré par l'horloge biologique interne dans les noyaux suprachiasmatiques de l'hypothalamus qui déterminent une période endogène d'une longueur d'environ 24 heures (Ralph et al., 1990; Dunlap, 2004). Chez l'humain, la période endogène interne moyenne est estimée à 24.2 heures (Czeisler et al., 1999). La capacité

rythmique des noyaux suprachiasmatiques s'exprime au niveau de chaque neurone (Welsh et al., 1995). Les mécanismes moléculaires des neurones des noyaux suprachiasmatiques fonctionnent selon le principe de rétroaction : lorsque les gènes de l'horloge sont exprimés, ils engendrent des produits qui régulent leur propre fonctionnement, ce qui forme ainsi des cycles d'expression de gènes et d'abondance de protéines (Cermakian et Boivin, 2003; Lowrey et Takahashi, 2004 pour revues). La synchronisation des neurones des noyaux suprachiasmatiques engendre la coordination de leurs signaux circadiens, ce qui finalement régule les rythmes physiologiques et comportementaux via des projections dans plusieurs zones principalement hypothalamiques (dont les noyaux paraventriculaires et l'hypothalamus dorsomédian) et thalamiques (Welsh et al., 1995; Moore et al., 2002; Panda et Hogenesch, 2004). L'activité des noyaux suprachiasmatiques est plus grande le jour que la nuit; de plus, certaines cellules des noyaux suprachiasmatiques réagissent à la lumière principalement via la voie rétino-hypothalamique et permettent aux rythmes circadiens de se maintenir en phase avec le cycle lumière-obscurité de l'environnement (Hastings et Herzog, 2004; Silver et Schwartz, 2005).

La localisation anatomique du processus S est beaucoup moins claire et pourrait être diffuse Dijk et Lockley, 2002). Le processus S a souvent été interprété comme une sorte de fatigue cellulaire ou métabolique (Kong et al., 2002) : plus un réseau neuronal est utilisé, plus la pression homéostatique s'accumule. En ce sens, l'utilisation et la non-utilisation d'un réseau neuronal cortical produisent respectivement une augmentation et une diminution de l'intensité du sommeil dans la zone corticale concernée (Kattler et al., 1994; Huber et al., 2004, 2006). De nombreuses hypothèses concernant le fondement neurochimique du processus S ont été émises (Borbély et Tobler, 1989; Kovalzon et Strekalova, 2006; Steiger, 2006). Ainsi, plusieurs molécules se sont avérées des candidates potentielles; leur accumulation ou leur déplétion pouvant agir comme médiateur de la pression homéostatique dans les cellules du système nerveux central. Krueger et Obál (1993) ont proposé un rôle important des interleukines et de l'hormone de relâche de l'hormone de croissance dans la théorie humorale de la régulation du sommeil. Parallèlement, il a été proposé que l'adénosine, molécule relâchée par les neurones et la glie du système nerveux central pendant les périodes de haute activité métabolique, puisse jouer un rôle dans l'intensité du sommeil (Ticho et Radulovacki, 1991; Benington et Heller, 1995). Depuis, de nombreuses évidences se sont accumulées en faveur d'un rôle de l'adénosine comme signal

homéostatique. Dans le prosencéphal basal, le niveau d'adénosine augmente pendant l'éveil et une administration d'adénosine dans cette région engendre le sommeil (Porkka-Heiskanen et al., 1997, 2000; Basheer et al., 2000; Murillo-Rodriguez et al., 2004). Aussi, il a été démontré récemment que l'adénosine active des neurones promouvant le sommeil dans le noyau préoptique ventrolatéral de l'hypothalamus (Gallopin et al., 2005). La caféine, antagoniste de l'adénosine, atténue les marqueurs du processus S en sommeil ainsi qu'à l'éveil (Landolt et al., 1995, 2004). Quoi qu'il en soit, il semble que le siège du processus S soit, du moins en partie, cortical étant donné la nature neurophysiologique de l'intensité du sommeil (Amzica et Steriade, 1998; Amzica et Massimini, 2002) ainsi que la spécificité topographique de ce marqueur en réponse à une stimulation (Kattler et al., 1994; Huber et al., 2004). De plus, la privation de sommeil affecte principalement des habiletés cognitives de haut niveau reconnues pour dépendre du lobe frontal du cortex (Horne, 1993; Drummond et al., 1999).

La façon dont les processus de régulation du sommeil et de la vigilance interagissent est encore mal comprise. Les modèles contemporains dérivés du modèle original de Borbély postulent que les processus C et S sont indépendants et donc que leurs effets sont additifs (Beersma, 1998). L'indépendance de C et S a été appuyée par des études de privation de sommeil chez les animaux où la réponse homéostatique demeure intacte lors de lésion des noyaux suprachiasmatiques et de la conséquente abolition de la rythmicité circadienne (Mistlberger et al., 1983; Tobler et al., 1983; Trachsel et al., 1992). Mais plusieurs résultats s'expliquent mal par l'indépendance des deux processus de régulation du sommeil et de la vigilance (Putilov, 1995; Jewett et Kronauer, 1999) Entre autres, l'activité neuronale pendant les stades de sommeil, dont l'activité à ondes lentes, affecte l'activité électrique des noyaux suprachiasmatiques (Deboer et al., 2003). À l'heure actuelle, de plus en plus de scientifiques mettent en doute l'indépendance structurale et/ou moléculaire des processus C et S (Gillette, 2004; Turek, 2004; Dijk et Franken, 2005; Gillette et Sejnowski, 2005).

1.3 Marqueurs circadiens et homéostatiques

1.3.1 Marqueurs circadiens

Chez l'humain, l'oscillation circadienne de l'horloge biologique ne peut malheureusement pas être mesurée directement. Il faut donc utiliser des indices indirects de la rythmicité circadienne pour l'étude de la régulation du sommeil. Les marqueurs du processus C sont des paramètres physiologiques dont la rythmicité d'environ 24 heures persiste en conditions constantes. À ce jour, trois marqueurs circadiens sont couramment utilisés comme indicateurs de l'horloge biologique chez l'humain : la sécrétion de mélatonine, la température corporelle et la sécrétion de cortisol (Klerman et al., 2002).

À ce jour, le rythme de la sécrétion de mélatonine est considéré comme le meilleur marqueur circadien, surtout en raison de sa grande stabilité et de la robustesse de son rythme (Voultsios et al., 1997; Lewy et al., 1999; Klerman et al., 2002; Waterhouse et DeCoursey, 2004; Benloucif et al., 2005). La mélatonine est sécrétée pendant la nuit (est indétectable pendant le jour) par la glande pinéale, sous le contrôle direct des noyaux suprachiasmatiques. L'épisode de sécrétion de mélatonine reflète le moment au cours duquel l'organisme perçoit la nuit (appelée nuit subjective) telle que déterminée par l'horloge endogène. La sécrétion de la mélatonine peut être mesurée dans le plasma, dans la salive ou encore via son métabolite dans l'urine (Arendt, 2003; Waterhouse et DeCoursey, 2004). Un des marqueurs du rythme de sécrétion de la mélatonine couramment utilisé est l'heure du début de la sécrétion en soirée qui peut être évaluée à l'aide d'un seuil fixe ou d'une valeur relative à l'amplitude totale de la sécrétion (Klerman et al., 2002). Les patrons de sécrétion de mélatonine sont hautement reproductibles chez un même individu (Arendt, 2003; Selmaoui et Touitou, 2003; Benloucif et al., 2005). Cependant, les échantillons biologiques doivent être recueillis en lumière tamisée puisque l'exposition à la lumière supprime la sécrétion de mélatonine (Lewy et al., 1980; Bojkowski et al., 1987; Zeitzer et al., 2000). De plus, la concentration de mélatonine peut être affectée par les changements de posture (Deacon et Arendt, 1994; Cajochen et al., 2003). Lorsque les conditions expérimentales sont bien contrôlées, la mélatonine s'avère particulièrement utile pour l'étude de la phase circadienne chez les chronotypes (Griefahn, 2002).

Pour sa part, le rythme de la température corporelle est sans doute le marqueur circadien le plus ancien en chronobiologie humaine (Kleitman, 1963). La température corporelle est élevée pendant la journée et basse pendant la nuit. Au départ, l'acrophase (l'heure du maximum) du rythme de température était utilisée comme marqueur; cependant, depuis environ 20 ans, l'heure du minimum circadien de la température est favorisée. Le rythme de la température corporelle est une mesure particulièrement utile car elle suit de très près le rythme circadien de la propension à l'éveil (Shochat et al., 1997). De plus, le minimum de la température pendant la nuit marque le point pivot de la courbe de réponse de phase à la lumière (Minors et al., 1991). Un problème notoire perdure néanmoins avec la température car elle est modifiée par l'activité, le sommeil et la prise alimentaire (Waterhouse et DeCoursey, 2004). Bien qu'il soit possible de corriger l'influence du sommeil sur la mesure de la phase de la température (Carrier et Monk, 1997), il semble que le rythme de température soit une mesure circadienne moins précise que celui de la sécrétion de mélatonine (Klerman et al., 2002; Benloucif et al., 2005).

Le rythme de sécrétion du cortisol est un marqueur circadien relativement nouveau. La sécrétion de cortisol augmente pendant la nuit, atteint un maximum au lever, puis diminue par la suite au cours de la journée pour atteindre un minimum peu après le coucher (Selmaoui et Touitou, 2003; Waterhouse et DeCoursey, 2004). Bien que le rythme de sécrétion du cortisol soit un marqueur robuste en conditions constantes, un problème avec cette hormone provient de son association à la réponse au stress (Dekker et al., 1996). Effectivement, une étude combinant mesures circadiennes et tâche de performance, par exemple, risque de voir son marqueur circadien de cortisol affecté par le stress provenant de la nécessité de bien performer.

Bien qu'étant peu utilisés comme marqueurs circadiens, plusieurs paramètres mesurés au cours du sommeil et de l'éveil subissent une forte influence circadienne. Pour le sommeil, la latence à l'endormissement, la latence au sommeil paradoxal, la quantité de sommeil paradoxal et le temps total de sommeil montrent une influence circadienne prépondérante (Webb et Agnew, 1977; Carskadon et Dement, 1980; Czeisler et al., 1980a, b; Richardson et al., 1982; Strogatz et al., 1986; Dijk et Czeisler, 1994, 1995; Shochat et al., 1997; Lavie, 2001). L'activité électrique corticale enregistrée à l'aide de l'électroencéphalogramme (EEG) peut être analysée quantitativement par analyse spectrale (Borbély, 1986). L'activité

spectrale dans certaines fréquences de l'EEG en sommeil lent (stades 2, 3 et 4 du sommeil) montre aussi une influence circadienne considérable. L'activité dans les bandes de fréquences 12-13 Hz et 14-15 Hz montre respectivement un maximum et un minimum en phase avec le maximum de la sécrétion de mélatonine (Aeschbach et al., 1997; Dijk et al., 1997; Knoblauch et al., 2003). Pendant l'éveil, plusieurs mesures de vigilance sont affectées par le processus C (Dijk et al., 1992; Wyatt et al., 1997, 1999). Cependant, la qualité globale de l'éveil semble dépendre grandement de la combinaison des influences circadiennes et homéostatiques (Dijk et al., 1992). Par contre, l'analyse spectrale de l'EEG enregistré à l'éveil a de fortes variations circadiennes dans l'alpha (8-12 Hz) sans influence homéostatique notable (Aeschbach et al., 1997, 1999; Dumont et al., 1999).

1.3.2 Marqueurs homéostatiques

Les marqueurs du processus S peuvent être retrouvés à l'éveil et en sommeil. Depuis l'élaboration du modèle de régulation du sommeil à deux processus, les modèles qui en découlent ont presque toujours utilisé les stades de sommeil lent profond (SLP, stades 3 et 4 de sommeil) et l'activité à ondes lentes (AOL; entre 0.5-5 Hz) durant le sommeil lent comme index du processus S (Achermann et al., 1993; Beersma, 1998; Borbély et Achermann, 1999). Puisque le processus S contrôle l'intensité du sommeil en fonction de la durée de l'éveil, il va de soi que la quantité de sommeil lent profond et d'AOL, indices de la profondeur du sommeil, constituent les piliers du modèle. L'augmentation de la durée de l'éveil engendre une augmentation proportionnelle de la quantité de SLP et d'AOL (Webb et Agnew, 1977; Borbély et al., 1981; Åkerstedt et Gillberg, 1986a, b; Dijk et al., 1987, 1990a, b, 1993). Aussi, le SLP et l'AOL se dissipent au cours de la période de sommeil (Aeschbach et Borbély, 1993; Dijk et al., 1993, 1997; Dijk et Czeisler, 1995; Aeschbach et al., 1997; Werth et al., 1997; Beersma, 1998), reflétant la diminution de la pression homéostatique. Le marqueur homéostatique que constitue l'AOL prédomine dans les régions antérieures du cortex cérébral tout autant que sa réponse homéostatique à un changement de la durée de l'éveil (Cajochen et al., 1999a; Finelli et al., 2000, 2001a, b; Knoblauch et al., 2002; Tinguely et al., 2006).

L'activité spectrale retrouvée dans les fréquences des fuseaux de sommeil, nommée activité sigma (12-16 Hz), est aussi maintenant souvent considérée comme un marqueur homéostatique (Landolt et al., 2004; Tinguely et al., 2006). L'activité dans diverses

divisions de la bande de fréquence sigma en sommeil lent est inversement associée à la pression homéostatique puisqu'elle augmente au cours du sommeil et montre une diminution suite à une privation de sommeil (Aeschbach et Borbély, 1993; Dijk et al., 1993, 1997; Aeschbach et al., 1997; Dijk et Czeisler, 1995; Landolt et al., 2000; Knoblauch et al., 2002). De plus, une corrélation négative a été rapportée entre l'AOL et l'activité sigma (Aeschbach et Borbély, 1993; Finelli et al., 2000). En sommeil lent, l'AOL et l'activité sigma sont donc les marqueurs du processus S.

Au cours de l'éveil, comme mentionné dans la section précédente, plusieurs variables neuro-comportementales sont sous l'influence conjointe des processus C et S. Cependant, l'analyse spectrale de l'EEG enregistré à l'éveil a permis de faire ressortir des indices de la régulation homéostatique à l'éveil. Notamment, l'activité spectrale dans les fréquences delta (0-4 Hz), thêta (4-8 Hz), et bêta (12-25 Hz, certains auteurs définissent le bêta à partir de 12 Hz) augmentent avec la durée de l'éveil, particulièrement pendant une privation de sommeil (Åkerstedt et Gillberg, 1990; Corsi-Cabrera et al., 1992; Cajochen et al., 1995, 2001, 2002; Aeschbach et al., 1997, 1999; Dumont et al., 1999; Finelli et al., 2000; Chapotot et al., 2003; Strijkstra et al., 2003; Danilenko et Putilov, 2005). De ces activités spectrales, seul le bêta a été rapporté comme étant libre d'influence circadienne significative (Corsi-Cabrera et al., 1992; Aeschbach et al., 1997, 1999; Dumont et al., 1999; sauf dans Cajochen et al., 2002). Bien que l'activité thêta soit influencée par le processus C, elle est à ce jour considérée comme un marqueur homéostatique fiable (Finelli et al., 2000; Taillard et al., 2003; Landolt et al., 2004).

1.4 Méthodes d'étude de la régulation du sommeil

Puisque les processus C et S varient ensemble au cours d'une journée normale, il est difficile de séparer l'influence de ces deux mécanismes sur les variables étudiées. Cependant, en chronobiologie humaine, plusieurs protocoles de recherche ont été développés pour permettre l'étude indépendante des deux processus de régulation du sommeil et de la vigilance. Les principaux protocoles sont les suivants : l'étude en libre cours, les protocoles de désynchronisation forcée, de cycle éveil-sommeil ultracourt et de

siestes répétées, la routine constante, et finalement le protocole de fragmentation du sommeil.

Les études en libre cours ("free-running") consistent à laisser les sujets choisir les moments de coucher et de lever en ne leur donnant aucun indice de l'heure de la journée. Lors des études de ce type, le cycle circadien endogène (le plus souvent indiqué par le rythme de la température corporelle) adopte la périodicité spontanée de l'oscillateur circadien du sujet, ce qui permet ainsi de la mesurer. Toutefois, l'intensité de la lumière durant la période d'éveil peut modifier la période mesurée (Klerman et al., 1996). Lorsque le protocole de libre cours est maintenu pendant une longue période (généralement plus de 3 semaines) il se produit souvent un désynchronisation spontanée entre le cycle éveil-sommeil et le cycle circadien endogène des individus; c'est-à-dire que la relation de phase (angle de phase) entre les deux cycles est modifiée. Le sommeil a donc la possibilité de se retrouver à différents moments circadiens. À l'aide de ce protocole, il a pu être observé que l'horloge circadienne contrôle des moments de faible et de forte propension pour le sommeil (Strogatz et al., 1987). Cependant, ce genre de protocole n'engendre pas toujours de désynchronisation interne (Monk et al., 1983) et il est maintenant très peu utilisé.

Vers la fin des années 1980, le protocole de désynchronisation forcée fit son apparition. Ce protocole vise à provoquer la désynchronisation entre le cycle circadien endogène et le cycle éveil-sommeil en obligeant le sujet à adopter un cycle éveil-sommeil très court ou très long. Effectivement, l'horloge biologique est incapable de se synchroniser à une périodicité de moins de 22 heures ou de plus de 26 heures environ. Dans ces conditions, elle adopte sa périodicité endogène, considérée par plusieurs comme étant la véritable longueur de la période endogène (Czeisler et al., 1999), et il en résulte une dissociation entre le cycle circadien et le cycle éveil-sommeil. Généralement, les cycles éveil-sommeil adoptés lors de l'utilisation d'un protocole de désynchronisation forcée sont de 20 heures ou de 28 heures (Dijk et al., 1992; Dijk et Czeisler, 1994; Hull et al., 2003; Wyatt et al., 2006), et ils engendrent des épisodes de sommeil et d'éveil à toutes les positions de phase circadienne. Toutefois, ce protocole est extrêmement coûteux, nécessite des installations complexes d'isolation temporelle et exige une très grande disponibilité des sujets (2 à 8 semaines en laboratoire). Pour ces raisons, très peu de chercheurs sont en mesure de l'utiliser.

D'autres stratégies analogues ont été développées pour l'étude de la régulation du sommeil. Les cycles éveil-sommeil ultracourts permettent également de répartir le sommeil sur plus d'un cycle circadien. Ces protocoles tentent de respecter le ratio veille-sommeil normal afin d'éviter une privation de sommeil et de maintenir en quelque sorte la pression homéostatique constante (Carskadon et Dement, 1975; Lavie et Segal, 1989; Lavie et Zvuluni, 1992; Liu et al., 2000). Cette méthode a pour avantage de pouvoir évaluer la propension au sommeil, la propension à certains stades de sommeil et la composition spectrale du sommeil à toutes les positions de phase circadienne mais rend impossible l'analyse de la structure d'un épisode de sommeil. De manière semblable, un jeune chercheur a développé récemment un protocole de "siestes" qui respecte aussi le ratio veille-sommeil normal mais qui, lorsque comparé à une privation de sommeil, permet de mesurer l'effet d'une faible ou d'une forte pression homéostatique à toutes les positions de phase circadienne (Cajochen et al., 2001, 2004; Knoblauch et al., 2002, 2003).

La routine constante consiste en un séjour de plus d'un cycle circadien en conditions constantes. La routine constante implique le contrôle du plus grand nombre de variables possible pendant toute la durée du protocole : température, humidité, activité, éclairage (généralement lumière tamisée), posture (généralement semi-assise), ingestion d'eau et d'aliments (généralement même volume et calories aux heures), isolation temporelle, etc. (Duffy et Dijk, 2002). La routine constante a pour avantage considérable de pouvoir évaluer précisément la position de la phase circadienne à l'aide d'un séjour de courte durée (généralement 28 à 40 heures). Cependant, bien que la routine constante permette une évaluation circadienne correcte, elle engendre une augmentation constante de la pression homéostatique puisqu'elle implique une privation de sommeil et ne peut donc pas être utilisée pour l'évaluation simultanée du sommeil. De plus, la routine constante pourrait affecter la phase de l'oscillateur circadien par un délai (Van Dongen et al., 1998; Cajochen et al., 2003).

La majorité des protocoles décrits ci-dessus sont très utiles pour étudier l'aspect circadien de la régulation du sommeil ou les interactions circadiennes et homéostatiques. Pour étudier plus spécifiquement les mécanismes homéostatiques, il est essentiel de modifier la pression homéostatique sans changer la phase circadienne. Une des manières de moduler la pression homéostatique en maintenant la phase circadienne constante est d'augmenter la durée de

l'éveil à l'intérieur même de l'épisode de sommeil. En ce sens, la fragmentation du sommeil est une technique consistant à morceler le sommeil. Elle est généralement effectuée à l'aide de sons ou par stimulation vocale et peut nécessiter des éveils comportementaux ou simplement un allègement du sommeil (Bonnet, 1986; Glovinsky et al., 1990; Roehrs et al., 1994; Dumont et al., 2000). Quelle que soit la méthode de fragmentation ou les critères d'éveil, cette technique est efficace pour diminuer la valeur récupératrice du sommeil et augmenter la pression homéostatique (Wesensten et al., 1999; Stepanski, 2002) sans changer l'horaire habituel de l'épisode de sommeil.

1.5 Régulation du sommeil chez les chronotypes

Dans le cadre des modèles actuels de régulation du sommeil et de la vigilance, on peut émettre l'hypothèse que la différence dans la position de l'épisode de sommeil et de la phase circadienne chez les chronotypes provienne de différences dans l'un ou l'autre des deux grands processus de régulation du sommeil, ou encore de différences dans la nature de leur interaction.

Clodoré et collaborateurs (1986) ont soutenu que des différences homéostatiques seraient minimes puisque l'architecture du sommeil des chronotypes est identique (Foret et al., 1985; Taillard et al., 2003). Cependant, d'autres séries de travaux ont relié la matinalité-vespéralité à des différences dans le processus S de régulation du sommeil : certaines à l'aide de l'étude des marqueurs du processus S au cours de l'éveil (Ehlers et al., 1998; Taillard et al., 2003) et une autre en sommeil (Kerkhof, 1991). L'hypothèse est que les Types-M seraient des individus qui accumuleraient plus rapidement la pression homéostatique pendant l'éveil et qui la dissiperaient plus rapidement au cours du sommeil comparativement aux Types-S. Cette plus grande vitesse de la dynamique homéostatique favoriserait un horaire de sommeil hâtif. D'autres auteurs ont suggéré un ratio différent de l'influence des processus C et S chez les chronotypes (Natale et Cicogna, 1996; Natale et al., 2005) : chez les Types-M, la régulation de la vigilance subjective dépendrait plus du processus homéostatique tandis que chez les Types-S, les influences circadiennes seraient dominantes. Cette hypothèse pourrait être appuyée par l'observation d'une plus grande amplitude du cycle circadien de la température chez les Types-S que les Types-M (Baehr et

al 2000). Cependant, d'autres travaux n'ont pas trouvé de différence d'amplitude de ce signal circadien entre chronotypes (Gibertini et al., 1999; Griefahn, 2002). Un aspect environnemental a aussi été proposé comme étant une source de la typologie circadienne, à savoir, la saison de la naissance (Natale et Adan, 1999; Natale et al., 2002; Caci et al., 2005b). La photopériode à la naissance pourrait influencer la sensibilité du système circadien à la lumière ou le développement du système circadien de manière à modifier la longueur de la période endogène (voir article complémentaire à l'annexe 8.4 : Mongrain et al., 2006c).

En 1973, à l'aide de l'observation des rythmes de la température orale et d'ingestion d'aliments chez les chronotypes, Östberg a formulé une des premières hypothèses circadiennes sur l'origine des chronotypes :

> ''The morning group subjects showed an autonomous 24-hour-periodicity, while the evening group subjects showed signs of entrainment from a longer-than-24-hour-periodicity''[1]

Dès lors, cette hypothèse, concernant des différences endogènes dans la période de l'oscillateur circadien, s'est vue appuyée par les résultats d'une longue série d'études. Selon la théorie classique de l'oscillateur (Pittendrigh et Daan, 1976; Roenneberg et al., 2003a, b), lorsque placée dans un même cycle lumière-obscurité de 24 heures, une personne ayant une période courte tendra à avancer sa phase circadienne tandis qu'une personne à période longue retardera la sienne. Ainsi, une personne ayant une période endogène plus courte que 24 heures a quotidiennement tendance à avancer son heure de coucher et doit à chaque jour se synchroniser au cycle lumière-obscurité par un délai. L'inverse est vrai pour une personne qui a une période plus longue que 24 heures. De ce fait, suite à la suggestion effectuée par Östberg (1973a), une étude a trouvé une corrélation entre la longueur de la période de la vigilance subjective et la position de la phase de vigilance subjective et a souligné l'importance de ces résultats dans la typologie circadienne (Monk et al., 1983). Plus d'une décennie plus tard, cette hypothèse de travail a été reprise (Kerkhof et Van Dongen, 1996) et est encore valide aujourd'hui. Une corrélation entre la longueur de la période endogène et la position de la phase circadienne, associant période courte à phase

[1] Östberg O (1973a) Circadian rhythms of food intake and oral temperature in 'morning' and 'evening' groups of individuals. Ergonomics 16:203-209, p. 208.

hâtive, a été démontrée (Duffy et al., 2001). Ces mêmes travaux ont aussi montré une association entre période plus courte et plus grande matinalité selon le pointage au QMV. De plus, un période très courte a aussi été mesurée chez un patient atteint du syndrome du sommeil en avance de phase, considéré comme un extrême matinal du continuum de matinalité-vespéralité (Jones et al., 1999). Ces données suggèrent fortement que la typologie circadienne est déterminée par la longueur de la période de l'horloge biologique interne. Cependant, une étude récente n'a pas trouvé d'association significative entre longueur de la période et position de la phase ou pointage de chronotype (Wright et al., 2005), laissant planer un doute sur la force de la relation entre la longueur de la période endogène et la typologie circadienne.

Plusieurs données concernant l'entraînement chez les chronotypes appuient cependant fortement l'hypothèse de déterminisme selon la période. L'entraînement est la relation stable par laquelle s'ajuste la période endogène à la période de l'environnement (Roenneberg et al., 2003a). L'entraînement est caractérisé par un angle de phase qui représente la façon dont une personne est ajustée au cycle lumière-obscurité. L'angle de phase se définit comme la relation temporelle entre la position de la phase circadienne telle qu'évaluée par des marqueurs circadiens et le cycle éveil-sommeil (voir figure 2A ci-après). L'angle de phase est généralement exprimé en fonction de l'heure du lever, particulièrement en relation avec le minimum thermique (Duffy et al., 1999; Baehr et al., 2000). Cette relation temporelle est importante puisqu'elle s'avère le reflet fidèle de la longueur de la période endogène (Pittendrigh et Daan, 1976; Aschoff, 1981; Lewy et al., 2001; Wright et al., 2005). En effet, une personne avec une longue période circadienne, aura non seulement une phase circadienne tardive mais sera également entraînée avec un angle de phase plus court (intervalle plus court entre le minimum thermique et l'heure du lever).

Tel qu'illustré à la figure 2B, la majorité des études ont effectivement rapporté que les Types-M ont un angle plus grand que les Types-S (Kerkhof, 1991; Duffy et al., 1999; Baehr et al., 2000; Liu et al., 2000), et une autre a trouvé une association entre phase hâtive et angle long (Martin et Eastman, 2002). Ces données appuient encore plus solidement l'hypothèse d'une différence dans la longueur de la période endogène entre les chronotypes. De plus, elles permettent d'expliquer pourquoi les Types-M sont plus

vigilants lors de leur lever puisque ceux-ci se lèvent à une phase circadienne plus tardive, donc à une température corporelle ainsi qu'à une propension circadienne pour l'éveil plus élevée.

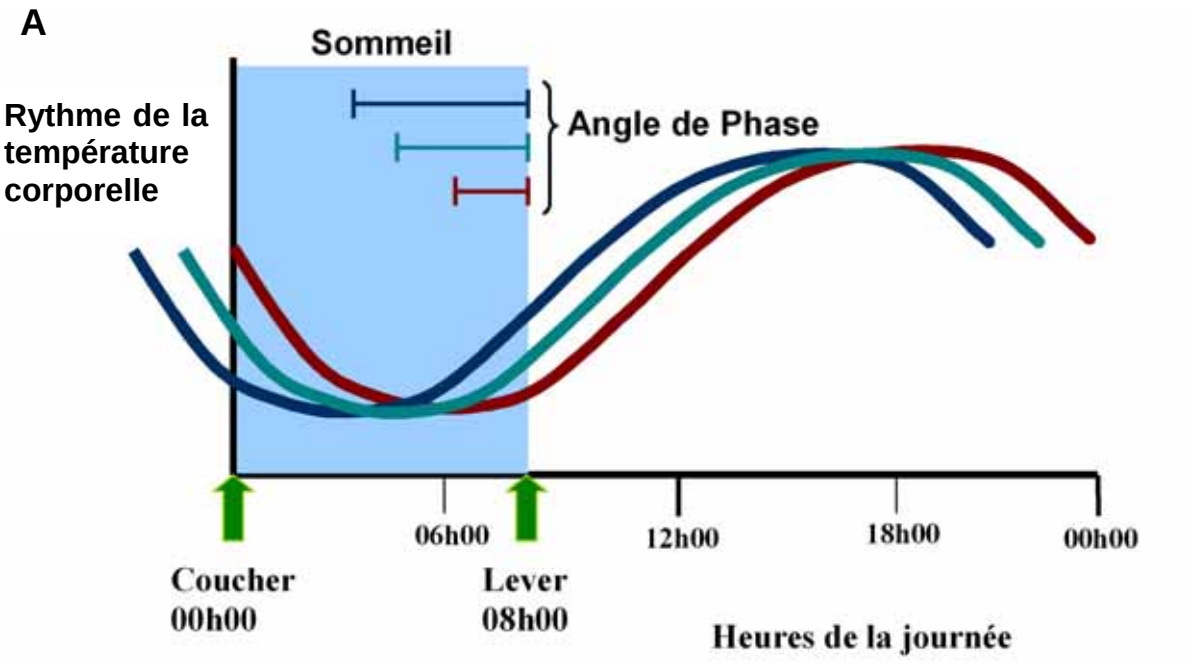

Figure 2 : Représentations schématiques de l'angle de phase. **A**, Relation de phase, nommée angle de phase, entre la phase circadienne (minimum du rythme de température = minimum circadien de propension à l'éveil) et l'épisode de sommeil (heure de lever). **B**, Relation de phase observée à plusieurs reprises chez les Types-M et les Types-S.

Plusieurs données moléculaires et génétiques appuient également l'hypothèse d'une différence dans la longueur de la période endogène comme origine du chronotype. Premièrement, dans les dernières années, plusieurs gènes de l'horloge ont été découverts

dont l'absence ou la modification affecte à la hausse ou la baisse la longueur de la période endogène chez l'animal (voir Albrecht, 2002; Cermakian et Boivin, 2003 pour revues). Ensuite, des travaux sur des populations de jumeaux supportent fermement l'héritabilité du chronotype (Hur et al., 1998; Vink et al., 2001). Or, la majorité des gènes de l'horloge ont été étudiés en fonction du chronotype et plusieurs polymorphismes ont été associés à la typologie circadienne (Katzenberg et al., 1998; Archer et al., 2003; Johansson et al., 2003; Mishima et al., 2005; Carpen et al., 2005; Pereira et al., 2005). Notamment, tandis qu'une souche de souris mutante pour le gène *mClock* engendre un phénotype similaire à la vespéralité (Sei et al., 2001), des travaux chez l'humain ont également associé un polymorphisme au niveau du gène *hClock* à la typologie circadienne (Katzenberg et al., 1998; Mishima et al., 2005). Le chronotype a aussi été lié à quelques reprises à un polymorphisme d'une répétition en tandem du gène *hPer3* (Archer et al., 2003; Pereira et al., 2005).

Le fait que les chronotypes semblent avoir des différences dans la longueur de leur période endogène, mais surtout l'observation qu'ils sont entraînés à des phases circadiennes différentes (différents angles de phase), rend cette population particulièrement intéressante pour l'étude de la régulation du sommeil. Effectivement, il est possible d'étudier chez cette population l'interaction entre les processus C et S en évaluant la qualité du sommeil et de l'éveil. Plus particulièrement, le modèle des chronotypes peut permettre d'étudier l'impact d'une phase circadienne différente sur la force du processus S. Ce modèle a pour avantage considérable d'utiliser des populations normales, sans pathologie, bien synchronisées au nycthémère avec des différences de phase circadienne, et ce sans changer l'horaire habituel de sommeil ni causer de privation de sommeil. Les chronotypes apparaissent donc comme un modèle intéressant pour l'étude de la régulation normale du sommeil et de l'éveil. C'est ce que nous avons voulu exploiter dans les travaux présentés dans cette thèse.

2. Objectifs et hypothèses

2.1 Objectifs généraux

Le but premier de ce programme de recherche était d'étudier l'interaction entre les processus circadien et homéostatique de régulation du sommeil. Pour ce faire, deux groupes de chronotypes ont été soumis à une fragmentation du sommeil pour vérifier l'effet d'une augmentation de la pression homéostatique sur le sommeil et la vigilance d'individus entraînés à des phases circadiennes différentes. Dans le cadre de ce programme de recherche trois objectifs généraux étaient visés :

2.1.1 Examiner le processus S à des phases circadiennes différentes, sans privation de sommeil ni allongement significatif de la durée du sommeil.

2.1.2 Mesurer les effets sur le sommeil et l'éveil d'une stimulation du processus S à des phases circadiennes différentes, sans changer la relation naturelle entre la phase circadienne et le sommeil.

2.1.3 Examiner la force de la récupération du sommeil et de l'éveil à des positions de phase différentes suite aux manipulations homéostatiques.

2.2 Objectifs spécifiques et hypothèses

Au cours de l'analyse du projet, des objectifs spécifiques ainsi que des hypothèses de recherche ont été élaborés dans le cadre de chaque article de recherche. Ils sont décrits ci-après :

2.2.1 Évaluer la relation temporelle entre la phase circadienne et l'épisode de sommeil chez les chronotypes lorsqu'étudiés selon leur horaire libre et spontané (premier article : Mongrain et al., 2004)

Dans un premier temps, pour atteindre ce premier objectif, il fallait évaluer l'horaire de sommeil ainsi que la position de la phase circadienne chez les deux groupes de chronotypes. Un sous-objectif complémentaire a aussi émergé à savoir la vérification de l'horaire de sommeil et de la phase circadienne chez les hommes et les femmes. Par la suite, l'évaluation des différences d'angle de phase entre les chronotypes a été effectuée.

Les hypothèses étaient que les Types-M auraient un horaire de sommeil et une phase circadienne plus hâtifs que les Types-S. De plus, il était attendu que les femmes auraient un horaire de sommeil et une phase circadienne plus hâtive que les hommes. Finalement, il était prédit que l'angle de phase des Types-M serait plus grand que celui des Types-S.

2.2.2 Évaluation des marqueurs du processus S en sommeil normal chez les chronotypes (second et troisième articles : Mongrain et al., 2005, 2006a)

Dans ce projet (premier article), aucune différence d'angle de phase n'a été observée entre les chronotypes. Par la suite, l'hypothèse de différences homéostatiques entre chronotypes a été proposée afin d'expliquer une différence dans l'horaire de sommeil. Les analyses suivantes de la présente thèse ont donc servi à évaluer les différences dans le processus S de régulation du sommeil et de la vigilance entre les chronotypes.

Dans un premier temps, l'évaluation des marqueurs de S en sommeil lent ainsi que l'évaluation des différences topographiques des marqueurs de S en sommeil lent et paradoxal étaient planifiées au cours d'une nuit normale de sommeil des chronotypes. Un sous-objectif a une fois de plus été énoncé suite aux résultats du premier article : l'évaluation des différences dans les marqueurs de S en sommeil lent selon le genre. Pour terminer, un dernier but était d'évaluer le décours de l'AOL en sommeil lent chez les groupes de chronotypes.

Les hypothèses étaient que les Types-M montreraient des indices du processus S plus élevés en particulier des valeurs plus élevées de SLP et d'AOL principalement en début de nuit et dans les régions antérieures du cortex cérébral. Il était attendu que les femmes

auraient également des valeurs plus élevées de SLP et d'AOL comparativement aux hommes. De plus, il était prédit que les Types-M auraient un décours nocturne d'AOL plus rapide que les Types-S.

2.2.3 Évaluation du décours de l'AOL chez les sous-catégories de chronotypes (quatrième article : Mongrain et al., 2006b)

Par la suite, nous avons voulu évaluer la dissipation de l'AOL pendant une nuit normale spécifiquement à l'intérieur de deux sous-catégories de chronotypes identifiées dans le premier article de cette thèse, à savoir les chronotypes à phase circadienne extrême et les chronotypes à phase circadienne intermédiaire. Aussi, nous avons voulu vérifier la relation entre les paramètres de la modélisation du décours de l'AOL et l'horaire de sommeil.

L'hypothèse était que les Types-M auraient une dissipation plus rapide d'AOL que les Types-S, plus particulièrement dans le sous-groupe de chronotypes à phase intermédiaire. Il était aussi prédit que, chez le sous-groupe intermédiaire, le taux de dissipation d'AOL serait associé à l'horaire de sommeil.

2.2.4 Évaluation du sommeil et de l'éveil des chronotypes pendant et suite à une augmentation de la pression homéostatique

Ensuite, nous avons voulu faire l'évaluation des marqueurs du processus S en sommeil lent pendant et suite à deux nuits de fragmentation du sommeil chez les deux groupes de chronotypes (cinquième article : Mongrain et Dumont, soumis). Nous avions également pour objectif d'évaluer la vigilance et les marqueurs du processus S pendant l'éveil suite à la fragmentation du sommeil et à une nuit de récupération chez les chronotypes (sixième article : Mongrain et al., en préparation). Finalement, le dernier objectif de la présente thèse était de vérifier le décours de l'AOL au cours d'une nuit de récupération chez les chronotypes (Mongrain et Dumont, en préparation : article non inclus dans la thèse).

Les hypothèses étaient que les Types-M montreraient une réponse à la fragmentation plus forte tel que mesurée avec les indices du processus S pendant le sommeil lent et l'éveil. Il était aussi attendu que les Types-M auraient une plus grande augmentation du niveau initial d'AOL que les Types-S à la nuit de récupération ainsi qu'une meilleure vigilance suite à la nuit de récupération.

3. Méthodologie

Les résultats des articles présentés dans cette thèse proviennent des même sujets étudiés dans le même protocole de recherche. Il s'agissait d'un projet d'envergure qui s'est déroulé sur une période de 3 ans et qui permettait d'aborder différents aspects de la régulation du sommeil et de la vigilance chez les chronotypes. Chaque article inclus dans la thèse présente la section de la méthodologie pertinente aux résultats analysés. Afin de faciliter une vision d'ensemble du projet, nous présentons ici les grandes lignes de la méthodologie globale du protocole de recherche utilisé.

3.1 Participants

Dans ce projet de recherche, 12 personnes matinales ont été comparées à 12 personnes vespérales, appariées pour l'âge et le sexe. Les volontaires ont été sélectionnés à l'aide du Questionnaire de matinalité-vespéralité (QMV) de Horne et Östberg (1976). Les QMV ont été distribués à l'intérieur des classes de cours de premier cycle des départements de sciences biologiques et de psychologie de l'Université de Montréal. Les QMV étaient accompagnés d'une feuille de présentation où les numéros de téléphone étaient demandés aux étudiants intéressés à participer à une étude sur le sommeil. D'autres QMV (environ 20 %) ont été complétés suite à la réponse à des affiches apposées à l'Université de Montréal ou à des annonces dans le journal de l'Université de Montréal (le Forum) ou le journal Voir. Le pointage de quelques 1200 QMV a été calculé, et les Types-M et Types-S ont été contactés pour une explication sommaire de l'étude ainsi que pour vérifier l'admissibilité préalable à l'étude.

Le pointage au QMV varie de 16 à 86. Les individus ayant obtenus un pointage plus petit ou égal à 41 sont considérés comme des Types-S extrêmes à modérés et ont été contactés pour le dépistage téléphonique. Les individus avec un pointage plus grand ou égal à 59, Types-M modérés à extrêmes, ont aussi été contactés. Le dépistage téléphonique a servi à s'assurer de la disponibilité des sujets pour un séjour prolongé en laboratoire (5 nuits, 5 jours) ainsi que de l'admissibilité à certains critères d'inclusion tels : l'âge (18-35 ans),

l'indice de masse corporel normal (plus bas que 27), être non-fumeur et ne pas consommer de drogue, être en bonne santé générale et ne pas prendre de médicament sur une base quotidienne (sauf contraceptifs), ne pas avoir de problème de sommeil (difficulté à s'endormir, mauvaise qualité de sommeil, etc), avoir un horaire de sommeil assez régulier avec une durée de sommeil entre 7 et 9 heures par nuit, ne pas faire de sieste dans le jour, ne pas avoir travaillé de nuit dans la dernière année, ne pas avoir fait de voyage transméridien dans les derniers 3 mois et finalement chez les femmes, avoir un cycle menstruel régulier. Les sujets répondant à ces critères et étant intéressés à participer à l'étude étaient invités à une visite du laboratoire de chronobiologie.

La visite du laboratoire durait 1h30 à 2h environ. Lors de celle-ci, l'étude était expliquée en détail aux sujets et les outils de mesures leur étaient présentés. Ensuite, un formulaire d'information et de consentement était lu avec le ou la volontaire et signé par ce dernier si toujours intéressé à participer. Par la suite, les sujets devaient compléter plusieurs questionnaires pour vérifier leur admissibilité à d'autres critères d'inclusion : le QMV était complété à nouveau; un questionnaire général portant sur la santé, les antécédents médicaux et le sommeil; le questionnaire d'anxiété d'état et de trait de Spielberger; le questionnaire de dépression de Beck; et finalement, le questionnaire sur la qualité du sommeil de Pittsburgh. À la fin de cette visite, les sujets se voyaient remettre 7 jours d'agendas de sommeil à compléter de façon consécutive et à retourner au laboratoire par la poste. Lorsque les agendas de sommeil étaient reçus, les sujets répondant toujours aux critères de recherche étaient invités à un séjour d'environ 24 heures en laboratoire pour un dépistage des troubles du sommeil et de la vigilance.

Le dépistage en laboratoire consistait en une nuit d'enregistrement polysomnographique (PSG) en utilisant un montage référentiel incluant 4 électrodes EEG (C3, C4, O1, O2), 2 électrodes EOG, 3 électrodes EMG mentonnières et 4 électrodes EMG tibia-antérieur, 2 électrodes ECG et finalement une thermistance nasale. L'horaire de sommeil habituel des sujets, tel que déterminé par la moyenne des 7 jours d'agendas de sommeil en dépistage, était conservé pour cette nuit d'enregistrement en laboratoire. L'arrivée des sujets au laboratoire était prévue quelques heures avant leur coucher pour l'installation des électrodes. Au lever, les électrodes EMG tibia-antérieur ainsi que la thermistance nasale étaient retirées mais le reste du montage était conservé pour l'enregistrement de 4 tests

itératifs d'endormissement (TIDE) administrés à 2 heures d'intervalle durant la journée. Pour être invités à participer à l'étude, les sujets devaient avoir une efficacité de sommeil nocturne supérieure ou égale à 85 %, un index d'apnées/hypopnées ou de mouvements périodiques des jambes inférieur à 5 et un délai moyen d'endormissement au TIDE supérieur à 7 minutes.

Le groupe des Type-M était constitué de 6 hommes et 6 femmes âgés de 19 à 34 ans (24.7 ± 1.5 ans) et avaient un pointage au QMV de 59 à 71 (65.9 ± 1.1). Les 12 Types-S incluaient également 6 hommes et 6 femmes, âgés de 21 à 30 ans (23.4 ± 0.7 ans) et ayant un pointage au QMV de 27 à 40 (32.7 ± 1.2).

3.2 Protocole expérimental

Pour chaque sujet, un horaire de sommeil individuel a été déterminé pour correspondre le plus fidèlement possible à son horaire libre et spontané. Pour ce faire, les questions du QMV concernant les heures de coucher et de lever préférées ont été utilisées en combinaison avec l'horaire de sommeil des journées sans obligations rapporté dans des agendas de sommeil de dépistage. Finalement, la décision finale a été prise en discutant avec le sujet pour s'assurer que cet horaire de sommeil correspondait bien à sa préférence. Une fois l'horaire individuel déterminé, celui-ci était conservé pour la totalité de l'expérimentation qui commençait par une semaine d'enregistrements ambulatoires et d'agendas de sommeil. Au cours de cette semaine, les heures de coucher et de lever cibles, qui visaient une durée de sommeil de 8 heures, devaient être maintenues avec une précision de plus ou moins 30 minutes. Le niveau d'activité ainsi que la lumière reçue par les sujets étaient enregistrés par actigraphie pendant que les sujets vaquaient à leurs occupations habituelles.

Immédiatement après les 7 journées de mesures ambulatoires, les sujets étaient accueillis au laboratoire quelques heures avant leur heure habituelle de coucher pour un séjour de 5 nuits et 5 jours consécutifs. Le premier 24 heures (J1) servait à l'adaptation au laboratoire et aux mesures des marqueurs de la phase circadienne. Le second 24 heures (J2) permettait de mesurer le sommeil et la vigilance en conditions normales. Durant les 3^{e} et 4^{e} nuits, le

sommeil était perturbé par une fragmentation du sommeil et l'impact sur le sommeil et la vigilance de cette manipulation était mesuré au cours des troisième et quatrième périodes de 24 heures (J3 et J4). Le sommeil a été fragmenté de la façon suivante : à chaque 30 minutes, un technicien entrait dans la chambre du sujet sans ouvrir les lumières et le sujet devait interragir verbalement avec le technicien pendant 5 minutes; parallèlement, un autre technicien dans la salle de contrôle vérifiait le temps et l'éveil sur l'enregistrement PSG (Dumont et al., 2000). Le dernier 24 heures en laboratoire permettait d'évaluer la récupération suite à ces perturbations (J5). Une représentation schématique de l'expérimentation des volontaires est montrée à la figure 3 ci-après.

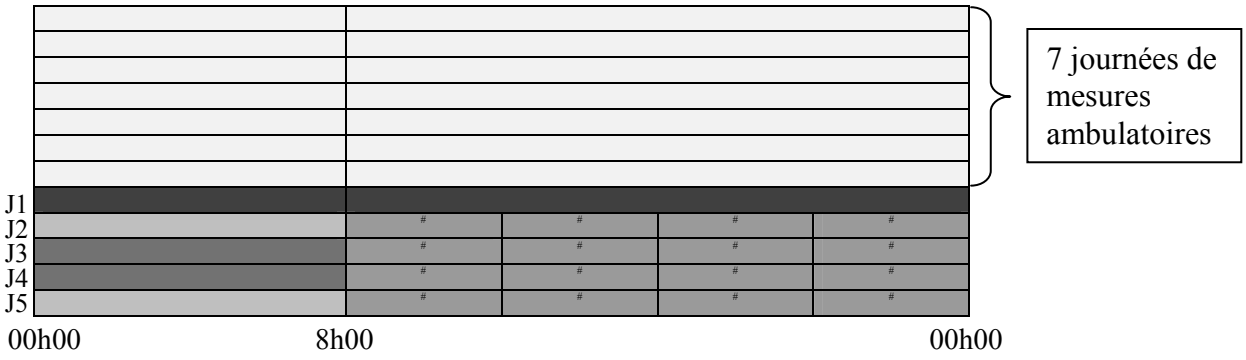

Figure 3 : Représentation schématique du protocole expérimental. Exemple montré pour un sujet se couchant à minuit et se levant à 8h00. Chaque bande horizontale représente une journée commençant par l'épisode de sommeil. Les journées en blanc représentent les jours de mesures ambulatoires. La journée foncée indique la journée de mesure de la phase circadienne. Les dièzes indiquent les séries de tests de vigilance.

3.3 Mesures circadiennes

Les mesures circadiennes recueillies lors de la première journée au laboratoire étaient 2 marqueurs physiologiques de la rythmicité circadienne : la température corporelle et la sécrétion de la mélatonine. La température rectale à été mesurée à toutes les minutes pendant 26 heures à l'aide d'une thermistance jetable (Yellowspring Co.) ayant été insérée 10 cm dans le rectum par les sujets eux-mêmes à leur arrivée au laboratoire, et reliée à un moniteur portatif (Mini-Logger, Mini-Mitter Co.). La mélatonine salivaire a été dosée à partir d'échantillons de salives recueillis à 6 reprises à toutes les 30 minutes suivant le lever du J1 et à 11 reprises toujours aux 30 minutes avant le coucher du J2. Lors des récoltes de salive, les sujets devaient demeurer dans une position assise constante ne se levant que pour

aller à la toilette et la luminosité devait être maintenue à 15 lux ou moins dans l'angle du regard. Les sujets devaient rincer leur bouche avant la récolte de l'échantillon et ne rien manger ni boire avant que l'échantillon ne soit récolté 5 minutes plus tard à l'aide de salivettes (Sarstedt Inc.).

3.4 Mesures de sommeil et de vigilance

De manière similaire à la nuit de dépistage, le sommeil a été enregistré pour les 5 nuits expérimentales en laboratoire. Les enregistrements de sommeil ont été effectués avec un montage référentiel aux oreilles liées à une résistance de 10 KΩ, en utilisant 20 électrodes appliquées sur le cuir chevelu selon le système 10-20 de Jasper (1958). Les signaux ont été enregistrés avec un système d'acquisition Grass Modèle 15 jumelé au logiciel Harmonie (Stellate Systems). L'acquisition et la numérisation des signaux d'EEG ont été effectuées à 256 Hz avec un gain de 10 000 et une bande passante de 0.3-100 Hz.

Les mesures de vigilance des journées 2, 3, 4 et 5 consistaient en 4 séries de tests. La première série avait lieu 1.5 heures après le lever et les autres à toutes les 4 heures. Chaque série, d'une durée approximative d'une heure, comprenait 5 tests administrés dans l'ordre suivant : **1)** une évaluation subjective de la vigilance avec une échelle visuelle analogique (EVA) : une ligne horizontale de 10 cm dont les extrémités sont "très endormi(e)" et "très éveillé(e)" (McCormack et al., 1988); **2)** une évaluation de l'humeur avec la version française du "Profile of Mood States" (POMS; McNair et al., 1971); **3)** une évaluation de l'attention soutenue par une tâche visuelle psychomotrice de 10 minutes ("Psychomotor Vigilance Task", PVT; Dinges et al., 1997); **4)** un enregistrement EEG à l'éveil pendant 2 minutes les yeux ouverts (en fixant une croix au mur) et 2 minutes les yeux fermés; **5)** une mesure de la propension au sommeil avec les TIDEs d'une durée maximale de 25 minutes (Carskadon et Dement, 1982). Les enregistrements EEGs à l'éveil et TIDEs ont été effectués avec la même technique que les enregistrments de sommeil.

3.5 Origine des articles de recherche

Le premier article de recherche de la présente thèse (Mongrain et al., 2004) découle de l'analyse des marqueurs de la phase circadienne mesurés à J1 ainsi que de l'horaire de sommeil des sujets calculé pour la semaine de mesures ambulatoires et de la relation entre les deux.

Le second article (Mongrain et al., 2005) provient de l'analyse des stades de sommeil et l'analyse spectrale de l'EEG du sommeil lent enregistré à la dérivation C3 durant la nuit J2. Le troisième article (Mongrain et al., 2006a) provient de l'analyse spectrale de l'EEG du sommeil paradoxal et du sommeil lent enregistré aux dérivations Fz, Cz, Pz, et Oz de l'enregistrement de la nuit J2. Le quatrième article de recherche (Mongrain et al., 2006b) provient de la modélisation plus avancée des données d'analyse spectrale de l'EEG en sommeil lent enregistré aux dérivations Fz, Cz, Pz, et Oz lors de la nuit J2, ainsi que de la relation avec une mesure de phase de J1.

Le cinquième article de cette thèse (Mongrain et Dumont, soumis) concerne l'analyse des stades de sommeil et l'analyse spectrale de l'EEG en sommeil lent enregistré aux dérivations Fz, Cz et Pz pour les nuits d'enregistrement J2, J3, J4 et J5. Le sixième article (Mongrain et al., en préparation) résulte de l'analyse des mesures de vigilance des journées J2, J3, J4 et J5.

4. Résultats : Articles de recherche

4.1 Premier article

Phase relationship between sleep-wake cycle and underlying circadian rhythms in morningness-eveningness

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(voir lettre de confirmation en annexe)

PHASE RELATIONSHIPS BETWEEN SLEEP-WAKE CYCLE AND UNDERLYING CIRCADIAN RHYTHMS IN MORNINGNESS-EVENINGNESS

Valérie MONGRAIN[1,3], Suzie LAVOIE[1,2], Brahim SELMAOUI[1], Jean PAQUET[1] and Marie DUMONT[1,2,3]

[1]Chronobiology Laboratory, Sacré-Cœur Hospital of Montreal; [2]Department of Psychiatry and [3]Department of Neurosciences, Université de Montréal, Québec, Canada

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Running title: Phase angles in Morningness/Eveningness

Corresponding author: Marie Dumont, Ph.D.

Chronobiology Laboratory

Sacré-Cœur Hospital

5400 blvd Gouin W.

Montréal (Québec)

Canada, H4J 1C5

Tel: (514) 338-2222 ext. 2246

Fax: (514) 338-2531

Email: m-dumont@crhsc.umontreal.ca

ABSTRACT

A shorter phase angle between habitual wake time and underlying circadian rhythms has been reported in evening-types (E-types) compared to morning-types (M-types). In this study, phase angles were compared between 12 E-types and 12 M-types to verify if this difference was observed when the sleep schedule was relatively free from external social constraints. Subjects were selected according to their morningness-eveningness score (MEQ score). There were 6 men and 6 women in each group (19 to 34 y.) and all had an habitual sleep duration between 7 and 9 hours. Sleep schedule was recorded by actigraphy and averaged over 7 days. Circadian phase was estimated by the hour of temperature minimum (Tmin) in a 26-h recording, and by the timing of the onset of melatonin secretion (DLMO) measured in saliva samples. Phase angles were defined as the interval between phase markers and averaged wake time. Results showed that, in our experimental conditions, phase angles were very similar in the two groups of subjects. However, our results confirmed the previously reported correlation between phase and phase angle, showing that a later circadian phase was associated with a shorter phase angle. Gender comparisons showed that for a same MEQ score, women had an earlier DLMO and a longer phase angle between DLMO and wake time. Despite a significant difference in the averaged circadian phases between E-type and M-type groups, there was an overlap in the circadian phases of the subjects of the two groups. Further comparisons were made between the two circadian types, separately for the sub-groups with overlapping or non-overlaping circadian phases. In both sub-groups, the significant difference between MEQ scores, bedtimes and wake times were maintained in the expected direction. In the sub-group with non-overlapping circadian phases, phase angles were shorter in E-types subjects, in accordance with previous studies. However, in the overlapping sub-group, phase angles were significantly longer in E-types compared to M-types. Our results suggest that the morningness-eveningness preference identified by the MEQ score refer to two distinct mechanisms, one associated with a difference in circadian period and phase of entrainment, and the other associated with chronobiological aspects of sleep regulation.

Key words: Morningness-eveningness, circadian phase, phase angle, sleep schedule, salivary melatonin, rectal temperature, gender differences, entrainment.

INTRODUCTION

Morningness-eveningness refers to individual preferences in the timing of daily activities. This characteristic is usually evaluated with a questionnaire, the most widely used being the morningness-eveningness questionnaire (MEQ) of Horne and Östberg (1976). Scores on the MEQ vary from 16 to 86 and approximate a normal distribution in the population (Posey and Ford, 1981). High scores (59 to 86) identify Morning-type individuals (M-types), low scores (16 to 41) correspond to Evening-types (E-types), whereas scores from 42 to 58, found in more than 60% of the population, refer to an intermediate type ("neither-types"). Compared to E-types, M-types tend to adopt an earlier sleep schedule and to present earlier diurnal peaks of alertness and performance (Foret, 1982; Kerkhof, 1991; Lack and Bailey, 1994; Natale and Cicogna, 1996; Natale and Cicogna, 2002). Physiological markers of the endogenous circadian rhythmicity, including body temperature and melatonin secretion rhythms, also show an earlier phase in M-type individuals compared to E-types (Lack and Bailey, 1994; Duffy et al., 1999; Baehr et al., 2000; Bailey and Heitkemper, 2001). An interaction between morningness-eveningness and gender is possible since higher MEQ scores and earlier circadian phases have been reported in women compared to men (Tankova and al., 1994; Gibertini and al., 1999; Baehr and al., 2000; Adan et Natale, 2002).

Since sleep propensity is largely under circadian control (Strogatz et al., 1986; Dijk and Czeisler, 1994; Lavie, 2001), it could be expected for M-types and E-types to go to bed and to wake up at the same circadian time. Contrary to these expectations, however, studies have reported that E-types wake up at an earlier circadian time compared to M-types (Kerkhof, 1991; Duffy et al., 1999; Baehr et al., 2000). Therefore, the phase angle, defined as the interval between the trough of the temperature rhythm and habitual wake time, was shorter in E-types compared to M-types in these studies. Similarly, Liu et al. (2000) found that both melatonin peak and sleep propensity onset occur later in relation to the sleep episode in E-type individuals, also resulting in a shorter phase angle compared to M-types.

Patients suffering from delayed sleep phase syndrome (DSPS) can be considered as very extreme E-types (Weitzman et al., 1981; Regestein and Monk, 1995; Terman et al., 1995). Differences in phase angles were also found in these patients compared to normal controls. In contrast to E-types, however, DSPS patients were reported to wake up at a later circadian phase and thus to have a longer phase angle when compared to healthy control subjects (Ozaki et al., 1996; Shibui et al., 1999; Uchiyama et al., 2000). From another perspective, aging is associated with a transition toward morningness (Tankova et al., 1994; Carrier et al., 1997), but older people were shown to wake up at an earlier circadian phase and thus to have shorter phase angle, in opposition to the characteristics associated with morningness in young people (Duffy et al., 1998, 1999).

A possible explanation for these contrasting observations could be the differences in the constraints placed upon the sleep schedule in these populations. Social constraints are quite different for E-type and M-type individuals. E-types usually have to go to bed and to wake up earlier than what they would do spontaneously. Conversely, M-types tend to delay their bedtime to meet their social needs (Ishihara et al., 1988; Taillard et al., 1999; Bailey and Heitkemper, 2001; Roenneberg et al., 2003). Social pressures may therefore have contributed to the presence of the shorter phase angle reported in E-types compared to M-types. These social constraints could be smaller for DSPS patients who have such a late circadian phase that they may give up trying to adjust to a socially acceptable schedule. Older individuals may have less social constraints in the evening and therefore less pressure to delay their bedtimes compared to younger people. These observations suggest that to explore differences in phase angles between M-types and E-types, they must be recorded when the sleep schedule is chosen with minimal external social constraints. If this is not done, the results are biased.

The aim of this study was to compare the temporal relationship (the "phase angle") between sleep schedule and circadian phase in M-type and E-type subjects. Great care was taken to ensure that subjects were free to adopt a sleep schedule as spontaneous as possible. Under these conditions, a difference in phase angle was expected to reflect a

physiological difference in the regulation of the sleep-wake cycle in association with morningness-eveningness instead of a direct influence of external social constraints.

METHODS

Subjects

M-type and E-type volunteers, aged 19 to 34 y, were recruited using a French version of the Horne and Östberg (1976) questionnaire. A total of 24 subjects completed the study, including 12 M-types (MEQ scores 59 to 71) and 12 E-types (MEQ scores 27 to 40). There were 6 women and 6 men in each group. Age was similar in the two groups (M-types: 24.7 y ± 1.5; E-types: 23.4 y ± 0.7). Most subjects were students without a summer job (11 E-types and 7 M-types), the others were between jobs (2 M-types), working in the afternoon (1 E-type) or worked at home with their own schedule (3 M-types). One subject in each group had young children at home.

All selected subjects had an habitual sleep duration of 7 to 9 h, as assessed by questionnaires and 7 days of sleep diary at screening. Volunteers with a large day-to-day variability in their sleep schedule (more than 2 hours) were excluded from the study. Subjects had no sleep or vigilance complaints and screening polysomnography confirmed the absence of sleep disorders. All subjects were in good physical and psychological health, as assessed by questionnaires and interview. Volunteers with night work experience in the past year or transmeridian travel in the past three months were excluded. All subjects were non-smokers and reported not using drugs or medications, except oral contraceptives in 5 women (3 M-types and 2 E-types). Women not using hormonal contraception were studied during the follicular phase of their menstrual cycle. Each subject signed an informed consent form approved by the hospital's ethics committee and received a financial compensation.

Procedures

For the study, individual sleep schedules were determined according to each subject's preferred bedtime and wake time. Criteria for these preferences included sleep schedules adopted on free days in the one-week screening sleep diary, and preferred wake time and bedtime reported in the MEQ. The final decision for the study sleep schedule was made after discussion with the subject to ensure that it was as close as possible to the schedule that he/she would spontaneously adopt. Since all volunteers with very irregular schedules were excluded from the study, the selected study sleep schedules were always close to the habitual sleep schedules of the subjects. The study was conducted in the summer, at a time when the subjects were free to choose a sleep schedule without external work/school constraints. Target bedtimes and wake times (± 30 min) were determined for a sleep duration of about 8 h, with a permitted range of 7 to 9 h. Subjects were requested to follow their selected sleep schedule for 7 days prior to laboratory admission and not to engage in any activity that would affect their sleep schedule. Compliance was verified by sleep diaries and by 24-h ambulatory measures of activity and light exposure (Actiwatch-L, Mini-Mitter Co, Bend, OR).

Circadian phase was estimated in the chronobiology laboratory using two markers: core body temperature minimum (Tmin) and dim light melatonin onset (DLMO). Subjects were admitted to the laboratory 4 h before their scheduled bedtime. Temperature recording started on admission and continued for the next 26 h. Temperature was measured with a disposable rectal probe (Yellowspring Co.) and recorded every minute with a portable monitor (Mini-Logger, Mini-Mitter Co., Bend, OR). During the night, subjects slept for 8 h according to their individual sleep schedule. Temperature data were smoothed by averaging the values every 10 min and fitted with a 24-h cosine curve (cosinor analysis; Nelson et al., 1979). Tmin was the clock time of the through and temperature amplitude was half the difference between the peak and through of the fitted cosine curve.

To determine the onset of melatonin secretion, saliva samples were collected every half-hour on the second day, starting 5 h before scheduled bedtime (total of 11 samples). Saliva was collected in dim light (< 15 lux) using the Salivette devices (Sarstedt Inc), centrifuged

and immediately frozen. During sampling time, subjects were seated and allowed to do quiet activities such as reading and watching TV. Melatonin concentration in the saliva was determined in duplicate using direct radioimmunoassay with a 125-iodine tracer (Bühlmann Laboratories AG, Switzerland). Intra-assay coefficients of variation (CVs) were lower than 10% and inter-assay CVs lower than 13%. The reported minimum detectable concentration of melatonin for the assay is 0.65 pg/ml. DLMO was found by interpolation, the onset being defined as twice the minimum detection limit of the assay (i.e., 1.3 pg/ml) when followed by a further increase in melatonin concentration (Deacon and Arendt, 1994).

Data Analysis

For each of the 7 days preceding the admission, bedtime and wake time were estimated as precisely as possible using both subject's sleep diary and the output of activity and light ambulatory recordings. For each subject, the estimates were then averaged over the 7 days. Phase angles were defined in relation to wake time. For temperature, phase angle (Tangle) was defined as the interval between Tmin and averaged wake time; for melatonin, phase angle (DLMOangle) was the interval between DLMO and averaged wake time. Because of the possibility of an interaction between morningness-eveningness and gender, all statistical comparisons were conducted with Group-by-Gender (2X2) ANOVAs. Relationships between variables were quantified with Pearson correlations. Statistical significance was set to 0.05.

RESULTS

Comparisons between M-type and E-type subjects

Sleep schedules are shown in Table 1. Wake times and bedtimes were significantly different between the two groups. On average, bedtime was about 2.5 h earlier and wake time 2.8 h earlier in M-types than in E-types. Sleep duration was similar in the two groups. DLMO was observed before bedtime in all participants. Because of missing data, temperature recordings were not usable in two M-type men, reducing the number of

subjects in all analyses including temperature data. Circadian phases were significantly earlier in M-types than in E-types, with a difference of 2.7 h for DLMO and 2.0 h for Tmin. Mean phase angles were very similar in M-type and E-type subjects (Table 1). They differed by only 4 min when using DLMO and by 47 min when using Tmin. DLMO to bedtime intervals were 2:49 h (± 23') and 2:39 h (± 15') in M-types and E-types, respectively. Amplitude of the temperature rhythm was also similar in the two groups (Table 1).

Circadian phase and sleep schedule

The phase markers were significantly correlated with the timing of the sleep schedule ($p< 0.001$ for all correlations). DLMO was correlated both with habitual wake time ($r= 0.75$) and habitual bedtime ($r= 0.84$). A similar association was found between Tmin and wake time ($r= 0.80$) and between Tmin and bedtime ($r= 0.81$). An association between sleep duration and phase angle was also found, showing that a longer sleep duration tended to be associated with a longer phase angle. This correlation was significant when phase angle was calculated with DLMO ($r= 0.60$, $p< 0.01$) and approached significance when calculated with Tmin ($r= 0.39$, $p= 0.07$).

Association between circadian phases and phase angles

It was not possible to compute correlations between MEQ scores and phase angles since our selection criteria produced a discontinuity in the distribution of MEQ scores. However, circadian phases showed a distribution that was both continuous and normal. A later circadian phase was significantly correlated with a shorter phase angle. For melatonin data, the correlation between DLMO and DLMOangle was significant when computed on all 24 subjects ($r= -0.55$, $p< 0.01$). The same correlation was also high when computed within the M-type group ($r= -0.90$, $p< 0.001$) and within the E-type group ($r= -0.68$, $p< 0.01$). When using available temperature data, the correlation between Tmin and Tangle approached statistical significance for the 22 subjects ($r= -0.37$, $p= 0.09$) and was significant within both the M-type (-0.81, $p< 0.01$) and E-type ($r= -0.74$, $p< 0.01$) groups.

Correlations between phase markers and their respective phase angles are illustrated in Fig. 1. Regression fittings showed that the general relationship between circadian phase and phase angle was similar for both M-type and E-type subjects, with a later circadian phase associated with a shorter phase angle. However, the scatter plots also illustrate that the phase angles predicted by a given circadian phase were different between the two circadian types. For example, according to the regression formulae, a M-type subject having a DLMO at 22:00 h should wake up approximately 9.5 h later, whereas an E-type subject with the same DLMO would show a longer phase angle of about 11.6 h as a result of a later sleep schedule. Therefore, in our experimental conditions, the relationship between circadian phase and phase angle gave two contrasting results: M-type and E-type groups had significantly different circadian phases but similar phase angles, while there was a significant correlation between circadian phase and phase angle.

Overlapping vs nonoverlapping circadian phases

Despite the significant difference between the two groups, there was a large overlap in the circadian phases of E-type and M-type subjects. In an attempt to understand the apparent contrasts in the results, further comparisons were made between the two circadian types, separately for those with overlapping and with non-overlapping circadian phases. To make these comparisons, the subjects were divided into two sub-groups, each including the same number of M-type and E-type subjects. The 6 M-type subjects with the earliest DLMOs (range: 17:25 to 20:59) and the 6 E-type subjects with the latest DLMOs (range: 23:57 to 01:35) were included in the sub-group of non-overlapping phases. The remaining 12 subjects with intermediate DLMOs (21:07 to 23:04) were included in the sub-group of "overlapping" circadian phases (M-types: 21:07 to 23:03; E-types: 21:18 to 23:04). To make this selection, DLMO was preferred to Tmin as a phase marker because masking effects were expected to be larger for Tmin than for DLMO in our recording conditions, and because Tmin was missing for two subjects. In the sub-group of non-overlapping phases, E-types included more men and tended to be younger (21.8 ± 0.3 y) than M-types (26.2 ± 2.1 y; $p= 0.07$). None of the subjects had children at home. In the sub-group with overlapping phases, E-types included more women than M-types (see Table 2), but age

was similar (23.3 ± 2.2 y versus 25.0 ± 1.2 y, respectively). Both M-types and E-types included one woman with young children at home.

Results are presented in Table 2. T-tests were used within each sub-group to compare sleep and circadian variables between M-types and E-types. Due to the sub-group selection, circadian phases were very similar between M-type and E-type subjects in the overlapping sub-group, but differed by 4 to 5 h in the non-overlapping sub-group. In both sub-groups, M-types had significantly higher MEQ scores and earlier bedtimes and wake times compared to E-types. However, sleep duration tended to be longer in E-types included in the overlapping sub-group ($p= 0.06$), but not in those of the non-overlapping sub-group. Temperature amplitude tended to be lower in M-types than in E-types of the non-overlapping subgroup ($p= 0.08$), but there was no difference between M-types and E-types with overlapping phases.

In the sub-group with non-overlapping phases, DLMOangle was significantly longer in M-type subjects compared to E-types. A non-significant difference in the same direction was observed for Tangle (Table 2). This is in contrast with the results observed in the sub-group with overlapping phases where both phase angles were significantly shorter in M-types compared to E-types. These differences are illustrated in Fig. 2.

Gender differences

MEQ scores were similar between men and women (Table 3). Sleep duration was also similar. However, sleep schedule tended to be later in the men, especially for bedtime. There was a significant main effect of gender for the circadian phase estimated with DLMO ($F_{1,20} = 8.90$, $p < 0.01$) and for DLMOangle ($F_{1,20} = 5.86$, $p < 0.05$). As shown in Table 3, DLMO was 1.6 h earlier and DLMOangle 1.1 h longer in women compared to men. Similarly, the DLMO to bedtime interval was longer in women (3:10 ± 10') than in men (2:17 ± 23'; $p < 0.05$). The same tendencies, but not statistically significant, were

observed in analyses using Tmin (data missing for two men). Temperature amplitude tended to be larger in men than in women.

DISCUSSION

In this study, M-type and E-type subjects were studied in a situation where their sleep schedule was relatively free of social constraints. Contrasting results were found regarding the phase relationship between circadian phase and habitual wake time in relation to morningness-eveningness. On the one hand, our results confirmed previous reports showing an association between a later circadian phase and a shorter phase angle. On the other hand, M-type and E-type groups showed identical phase angles despite having significantly different circadian phases.

Inspection of M-types and E-types with or without overlapping circadian phases (Table 2 and Fig. 2) may help to understand the apparent paradox in the results. When comparisons were conducted only between M-type and E-type subjects with non-overlapping circadian phase (as it was specifically done in Kerkhof study), a longer phase angle was found in M-types, in accordance with previous reports (Kerkhof, 1991; Duffy et al., 1999; Baehr et al., 2000; Liu et al., 2000). However, when M-types and E-types with overlapping circadian phases were compared, bedtimes and wake times were still significantly different, in the range expected for morning and evening subjects, despite a similar circadian phase. This resulted in a significant difference in the phase angles, but showing this time a shorter phase angle in M-types compared to E-types. This is the first time that such an observation is reported in morningness-eveningness. It seems therefore that the MEQ identifies correctly the individual preference for morning or evening activity, but that this preference refers to two different circadian patterns. In one case (as in the "non-overlapping sub-groups"), this preference is closely linked to the endogenous circadian phase, as it is usually expected. But in the other case (as in the "overlapping" sub-groups), this preference is independent of the endogenous circadian phase and should be related to some other characteristic of the regulation of the sleep-wake cycle.

The longer phase angle in the "non-overlapping" M-types is consistent with the negative correlation found between circadian phase and phase angle. Such a correlation has been reported before (Martin and Eastman, 2002). It cannot be due to a dichotomy in the circadian physiology of M-types and E-types since the correlation was even stronger when computed within the M-type and E-type groups. A number of reasons may explain this association between circadian phase and phase angle. As mentioned in the introduction, the most obvious reason relates to social constraints. However, in the present study, great care has been taken to ensure that subjects were sleeping at their preferred time of day, relatively free from external social constraints. It cannot be totally excluded that the choice of the study sleep schedule was affected by some unspecified external influences. However, a more likely possibility would be that the different circadian phases of the "non-overlapping" M-types and E-types reflect different endogenous periods. In a study where both phase and period were estimated in 9 subjects, a strong correlation ($r= 0.72$) was found between the two circadian parameters (Duffy et al., 2001). In both animals and humans, mechanisms of entrainment are consistent with the prediction that individuals with shorter periods will have circadian phases earlier within their sleep-wake / light-dark cycle, resulting in a longer interval between circadian phase and wake time (Pittendrigh and Daan, 1976; Aschoff, 1981; Lewy et al. 2001; Roenneberg et al., 2003). Our results suggest that this mechanism of entrainment was at work in both M-type and E-type subjects.

M-types and E-types of the overlapping sub-group had similar circadian phases, but different phase angles. A shorter phase angle in M-types compared to E-types having a similar circadian phase has never been reported before. However, it is reminiscent of the results reported in Duffy et al (1999) concerning the comparisons between younger and older subjects. Analogously to the characteristics of our "overlapping" M-types, their older subjects had a shorter phase angle and a shorter sleep duration compared to the young. The circadian phase (Tmin) of their older M-type subjects was also relatively late (05:18) for morning types, but similar to the Tmin of our "overlapping" M-types (05:06). The authors suggested that the chronobiological features associated with self-reported morningness are quite different in young and older subjects. We suggest that such differences in

chronobiological features are not exclusive to aging but can also be observed in younger subjects. The nature of this difference is still unknown in the elderly, but it could be related to a change in the interaction between homeostatic and circadian processes of sleep regulation (Duffy et al., 1998; Dijk and Duffy, 1999).

A similar interpretation may apply to our results. For example, a faster decline of the homeostatic sleep pressure (or sleep need) during sleep would be consistent with the observation that M-types of the overlapping sub-group tended to have a shorter sleep duration than E-types. Such a mechanism would also explain the positive correlation between phase angle and sleep duration because if sleep pressure decreased more rapidly, then awakening is expected to happen at an earlier circadian phase. Conversely, a faster build-up of the homeostatic sleep pressure during waking would be consistent with a sleep onset closer to DLMO since sufficient sleep pressure would be accumulated at an earlier circadian time. This interpretation is supported by a recent study where M-types were shown to have a faster build-up of subjective sleepiness and a shorter time constant in the increase of sleep pressure represented by theta-alpha activity in the waking EEG, compared to E-types (Taillard et al., 2003). However, further studies will be necessary to determine the nature of the mechanisms involved and to establish their analogy with age-related processes.

We found significant differences between men and women. Both men and women had the same entrained sleep-wake schedule and similar MEQ scores, but women had an earlier circadian phase and thus a longer phase angle compared to men. In other words, for a given circadian phase, women seem to go to sleep and wake up later compared to men. An earlier circadian phase in women has already been reported in previous studies (Gibertini et al., 1999; Baehr et al., 2000) and a longer phase angle in women has also been observed (Baehr et al., 2000). Women also tended to have a longer sleep duration than men, which has also been reported in other studies (Carrier et al., 1997; Roenneberg et al., 2003). It is therefore possible that a gender difference exists in the regulation of the sleep-wake cycle, similar to the difference observed between circadian types with overlapping circadian phases. In fact, in the sub-group of subjects with overlapping circadian phases, most E-

types were women whereas M-types were mostly men (Table 2). The number of subjects in each sub-group is too small to make any definite conclusion, but since there was no significant interaction between circadian type and gender in our data, the effects of circadian type and gender on phase angle are expected to be additive and, more speculatively, to add also with those of aging.

Our findings indicate that some caution should be used before assuming that M-types have a shorter phase angle than E-types. It should be noted that not all studies found a difference in phase angles between the two chronotypes (Bailey and Heitkemper, 2001) and that the differences that were reported were not always very robust. For example, in Kerkhof's study (1991) M-type and E-type phase angles were no longer different when using ambulatory wake times instead of laboratory ones, and in Duffy et al. (1999) the difference was significant when using Tmin, but fell short of statistical significance when using melatonin as phase marker. It is possible that variations between studies came in part from differences in the criteria used for subjects' selection. In our study, volunteers with high day-to-day variability (more than 2 hours) in their sleep schedules, or with habitual sleep duration longer than 9 hours, were excluded. These criteria led to the exclusion of many E-type individuals (no M-type was excluded because of these criteria). Therefore, it is possible that our E-type subjects did not compare entirely with E-types included in previous studies. Subjects' selection might also explain why there was no significant difference in temperature amplitude, but the tendency towards a larger amplitude in E-types compared to M-types in the subgroup of subjects with non-overlapping circadian phases was consistent with previous observations (Baehr et al., 2000). It is also possible that the chosen methodology in the present study caused a lack of precision in the phase estimates: DLMO estimates could have been distorted by unknown differences in the amplitude of melatonin secretion (Lewy et al., 1999) and Tmin estimates were partly masked by the influence of sleep and posture (Duffy and Dijk, 2002). However, it is unlikely that our results can be explained by those potential biases since the two phase markers gave consistent results and because both were within the ranges previously reported for M-type and E-type individuals (Kerkhof and Van Dongen, 1996; Hall et al., 1997; Duffy et al., 1999; Baehr et al., 2000; Griefahn, 2002). We rather suggest that two

types of mechanisms are simultaneously at work in the expression of morningness-eveningness, as assessed with the MEQ. These mechanisms have opposite effects on the relationship between circadian phase and the sleep-wake schedule, and their combined action can explain the absence of difference in the phase angles of our M-type and E-type groups. Our results further imply that the MEQ score is not a good predictor of the endogenous circadian phase, which is in accordance with the relatively low correlations previously reported between these two variables (Duffy et al., 1999; Baehr et al., 2000; Martin and Eastman, 2002). They also indicate that, with a regular sleep schedule as the one used in our experimental conditions, a variation of 1 to 6 hours can be expected when using wake time to predict circadian phase.

The observations reported in this study may help to elucidate some of the interindividual variations in the circadian regulation of the sleep-wake cycle, in normals and in circadian disorders such as DSPS. If DSPS patients can be seen as extreme E-types on the morningness-eveningness continuum, our results suggest that this continuum can run on two parallel paths: one related to an extremely late circadian phase and long endogenous period, and another related to an abnormal phase angle caused by some deficiency in sleep-wake regulation. Clearly, these two sources of pathophysiology require the development of distinct treatment strategies.

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FIGURE LEGENDS

Figure 1. Scatter plots of phase angles relative to circadian phase for melatonin onset (A) and temperature minimum (B). Regression lines are also shown, computed separately for E-types (solid lines: melatonin, r= -0.68; temperature, r= -0.74) and M-types (broken lines: melatonin, r= -0.90; temperature r= -0.81).

Figure 2. Schematic representation of phase angles between sleep schedule (rectangle) and temperature (downward triangle) or DLMO (upward pointing arrow), for M-types and E-types of the sub-groups with non-overlapping or overlapping circadian phases. Sleep schedules are aligned on the chosen study wake time. Dotted lines represent DLMO to bedtime interval. This interval was longer in M-types (3:39 ± 32') than E-types (2:14 ± 22') in the sub-group with non-overlapping phases (p= 0.05), but was shorter in M-types (1:58 ±17') than E-types (3:04 ± 15') in the sub-group with overlapping circadian phases (p= 0.02). See Table 2 for phases angles in relation to wake time.

Table 1. Circadian and sleep characteristics (mean ± SEM) in M-type and E-type subjects. Probabilities are from the Group factor in Group-by-Gender ANOVAs.

	M-types (n=12)	**E-types** (n=12)	**P**
MEQ score	65.9 ± 1.1	32.7 ± 1.2	---
Bedtime (clocktime, h:min)	23:29 ± 14'	02:02 ± 17'	< 0.0001
Wake time (clocktime, h:min)	07:16 ± 12'	10:04 ± 20'	< 0.0001
Sleep duration (h:min)	7:47 ± 9'	8:02 ± 12'	n.s.
DLMO (clocktime, h:min)	20:41 ± 27'	23:23 ± 25'	< 0.001
Tmin (clocktime, h:min) [a]	04:17 ± 23'	06:17 ± 29'	< 0.01
Temperature amplitude (°C) [a]	0.36 ± 0.03	0.39 ± 0.04	n.s.
DLMOangle (h:min)	10:36 ± 24'	10:40 ± 24'	n.s.
Tangle (h:min) [a]	3:00 ± 21'	3:47 ± 18'	n.s.

[a] Analyses on temperature data include only 10 M-type subjects (2 men missing).

Table 2. Comparisons (mean ± SEM) between M-type and E-type subjects with non-overlapping and overlapping circadian phases. The number of men and women in each sub-group is also indicated.

	Nonoverlapping Phases			**Overlapping Phases**		
	M-types (4 W / 2 M)	**E-types** (1 W / 5 M)	**p(t)**	**M-types** (2 W / 4 M)	**E-types** (5 W / 1 M)	**p(t)**
DLMO (clocktime, h:min)	19:33 ± 33'	24:36 ± 16'	---	21:48 ± 17'	22:10 ± 17'	---
Tmin (clocktime, h:min) [a]	03:28 ± 25'	07:19 ± 19'	---	05:06 ± 23'	05:15 ± 41'	---
Temperature amplitude (°C) [a]	0.30 ± 0.04	0.41 ± 0.04	0.08	0.42 ± 0.05	0.37 ± 0.07	n.s.
MEQ score	67.8 ± 1.5	31.8 ± 1.4	< 0.001	64.0 ± 1.4	33.7 ± 1.9	< 0.001
Bedtime (clocktime, h:min)	23:12 ± 25'	02:50 ± 15'	< 0.001	23:47 ± 9'	01:14 ± 13'	< 0.001
Wake time (clocktime, h:min)	07:11 ± 22'	10:35 ± 23'	< 0.001	07:21 ± 11'	09:32 ± 27'	0.001
Sleep duration (h:min)	8:00 ± 13'	7:45 ± 14'	n.s.	7:34 ± 11'	8:18 ± 17'	0.06
DLMOangle (h:min)	11:38 ± 25'	9:59 ± 34'	< 0.05	9:33 ± 15'	11:22 ± 26'	< 0.01
Tangle (h:min) [a]	3:45 ± 10'	3:16 ± 16'	n.s.	2:15 ± 30'	4:17 ± 28'	0.01

[a] Analyses on temperature data include only 5 M-type subjects (one man missing) in each sub-group[a].

Table 3. Circadian and sleep characteristics (mean ± SEM) in men and women. Probabilities are from the Gender factor in Group-by-Gender ANOVAs.

	Men (n=12)	**Women** (n=12)	**P**
MEQ score	49.5 ± 5.4	49.2 ± 4.9	---
Bedtime (clocktime, h:min)	01:06 ± 34'	24:25 ± 64'	0.06
Wake time (clocktime, h:min)	08:52 ± 33'	08:27 ± 70'	n.s.
Sleep duration (h:min)	7:46 ± 13'	8:03 ± 8'	n.s.
DLMO (clocktime, h:min)	22:49 ± 39'	21:14 ± 30'	< 0.01
Tmin (clocktime, h:min) [a]	06:04 ± 28'	04:48 ± 32'	0.11
Temperature amplitude (°C) [a]	0.43 ± 0.05	0.33 ± 0.02	0.10
DLMOangle (h:min)	9:51 ± 23'	10:58 ± 13'	< 0.05
Tangle (h:min) [a]	2:58 ± 20'	3:24 ± 19'	n.s.

[a] Analyses on temperature data include only 10 men.

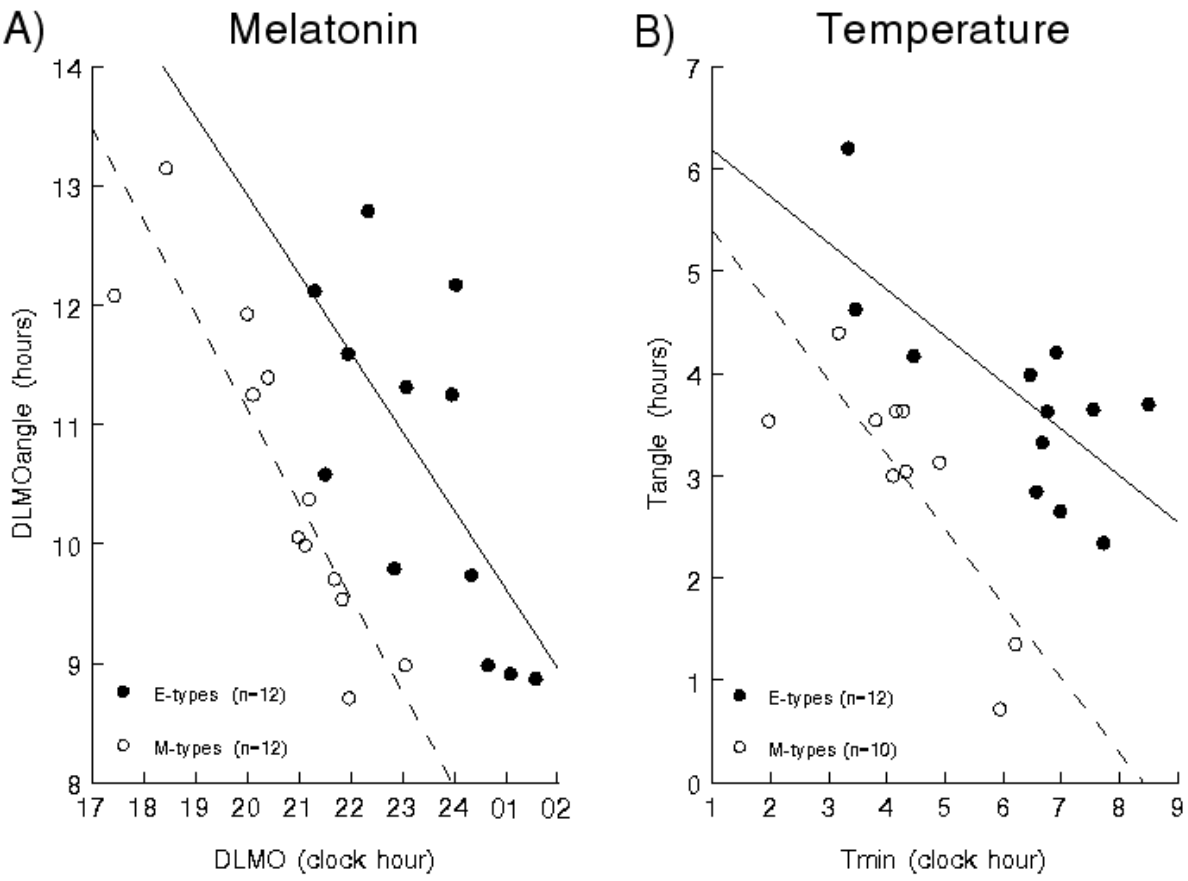

Figure 1

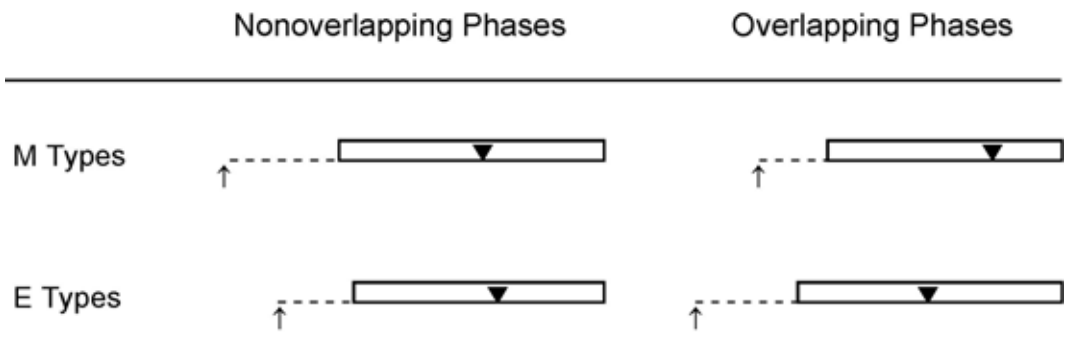

Figure 2

4.2 Second article

Chronotype and sex effects on sleep architecture and quantitative sleep EEG in healthy young adults

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(voir lettre de confirmation en annexe)

Chronotype and Sex Effects on Sleep Architecture and Quantitative Sleep EEG in Healthy Young Adults

Valérie MONGRAIN M.Sc.[1,2], Julie CARRIER Ph.D[1,2,3] and Marie DUMONT Ph.D.[1,2,4]

[1]Chronobiology Laboratory, Sacré-Cœur Hospital of Montréal, Québec, Canada, H4J 1C5; [2]Department of Neurosciences, [3]Department of Psychology and [4]Department of Psychiatry, Université de Montréal, Québec, Canada, H3C 3J7.

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Corresponding author: Marie Dumont, Ph.D.
Chronobiology Laboratory
Sacré-Cœur Hospital
5400 blvd Gouin W.
Montréal (Québec)
Canada, H4J 1C5
Tel: (514) 338-2222 ext. 2246
Fax: (514) 338-2531
Email: marie.dumont@umontreal.ca

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ABSTRACT

Study Objectives: To evaluate the influence of chronotype (morning types: M-types and evening types: E-types) on sleep stages and quantitative sleep EEG when constraints on the sleep schedule are minimal and when gender difference is taken into account.

Design: A 48-hour session in the laboratory, including 2 nights of polysomnography (PSG), preceded by 7 days of ambulatory actigraphy.

Setting: Chronobiology laboratory.

Participants: Twenty-four healthy young subjects: 12 M-types and 12 E-types selected by questionnaire. Each group included 6 men and 6 women.

Interventions: None

Measurements and Results: PSG night of 8-hour duration was recorded according to preferred sleep schedule. Sleep stage analysis revealed that M-types and E-types did not differ in sleep architecture. However, M-type men showed more % of stage 1 sleep and lower sleep efficiency than E-type men. EEG spectral analysis was conducted in NREM sleep for 6 frequency bands. M-types had more spectral power in low sigma (12-14 Hz) compared to E-types. The most robust difference between women and men was found in high sigma (14-16 Hz), and was not present between chronotypes. The decay rate of SWA (1-5 Hz) tended to be faster in M-types compared to E-types ($p = 0.06$). This rate was almost identical for women and men.

Conclusions: These results agree with the hypothesis that homeostatic sleep regulation differs between M-types and E-types, M-types showing indications of a higher rate of dissipation of sleep pressure during the night. Morningness-eveningness seems to affect sleep in a gender-specific manner, with men being more affected by their chronotype.

Key Words: morningness-eveningness, sleep, spectral analysis, circadian rhythms, human, gender difference, sleep regulation

INTRODUCTION

Circadian types, also called "chronotypes", classify individuals according to their preferred timing for activity and sleep, morning types (M-types) having an earlier sleep/wake schedule compared to evening types (E-types).[1,2,3,4] This individual difference has been linked to the entrained phase of the circadian pacemaker, which is earlier in M-types than in E-types, as estimated with temperature or melatonin rhythms.[3,4,5,6,7]

According to current models, sleep regulation depends on circadian and homeostatic processes.[8,9] A precise interaction between those two processes is necessary to ensure an optimal quality of both sleep and vigilance.[10] The circadian process (Process C) originates from the main biological clock, the suprachiasmatic nucleus of the hypothalamus. It generates a circadian rhythm of sleep/wake propensity.[10,11] Therefore, an earlier endogenous circadian phase, as found in M-types, will produce an earlier tendency for bedtime and for wake time. The circadian process also influences sleep onset, sleep duration, sleep architecture, and sleep EEG.[11,12,13,14] The homeostatic process (Process S) refers to the homeostatic sleep pressure that increases exponentially during wakefulness and decreases in the same way during sleep. The dynamics of process S is reflected by the propensity of slow wave sleep (SWS, sleep stages 3 and 4) and, more precisely, by the variation in the spectral power of slow-wave activity (SWA, 0.75-4.5 Hz) in the sleep EEG. Both SWS and SWA increase with increased homeostatic sleep pressure.[15,16] Other low frequency activities in the delta (0.75-3.5 Hz) and theta (4-8 Hz) range also express a similar influence.[14,16]

Sleep regulation may differ between M-types and E-types, but information on this topic is presently relatively scarce. Studies on sleep architecture gave conflicting results. Some found no difference between M-types and E-types,[17] whereas others found indications of better sleep quality in M-types[18,19,20,21] or in E-types.[19,22] Some differences in REM sleep latency or duration were also reported.[22,23] One of the main reasons that can explain this lack of consensus is that in most studies, the subjects were not studied according to their preferred sleep schedule. Even in studies where the subjects had to maintain a regular and/or habitual sleep schedule, the subjects had to take into account the timing of school or work obligations. The imposition of a sleep schedule too early or too late in regard to the

preferred sleep schedule modifies the position of the sleep episode in relation to circadian phase. This is likely to affect sleep architecture, especially sleep latency, REM sleep propensity, sleep duration and sleep consolidation.

Only one group studied quantitative sleep EEG in chronotypes.[2,24] This group found no difference in delta activity at the beginning of the night, but reported a difference in the time course of delta and theta activity between M-types and E-types. Compared to M-types, E-types tended to show a smaller decrease in the spectral power of these two EEG frequencies between the first and the second NREM/REM sleep cycles. These data support the hypothesis of a difference in the rate of dissipation of homeostatic sleep pressure between M-types and E-types. Studies on waking EEG also support the proposition of a different time course of the homeostatic process in M-types and E-types. Ehlers et al.[25] found that morningness was associated with more delta power before sleep onset, which is consistent with a higher level of homeostatic sleep pressure in M-types at bedtime. Finally, a recent study revealed that M-types have a faster increase in theta-alpha activity (6.25-9 Hz) during the daytime compared to E-types,[26] also suggesting a difference in the build-up of homeostatic pressure between the two chronotypes.

Women seem to have circadian characteristics similar to those of M-types. Many studies have reported an advanced sleep schedule[27,28,29] and an earlier circadian phase[6,30] in women compared to men. Women also show greater morningness tendencies on morningness/eveningness (M/E) scales.[27,29,31] In our previous analyses on circadian phase and sleep-wake cycle, we observed that women not only have an earlier circadian phase, but that they also show a longer phase angle between melatonin onset and the sleep episode compared to men.[4] We found a similar difference in phase angles between M-types and E-types when we compared chronotypes with the earliest and the latest circadian phases.[4] It is therefore possible that some differences in sleep regulation expected in M-types compared to E-types can also be observed in women compared to men. Some sleep studies have noted more SWS in women compared to men,[22,32] but most work found no difference in sleep stages.[33,34,35,36,37] However, quantitative EEG analysis in NREM sleep revealed more spectral power in delta, theta and sigma in women compared to men.[32,33,34,36,37,38,39] Concerning the time course of SWA, three studies found no gender difference[33,36,37] but another reported a larger decrease in delta power between the two halves of the night in

women compared to men.[35] Although no consensus has been reached at the present time on the interpretation of those gender differences in regard to the mechanisms of sleep regulation, it is of interest to examine them in association with morningness-eveningness.

This paper presents sleep architecture and quantitative sleep EEG in normal young adults in the context of chronotype and gender. Subjects were studied when they were allowed to follow their preferred sleep schedule, free from most social and work constraints. In those conditions, we expected to observe sleep characteristics when circadian and homeostatic influences are in a stable relationship. The aim was to verify if differences in sleep regulation between M-types and E-types would be present in those stable conditions and if women and M-types would show similar results in contrast to men and E-types.

METHODS

Subjects

Results presented in this paper come from a larger study on sleep regulation in morningness-eveningness. More details on subjects' recruitment, sleep-wake cycle and circadian phase can be found in our previous publication.[4] M-type and E-type participants (19 to 34 y) were recruited using a French version of the Morningness-Eveningness Questionnaire (MEQ) of Horne and Östberg.[40] Twenty-four subjects completed the study: 12 M-types (MEQ scores 59 to 71) and 12 E-types (MEQ scores 27 to 40). There were 6 women and 6 men in each group. Age was similar in the two groups (M-types: 24.7 y ± 1.5; E-types: 23.4 y ± 0.7). All subjects were in good physical and psychological health, as assessed with questionnaires and interview, and had no sleep complaints. Subjects who reported having more than 3 drinks of alcohol per week or drinking more than 2 caffeinated beverages per day were excluded from the study. Selected subjects had a regular sleep schedule with an habitual sleep duration between 7 and 9 h. A 24-h laboratory screening confirmed the absence of sleep and vigilance disorder by polysomnography and a multiple sleep latency test (MSLT).[41] Inclusion criteria were: sleep efficiency higher than 85 %, sleep latency lower than 30 min, apneas/hypopneas index and periodic leg movements index lower than 5 per hour of sleep, and mean sleep latency on the MSLT higher than 7 min. Subjects had no night work experience in the past year and no transmeridian travel in

the past three months. They were all non-smokers and reported not using drugs or medications, except oral contraceptives. Women not using hormonal contraception (3 M-types and 4 E-types) were studied during the follicular phase of their menstrual cycle. Each subject signed an informed consent form approved by the hospital ethics committee and received a monetary compensation.

Procedures

For the study, individual sleep schedules were determined according to each subject's preferred bedtime and wake time using information from screening sleep diaries during free days and preferred wake time and bedtime reported in the MEQ. The final decision for the study sleep schedule was made after discussion with the subject to ensure that it was as close as possible to the schedule that he/she would spontaneously adopt. Bedtime and wake time were determined for sleep duration of 8 hours. Subjects were requested to adopt their selected bedtime and wake time (± 30 min) for 7 days prior to laboratory admission. Compliance was verified by sleep diaries and by 24-h ambulatory measures of activity and light exposure (Actiwatch-L, Mini-Mitter Co, Bend, OR). During the study, subjects were asked to abstain from alcohol, caffeine, drugs and medications.

The selected bedtime and wake time used during the week of ambulatory recordings were also used in the laboratory, but with a fixed duration of 8 hours. Subjects were admitted to the laboratory 4 h before their scheduled bedtime and slept two nights according to their individual sleep schedule. Subjective sleep quality was assessed in the morning using a five-point scale. Sleep episodes were recorded with 25 EEG, 2 EOG and 3 chin EMG electrodes, using a referential montage with linked-ears. Signals were recorded using a polygraph Grass Model 15A54 amplifier system [Astro-Med Inc., West-Warwick, USA; gain 10000, bandpass 0.3 (effective roll-off –12 dB/octave, fall time constant 250 ms)-100 Hz (effective roll-off -24 dB/octave, rise time constant 3.5 ms)] and digitized at a sampling rate of 256 Hz with a commercial software (Harmonie 5.1, Stellate Systems, Montreal, Canada). A 60-Hz notch filter was applied during data acquisition. The first night was for adaptation and the second night was used for sleep analysis.

Circadian phase was estimated using two markers: core body temperature minimum (T_{min}) and dim light melatonin onset (DLMO). Technical details can be found in Mongrain et al.[4]

Briefly, rectal temperature recording started on admission and continued for the next 26 h including the adaptation night, using a portable monitor (Mini-Logger, Mini-Mitter Co., Bend, OR). T_{min} was estimated by cosinor analysis.[42] Temperature data are missing for two M-type men. To determine DLMO, 11 saliva samples were collected every half-hour on the day following the adaptation night, starting 5 h before scheduled bedtime. Saliva was collected in dim light (< 15 lux) using the Salivette devices (Sarstedt Inc). Melatonin concentration in the saliva was determined by radioimmunoassay (RIA) using commercial kits (ALPCO, Windham, NH, with reagents from Bühlmann Laboratories, Switzerland). DLMO was found by interpolation, the onset being defined as twice the minimum detection limit of the assay (i.e., 1.3 pg/ml) when followed by a further increase in melatonin concentration.[43]

Sleep Data Analysis

Sleep stages were visually scored from the C3 derivation, on 20-sec epochs, according to standard procedures.[44] NREM/REM sleep cycles were determined according to Feinberg and Floyd criteria.[45] Sleep latency was defined as the time from lights off to the first min of stage 1 or to the first epoch of any other sleep stage. Sleep period was the time from sleep onset to the final awakening. Sleep efficiency was the time spent asleep (including stage 1) divided by the sleep period, multiplied by 100. Spectral analysis was performed on the C3 derivation with a commercial software package (Sensa, Stellate Systems, Montreal, Canada). EMG artifacts were automatically detected[46] and further artifacts were identified by visual inspection. Spectral power was obtained by fast Fourier transforms (FFT) performed on 4-sec artifact-free sections using a cosine window tapering, with a 0.25 Hz spectral resolution. Spectral power in NREM sleep (stages 2, 3 and 4) was averaged for six frequency bands: slow-wave activity (SWA, 1-5 Hz), theta (4-8 Hz), alpha (8-12 Hz), low sigma (12-14 Hz), high sigma (14-16 Hz), and beta (16-24 Hz).

Statistical Analysis

Group-by-Gender ANOVAs were used for the statistical analyses of circadian phases, subjective sleep quality, sleep architecture variables and all-night EEG power spectra. Changes in absolute power spectra in each EEG frequency band were evaluated in NREM sleep (stages 2-3-4) over the first four NREM/REM sleep cycles of the night using Group-by-Gender-by-Cycle ANOVAs. Huynh/Feldt corrections were used to adjust significance

levels for repeated measures but the original degrees of freedom are reported. Significant interactions were decomposed using simple effect analysis. Statistical significance was set to 0.05. To further investigate the nighttime course of SWA, a nonlinear regression analysis was performed on relative data. The mean value of SWA within each cycle was expressed as the percentage of all-night SWA. The time of the midpoint within each cycle was determined for each subject and used as the independent variable. An exponential decay function was fitted to the data: $SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$, with t = time of the cycle midpoint, SWA_t = SWA activity averaged per cycle, $SWA\infty$ = horizontal asymptote for t = ∞, SWA_0 = intercept of the y axis if $SWA\infty$ were zero and r = slope of the decay.[33,47] Estimated values of $SWA\infty$, SWA_0 and r were compared using 95% confidence intervals. Individual estimates of r were also calculated, log-transformed to homogenize the variances and compared using Group-by-Gender ANOVA.

RESULTS

Circadian parameters

Mean (± SEM) MEQ scores were 65.9 (± 1.1) in M-types and 32.7 (± 1.2) in E-types. MEQ scores were similar between women and men: 49.2 (± 4.9) in women and 49.5 (± 5.4) in men. As reported previously[4], DLMO was more than 2.5 h earlier in M-types compared to E-types (20:41 ± 27' vs. 23:23 ± 25'; $F_{(1,20)}$=26.1, p<0.001) and 1.5 h earlier in women compared to men (21:14 ± 30' vs. 22:49 ± 39'; $F_{(1,20)}$=8.9, p<0.01). T_{min} was 2 h earlier in M-types compared to E-types (04:17 ± 23' vs. 06:17 ± 29'; $F_{(1,18)}$=10.1, p<0.01) but the difference between women and men was not statistically significant (04:48 ± 32' vs 06:04 ± 28', respectively; $F_{(1,18)}$=2.8, p=0.1). On average, elapsed time from DLMO to laboratory wake time (phase angle) was similar between M-types and E-types (10:27 ± 85' vs. 10:22 ± 61', p=0.9). However, phase angle was more than 1 h longer in women compared to men (10:58 ± 46' vs. 9:51 ± 80'; $F_{(1,20)}$=5.8, p=0.02). Phase angles calculated between T_{min} and wake time were not significantly different between M-types and E-types (2:53 ± 60' vs. 3:28 ± 66'. p=0.2) or between women and men (3:24 ± 67' vs. 2:58 ± 62'. p=0.3). There was no significant Group-by-Gender interaction for any circadian parameter, including circadian phases and phase angles.

Sleep schedule and sleep stages

Characteristics of sleep schedules and sleep stages for M-types, E-types, men and women are shown in Table 1, as well as probability results of Group-by-Gender ANOVAs. Characteristics of the preferred sleep schedules of the two groups during the week of actigraphic recordings were reported in a previous publication.[4] Laboratory sleep schedules were more than 2.5 h earlier in M-types compared to E-types, but they were similar in men and women. Subjective sleep quality tended to be better for M-types compared to E-types but did not differ between men and women. Sleep and REM latencies as well as total sleep time, sleep efficiency, duration and percentages of each sleep stage were all similar for M-types and E-types and for men and women. There was a significant Group-by-Gender interaction for sleep efficiency (Figure 1A; $F_{(1,20)}=7.7$, $p=0.01$), showing that M-type men had poorer sleep efficiency than E-type men (simple effect analysis: $p=0.03$) whereas there was no difference between chonotypes in women. We found a similar interaction for percentage of stage 1 sleep (Figure 1B; $F_{(1,20)}=4.2$, $p=0.05$), showing that M-type men had a greater percentage of stage 1 than E-type men (simple effect analysis: $p=0.04$) but that there was no difference in women. A similar trend was observed for the number of minutes of stage 1 sleep.

All night EEG power spectra

Group-by-Gender ANOVAs performed on results averaged over EEG frequency bands revealed a main Group effect in low sigma ($F_{(1,20)}=6.4$, $p=0.02$) with more power in M-type subjects. There were significant Gender effects in theta ($F_{(1,20)}=5.2$, $p=0.03$) and high sigma ($F_{(1,20)}=10.6$, $p<0.01$), women having more power than men. Finally, a significant Group-by-Gender interaction was found in the beta EEG frequency band ($F_{(1,20)}=4.8$, $p=0.04$), which showed that M-type men had more power than E-type men (simple effect analysis: $p=0.02$), while there was no difference between chronotypes in women (Figure 1C).

Time course of power spectra in the first four NREM/REM cycles

As shown in Table 2, cycle length and duration of NREM sleep episodes were equivalent between groups and between men and women. Power spectra averaged within the first 4 NREM/REM sleep cycles were compared using Group-by-Gender-by-Cycle ANOVAs for each EEG frequency bands. Significant results are summarized in Table 3. The main Group effect in low sigma was the same as the one found in all-night analyses, with more power in M-types compared to E-types. A tendency for a main Gender effect in low sigma revealed that women tended to have more activity than men. A significant Group-by-Cycle interaction was found in the theta band and tended to be significant for SWA. As illustrated in Figure 2A, there was no significant difference between M-types and E-types for any cycle, but simple effect analysis revealed a tendency for M-types to have more SWA than E-types in the first two cycles ($p=0.1$ for both). The initial SWA decrease was more accentuated in M-types compared to E-types. In M-types, there was a significant decrease from cycle 1 to cycle 2 and from cycle 2 to cycle 3 ($p<0.001$). In E-types, the decrease from cycle 1 to cycle 2 was statistically significant ($p<0.05$) but only a trend ($p<0.08$) was found in the decrease from cycle 2 to cycle 3 and from cycle 3 to cycle 4. Results were similar in the theta band (Figure 2B), with no significant difference between the two groups in the first sleep cycle ($p=0.2$). Theta activity decreased in M-types from cycle 1 to cycle 2 and from cycle 2 to cycle 3 ($p<0.001$). In E-types, it decreased significantly from one cycle to the other from cycle 1 to cycle 4 ($p<0.01$). We found significant Gender-by-Cycle interactions in SWA, theta and high sigma frequency bands. As shown in Figure 3, women tended to have more spectral power in these frequency bands compared to men, but the extent of this difference varied according to the NREM/REM sleep cycle. In women, SWA decreased from cycle 1 to cycle 2 and from cycle 2 to cycle 3 (simple effect analysis: $p<0.001$) while in men, the decrease was significant only from cycle 1 to cycle 2 and from cycle 3 to cycle 4 ($p<0.05$). Theta activity decreased from cycles 1 to cycle 2 and from cycle 2 to 3 in women ($p<0.0001$) and between each of the four cycles in men ($p<0.01$). High sigma activity decreased from cycle 1 to cycle 2 in women and increased afterwards (cycle 2 to cycle 3 and cycle 3 to cycle 4, $p<0.01$). In men, high sigma activity decreased from cycle 1 to cycle 2 ($p<0.01$) but did not change significantly in the following two cycles. There was no significant Group-by-Gender or Group-by-Gender-by-Cycle

interaction. The beta band was the only one not showing any significant changes over the 4 cycles.

Nonlinear regression analysis of all night SWA

Estimates of the parameters given by the exponential decay fits are shown in Table 4. The $SWA_{0,}$ $SWA\infty$ and r estimates did not differ significantly between groups as reflected by their overlapping confidence intervals. Women had a higher SWA_0 than men (nonoverlapping confidence intervals), but a similar r and a similar $SWA\infty$ (almost complete overlap of confidence intervals). Regression curves are illustrated in Figure 4. Group-by-Gender ANOVA on individuals r estimates revealed a trend for M-types to have higher r values than E-types ($F_{(1,20)}$= 3.9, p=0.06). No significant gender effect or interaction was observed.

DISCUSSION

In this study, M-type and E-type individuals were compared on sleep architecture and quantitative sleep EEG while taking into account possible gender differences. We did not find any main chronotype effect in the PSG recordings. Previous studies have reported mostly longer sleep latencies, shorter sleep durations and lower sleep efficiencies in E-type subjects. However, those studies often used a sleep schedule that was too early for E-types.[18,21] Even in studies in which subjects were following their habitual/regular sleep schedule, that schedule was not necessarily free from school/work influences.[19,22] A sleep schedule that is too early compared to the spontaneous sleep timing is expected to lengthen sleep latency and to increase both the number of awakenings and the occurrence of stage 1 sleep.[10,11] The only group that reported an absence of difference between M-types and E-types allowed their subjects to choose their wake time and bedtime.[17] On average, our M-types and E-types showed the same phase angle between their endogenous circadian rhythms and their sleep-wake schedule. It was therefore expected that their sleep architecture, especially sleep latency and REM sleep propensity, would not be differentially affected by their circadian phase.

Similarly to most previous studies,[33,34,35,36,37] we found few differences between men and women in sleep architecture. Men had more minutes of stage 1 sleep compared to women. However, a significant interaction between chronotype and gender was predominant for sleep efficiency and percentage of stage 1 sleep. No significant difference was found between the two chronotypes in women but, compared to E-type men, M-type men spent a larger proportion of their sleep episode awake or in the lightest sleep stage. This result can be related to the similar Group-by-Gender interaction found in the beta range with the quantitative analysis of all-night EEG. That analysis showed lower spectral power in the 16-24 Hz frequency band in E-type men compared to M-type men. Elevated fast-frequency activity during sleep has been interpreted as an indicator of cortical arousal.[48,49] Altogether, these results suggest that E-type men slept more soundly than their M-type counterparts. However, M-type subjects did not complain of decreased subjective sleep quality. One possible interpretation could be that sleep need, defined here as the sleep duration spontaneously needed to feel refreshed, differs between M-type and E-type men. Associations between morningness and shorter sleep duration have already been reported.[29,50] During subjects' recruitment for the present study, volunteers with short (< 7 h) or long (> 9 h) habitual sleep duration were specifically excluded from the study. Only E-type volunteers had to be excluded because of an habitual sleep duration longer than 9 hours, suggesting a tendency for a longer sleep duration in E-types compared to M-types. During the week of ambulatory recordings, the subjects had to follow their self-selected 8-h sleep schedule ± 30 min for bedtime and waketime, thus allowing for a possible sleep duration between 7 and 9 hours. During that week, M-type men were inclined to sleep less (m ± SEM: 7.60 ± 0.21 h) compared to E-type men (7.94 ± 0.37 h), M-type women (7.97 ± 0.21 h) and E-type women (8.12 ± 0.16 h). Therefore, contrary to the 3 other sub-groups who had an averaged sleep duration close to 8 hours, M-type men spontaneously slept about 24 minutes less. It is known that sleep extension decreases sleep efficiency.[51,52] In the laboratory M-type men slept for a longer duration than what they usually do, and this might have decreased their sleep consolidation. This effect was statistically significant but very small since M-type men still achieved an averaged sleep efficiency of 94.6%, not clinically different from the 96.8% observed in E-type men. Apparently, this small decrease was not sufficient to decrease subjective sleep quality.

As illustrated in Figure 2, we found a trend for a Group-by-Cycle interaction for SWA, which was statistically significant for theta activity. However, there was no significant difference between M-types and E-types in SWA ($p=0.1$) or theta activity ($p=0.2$) in the first NREM/REM sleep cycle, similar to the results reported for delta activity in the Kerkhof's study[2] ($p<0.1$). Group differences were essentially in the changes between successive sleep cycles. Nonlinear regression analyses performed on relative data revealed a difference close to statistical significance ($p=0.06$) in the slope of the SWA decay between M-types and E-types, showing that SWA tended to decrease faster in the former than in the latter. This observation can also be viewed in the light of recent results on the time course of EEG theta-alpha activity during wakefulness in M-types and E-types.[26] This frequency band reflects the changes in sleep pressure in the waking EEG[53,54] and it was found that M-types had a faster increase in sleep pressure during extended wakefulness, compared to E-types. Even if the build-up and the dissipation of sleep pressure are not necessarily controlled by the same mechanisms,[55,56] our results combined with those of Taillard et al.[26] suggest that homeostatic sleep regulation may differ in some ways between the two chronotypes. These differences might be related to differences in spontaneous sleep duration since, for an equivalent sleep pressure at the beginning of the night, a faster rate of dissipation would favor sleep satiation after a shorter period of time. This proposition has been ruled out in real short and long sleepers (sleep episodes <6 h and >9 h), for whom the rate of relative SWA dissipation was found to be similar.[55] However, variations of sleep length in the 7-9 h range in relation to morningness-eveningness may reflect different mechanisms. Therefore, more robust differences in the markers of homeostatic sleep regulation might have been found if no criteria for spontaneous sleep duration had been applied in our subjects' selection. Moreover, the use of EEG derivations known to be more sensitive to variations in homeostatic sleep pressure, especially the frontal ones,[57,58,59] might also increase the ability to detect group differences in future comparisons.

There were gender differences in absolute activity of slow EEG frequencies over the NREM/REM sleep cycles that were similar to the ones observed between M-types and E-types. In both cases, a significant interaction with the NREM/REM sleep cycles was found. However, contrary to the differences between chronotypes, nonlinear regression analysis on relative values revealed an almost identical rate of decrease of SWA over the night for women and men. This observation is consistent with previous reports[33,36] and suggests that

the homeostatic process of sleep regulation is similar in men and women. M-types and women were also similar for the spectral power in the low sigma range, which was higher in M-types compared to E-types and showed a similar trend ($p<0.06$) in women compared to men. The meaning of elevated low-sigma activity in M-types and in women remains unclear. This frequency band has been associated with homeostatic regulation,[14,59,60,61,62] as it decreases following sleep deprivation and increases over successive NREM/REM sleep cycles. However, we found no interaction with the sleep cycles for either chronotype or gender, which suggests that this difference does not necessarily reflect a difference in sleep regulatory mechanisms.

The most robust difference between men and women was found in the high sigma range, and it was not present between the chronotype groups. Compared to men, women showed higher spectral power in the 14-16 Hz frequency range in all-night analyses, and a significant Gender-by-Cycle interaction was found in the analyses over the first 4 NREM/REM sleep cycles. As illustrated in Figure 3, spectral power in high sigma frequency increased in women at the end of the night, which is consistent with some but not all previous reports.[35,37] This difference might be related to the fact that compared to men, women went to bed later and woke up later after their DLMO. It has been shown that spectral activity in the 13.75-15.5 Hz range is minimal when melatonin secretion is maximal, then increases afterwards.[14] Because the sleep schedule was later relatively to the circadian phase of melatonin in women, it might explain why spectral activity in high sigma increased earlier within their sleep episode. However, it is unlikely that a 1-hour difference in phase angle could entirely explain the large difference observed in high sigma activity in the present data.

Our results are consistent with the hypothesis that sleep regulation differ between M-types and E-types. M-types are showing indications of a faster dissipation rate of homeostatic sleep pressure during sleep compared to E-types. Our results also suggest that the morningness-eveningness dimension influences sleep in a gender-specific manner, men being more affected by their circadian typology. Although similarities were found in the sleep of women and M-types, our results do not support the hypothesis of similar characteristics of sleep regulation in these groups. Future investigations on the relationship

between morningness-eveningness and spontaneous sleep duration may help to unravel possible links between circadian typology and mechanisms of sleep regulation.

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FIGURE LEGENDS

Figure 1 : Illustration (means and SEM) of significant Group-by-Gender interactions found for A) Sleep efficiency, B) Percentage of stage 1 sleep, and C) All-night beta activity. Simple-effect analyses showed significant differences between M-type and E-type men, but not between the two chronotypes in women.

Figure 2 : Illustration (means and SEM) of significant Group-by-Cycle interactions in NREM sleep spectral power averaged over the first four NREM/REM sleep cycles. A) Slow Wave Activity (1-5 Hz); B) Theta activity (4-8 Hz).

Figure 3 : Illustration (means and SEM) of significant Gender-by-Cycle interactions in NREM sleep spectral power averaged over the first four NREM/REM sleep cycles. A) Slow Wave Activity (1-5 Hz); B) Theta activity (4-8 Hz); C) High sigma activity (14-16 Hz). Significant differences between men and women from simple effect decomposition of Gender-by-Cycle interactions: * $p \leq 0.05$; ** $p \leq 0.01$.

Figure 4 : Exponential decay function adjusted on relative NREM sleep SWA for all night cycles. Exponential decay fits: $SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$ (with 95% confidence intervals), A) M-types data; 61 cycles; B) E-types data; 69 cycles, C) Women data (64 cycles) and, D) Men data (66 cycles).

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Table 1 : Sleep schedule and sleep stages (mean ± SEM) for M-type and E-type individuals, and for men and women. Probabilities from Group-by-Gender ANOVAs are also indicated.

		Groups		Genders		p		
		M-types (n=12)	E-types (n=12)	Women (n=12)	Men (n=12)	Group effect	Gender effect	Interaction
Bedtime (clocktime, h:min)		23:08 ± 11'	01:45 ± 17'	00:12 ± 25'	00:40 ± 30'	<0.001	ns	ns
Subjective sleep quality		4.79 ± 0.1	4.25 ± 0.25	4.75 ± 0.1	4.29 ± 0.2	0.06	ns	ns
Sleep latency (min)		6.7 ± 0.8'	7.4 ± 1.5'	6.5 ± 1.3’	7.7 ± 1.0’	ns	ns	ns
REM latency (min)		70.7 ± 10’	66.8 ± 3’	74.4 ± 9.7’	63.1 ± 3.3’	ns	ns	ns
Total sleep time (min)		449.9 ± 4.5'	451.6 ± 3.0'	451.6 ± 4.7’	449.9 ± 2.7’	ns	ns	ns
Sleep efficiency (%)		95.9 ± 0.6	96.2 ± 0.6	96.5 ± 0.6	95.7 ± 0.4	ns	ns	0.01
Stage 1 sleep	min	27.1 ± 4.8’	22.5 ± 2.4’	19.0 ± 2.5’	30.7 ± 4.2’	ns	0.02	0.06
	%	6.1 ± 1.1	5.0 ± 0.6	4.2 ± 0.6	6.8 ± 0.9	ns	0.02	0.05
Stage 2 sleep	min	264.3 ± 7.1’	272.8 ± 7.9’	262.7 ± 7.4’	274.4 ± 7.4’	ns	ns	ns
	%	58.7 ± 1.4	60.4 ± 1.8	58.2 ± 1.5	61.0 ± 1.6	ns	ns	ns
Slow-wave sleep	min	42.4 ± 9.8’	38.2 ± 8.0’	48.0 ± 9.3’	32.6 ± 7.9’	ns	ns	ns
(stagse 3 and 4)	%	9.4 ± 2.1	8.4 ± 1.7	10.6 ± 2.0	7.2 ± 1.7	ns	ns	ns
REM sleep	min	116.1 ± 4.7’	118.1 ± 5.8’	121.9 ± 6.4	112.3 ± 3.4’	ns	ns	ns
	%	25.9 ± 1.1	26.1 ± 1.3	27.0 ± 1.4	25.0 ± 0.8	ns	ns	ns

Table 2 : Durations of sleep cycles and NREM/REM sleep episodes (mean ± SEM) for M-type and E-type individuals, and for men and women.

	Groups		**Genders**	
	M-types (n=12)	E-types (n=12)	Women (n=12)	Men (n=12)
Cycle 1 duration (min)	80.6 ± 11.0	79.9 ± 4.0	89.4 ± 10.3	71.1 ± 3.2
Cycle 2 duration (min)	109.1 ± 6.3	111.5 ± 8.5	113.7 ± 7.8	106.9 ± 7.0
Cycle 3 duration (min)	104.3 ± 7.1	103.8 ± 6.9	102.9 ± 5.6	105.1 ± 8.2
Cycle 4 duration (min)	104.7 ± 6.3	90.9 ± 6.1	97.3 ± 6.8	98.3 ± 6.3
NREM sleep episode 1 (min)	67.1 ± 10.1	64.5 ± 2.9	71.6 ± 9.7	60.0 ± 3.2
NREM sleep episode 2 (min)	76.7 ± 4.0	74.3 ± 4.9	76.5 ± 4.2	74.5 ± 4.7
NREM sleep episode 3 (min)	69.1 ± 4.0	69.8 ± 5.0	66.2 ± 4.8	72.6 ± 4.0
NREM sleep episode 4 (min)	58.7 ± 2.7	54.1 ± 4.1	57.3 ± 2.6	55.5 ± 4.2
REM sleep episode 1 (min)	13.5 ± 2.6	15.4 ± 2.1	17.9 ± 2.6	11.1 ± 1.5
REM sleep episode 2 (min)	32.4 ± 3.6	37.2 ± 7.1	37.1 ± 7.2	32.4 ± 3.5
REM sleep episode 3 (min)	35.2 ± 4.1	33.9 ± 4.4	36.7 ± 3.1	32.5 ± 5.1
REM sleep episode 4 (min)	46.0 ± 6.6	36.8 ± 3.7	40.0 ± 6.9	42.8 ± 3.6

Table 3: Significant effects of the Group-by-Gender-by-Cycle ANOVAs analysis of spectral activity in the different frequency bands. Adjusted probabilities are shown.

	Group Effects (df=1,20)	Gender effects (df=1,20)	Cycle effects (df=3,60)	Group-by-Cycle interaction (df=3,60)	Gender-by-Cycle interaction (df=3,60)
SWA (1-5 Hz)	ns	ns	F=40.1 p<0.001	F=2.8 p=0.08	F=4.3 p<0.05
Theta (4-8 Hz)	ns	ns	F=69.2 p<0.001	F=3.4 p<0.05	F=6.7 p<0.01
Alpha (8-12 Hz)	ns	ns	F=5.6 p<0.01	ns	ns
Low Sigma (12-14 Hz)	F=6.6 p<0.05	F=3.9 p=0.06	F=8.0 p<0.001	ns	ns
High Sigma (14-16 Hz)	ns	ns	F=13.8 p<0.001	ns	F=9.06 p<0.001
Beta (16-24 Hz)	ns	ns	ns	ns	ns

Table 4 : Parameters of the nonlinear regression analysis on the decay of SWA during sleep for M-types, E-types, men, and women. Values are mean and 95 % confidence intervals in parentheses. ($SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$)

	M-types	**E-types**	**Women**	**Men**
SWA∞ (%)	49.2	36.6	41.1	52.6
	(33.8 – 64.6)	(15.3 – 57.9)	(28.0 – 54.1)	(35.2 – 70.0)
SWA_0 (%)	183.3	155.7	197.0	141.0
	(155.9 – 210.7)	(137.8 – 173.6)	(176.4 – 217.6)	(120.2 – 161.8)
r (min^{-1})	0.0086	0.0052	0.0076	0.0069
	(0.0052 – 0.0120)	(0.0030 – 0.0073)	(0.0054 – 0.0099)	(0.0036 – 0.0101)
R^2	0.79	0.84	0.88	0.75

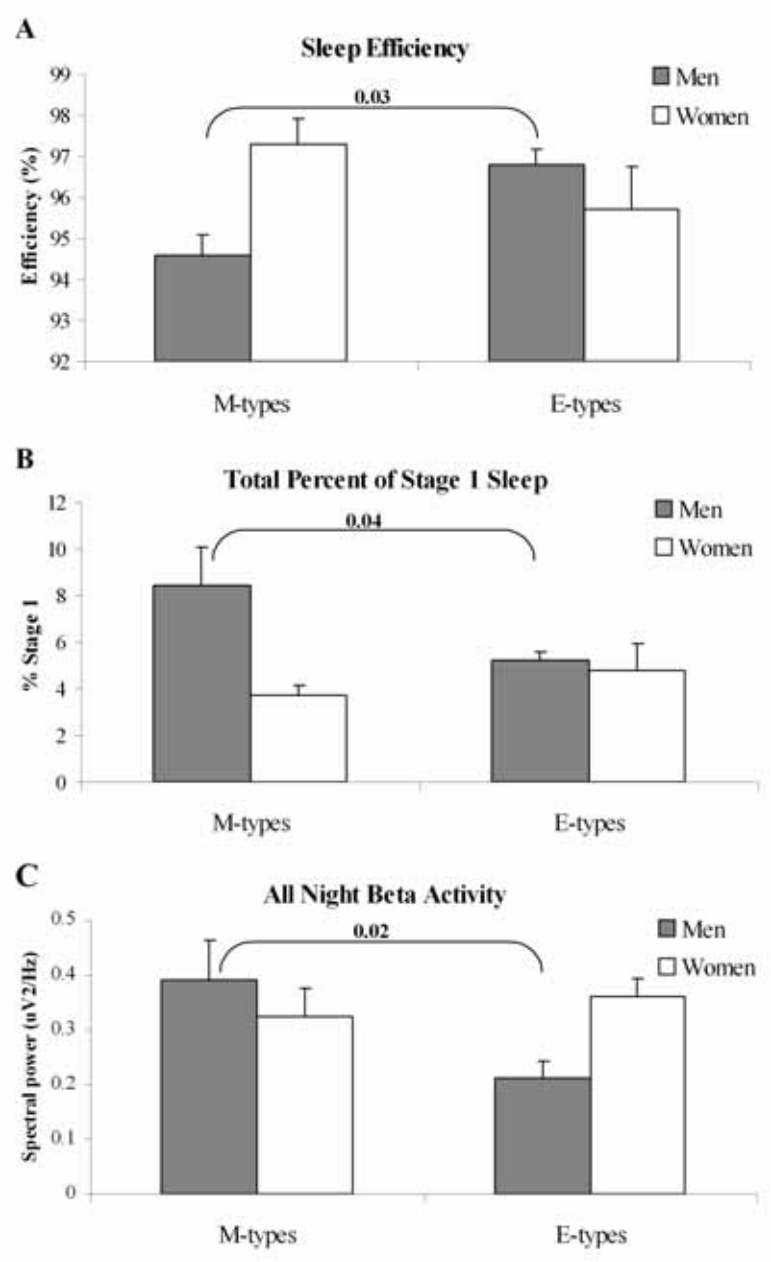
A
Sleep Efficiency
Men
Women
0.03
Efficiency (%)
99
98
97
96
95
94
93
92
M-types
E-types
B
Total Percent of Stage 1 Sleep
Men
Women
0.04
% Stage 1
12
10
8
6
4
2
0
M-types
E-types
C
All Night Beta Activity
Men
Women
0.02
Spectral power (uV2/Hz)
0.5
0.4
0.3
0.2
0.1
0
M-types
E-types

Figure 1

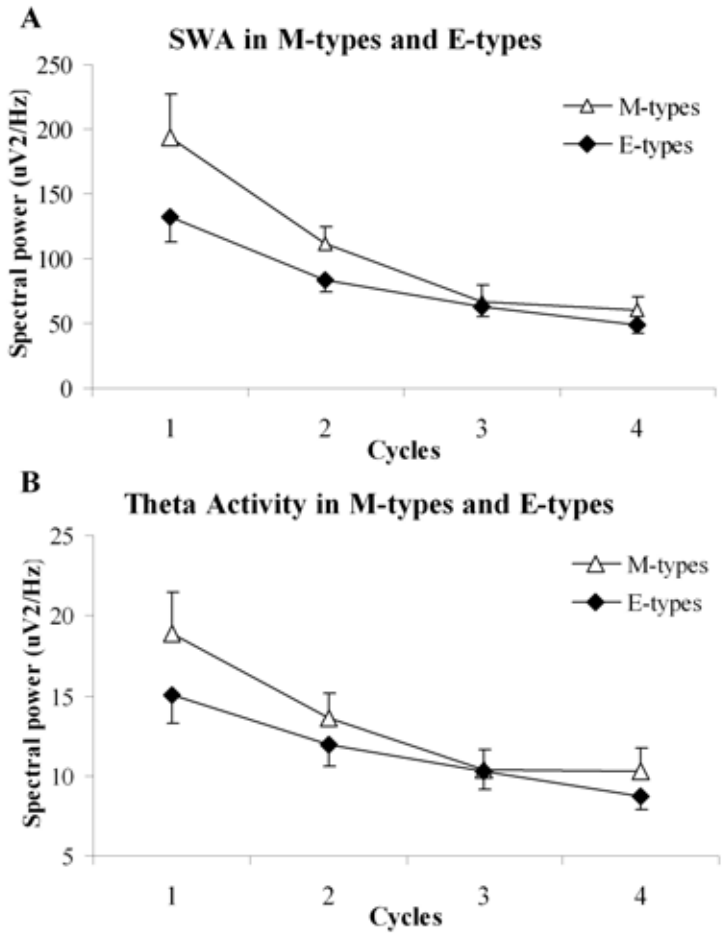
A
SWA in M-types and E-types
Spectral power (uV2/Hz)
250
200
150
100
50
0
M-types
E-types
1
2
3
4
Cycles
B
Theta Activity in M-types and E-types
Spectral power (uV2/Hz)
25
20
15
10
5
M-types
E-types
1
2
3
4
Cycles

Figure 2

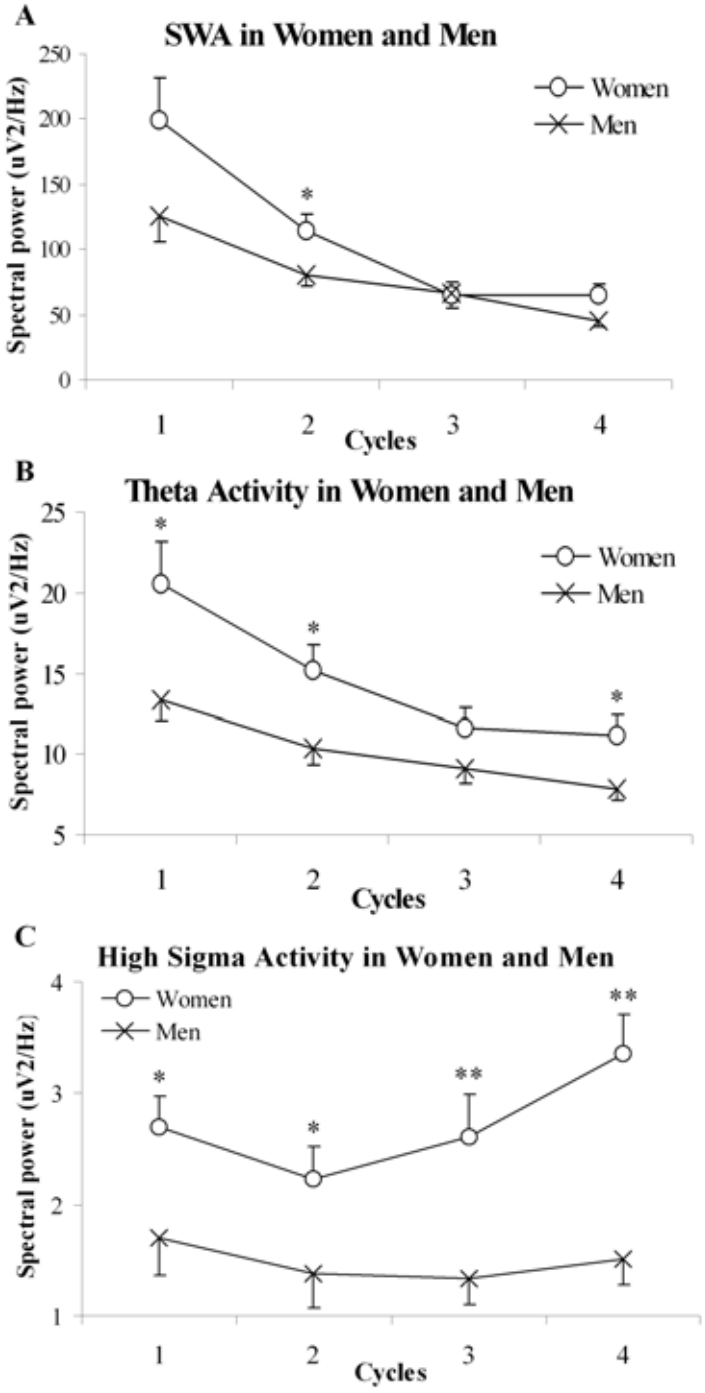
A
SWA in Women and Men
Spectral power (uV2/Hz)
250
200
150
100
50
0
Women
Men
*
1
2
3
4
Cycles
B
Theta Activity in Women and Men
Spectral power (uV2/Hz)
25
20
15
10
5
Women
Men
*
*
*
1
2
3
4
Cycles
C
High Sigma Activity in Women and Men
Spectral power (uV2/Hz)
4
3
2
1
Women
Men
*
*
**
**
1
2
3
4
Cycles

Figure 3

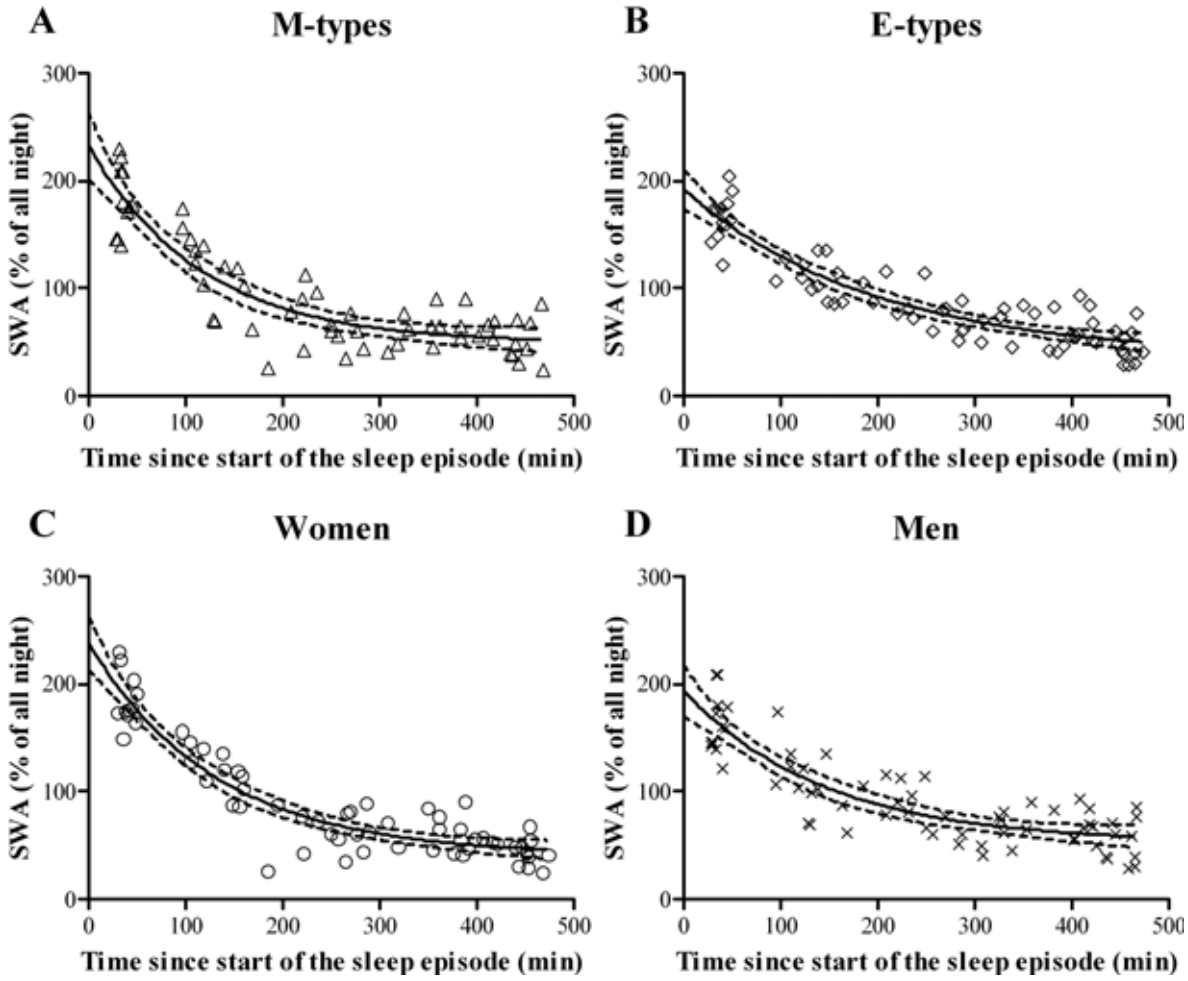
A
M-types
B
E-types
C
Women
D
Men
SWA (% of all night)
Time since start of the sleep episode (min)
0
100
200
300
400
500

Figure 4

4.3 Troisième article

Differences in sleep regulation between morning and evening circadian types as indexed by antero-posterior analyses of the sleep EEG

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DIFFERENCE IN SLEEP REGULATION BETWEEN MORNING AND EVENING CIRCADIAN TYPES AS INDEXED BY ANTERO-POSTERIOR ANALYSES OF THE SLEEP EEG

Valérie MONGRAIN[1,2], Julie CARRIER[1,3] and Marie DUMONT[1,4]

[1]Chronobiology Laboratory, Sacré-Cœur Hospital of Montréal, Québec, Canada, H4J 1C5; [2]Department of Neurosciences, [3]Department of Psychology and [4]Department of Psychiatry, University of Montréal, Québec, Canada, H3C 3J7.

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Running title: Sleep EEG topography in chronotypes

Corresponding author: Marie Dumont, Ph.D.
Chronobiology Laboratory
Sacré-Cœur Hospital
5400 blvd Gouin W.
Montréal (Québec)
Canada, H4J 1C5
Tel: (514) 338-2222 ext. 2246
Fax: (514) 338-2531
Email: marie.dumont@umontreal.ca

Keywords: morningness-eveningness, EEG spectral analysis, circadian rhythms, sleep, interindividual differences

ABSTRACT

Circadian types classify individuals according to their preferred timing for activity and sleep, morning and evening types showing respectively early or late preferences. This characteristic has been associated with corresponding differences in circadian sleep propensity. In this study, quantitative analysis of the sleep EEG in antero-posterior derivations was used to test the hypothesis that morning and evening types differ not only in the circadian aspect of sleep regulation, but also in the homeostatic aspect. Morning types and evening types (6 men and 6 women per group, aged 19-34 y.) were selected using the Morningness-Eveningness questionnaire. They were studied by polysomnography according to their preferred sleep schedule. Spectral activity in 4 midlines derivations (Fz, Cz, Pz, Oz) was calculated separately in non-rapid-eye movement (NREM) sleep and in rapid-eye-movement (REM) sleep. In NREM sleep, morning types showed a steeper decrease of slow-wave activity (SWA: 1-5 Hz) per sleep cycle in the fronto-central derivations and a steeper increase in 13-14 Hz activity in the parieto-occipital derivations compared to evening types. Nonlinear regression analysis revealed that the exponential decay rate of relative values of SWA in NREM sleep was faster in morning than evening types, in the frontal derivation. In REM sleep, morning types showed a steeper decrease of high sigma (14-16 Hz) and beta (16-24 Hz) activities across the night in centro-parietal derivations compared to evening types. These results show for the first time a clear difference between morning types and evening types in homeostatic sleep regulation.

INTRODUCTION

Circadian type, or chronotype, refers to the individual preference in the timing for activity and sleep, with about 60% of the people being of an intermediate type and extremes being classified as morning or evening types (Posey & Ford, 1981). Compared to evening types, morning types go to sleep and wake up about 2 hours earlier (Kerkhof, 1991; Natale & Cicogna, 2002). It is now well accepted that both the circadian timing system (CTS) and sleep homeostasis contribute to the regulation of sleep-wake behavior. Sleep homeostasis adjusts levels of sleep propensity and sleep intensity according to the duration of time awake, whereas the CTS gates the occurrence of daily phases of higher and lower sleep propensity (Borbély, 1982; Daan *et al*., 1984). Studies have shown repeatedly that the signal from the CTS is earlier in morning types compared to evening types (Duffy *et al.*, 1999; Liu *et al.*, 2000; Baehr *et al.*, 2000; Mongrain *et al.*, 2004). The possibility of a difference in homeostatic sleep regulation between chronotypes has also been proposed. In NREM sleep, the dissipation of homeostatic pressure is reflected by the diminution of spectral power in low frequencies across the night (Beersma, 1998). One study on quantitative sleep EEG in chronotypes reported that morning types tended to have a larger decrease in delta and theta activity across the first sleep cycles compared to evening types (Kerkhof, 1991; Lancel & Kerkhof, 1991). We also recently reported a tendency for a faster decay rate of slow wave activity (SWA, 1-5 Hz) during sleep in morning than in evening types (Mongrain *et al*., 2005).

Those previous results were obtained exclusively from the central derivations of the sleep EEG. However, other EEG derivations are known to be more sensitive to changes in frequency activity associated to homeostatic pressure. For instance, the increase of SWA with enhanced time awake is predominant in fronto-central derivations (Cajochen *et al*., 1999; Finelli *et al*., 2000, 2001a, b; Knoblauch *et al*., 2002). It is therefore likely that differences in markers of homeostatic sleep regulation between morning and evening types could be revealed more clearly in anterior compared to posterior derivations. Indications of homeostatic sleep regulation can also be identified in REM sleep EEG **as** SWA and theta activity also decrease across the night and increase with increasing time awake (Borbély *et*

al., 1981; Dijk *et al*., 1990a, b, 1997; Aeschbach & Borbély, 1993; Merica & Blois, 1997). However, spectral analysis of REM sleep EEG has never been reported in chronotypes.

In this paper, we sought to verify whether morning-type individuals would show indications of a faster rate of dissipation of homeostatic sleep pressure compared to evening types. We hypothesized that a difference in SWA would be more easily observed using the EEG recorded in frontal derivation instead of the central one. Other potential markers of homeostatic sleep regulation were also examined in the EEG of NREM and REM sleep and compared between the two circadian types.

METHODS

Subjects

Morning-type and evening-type participants, aged 19 to 34 y, were recruited using a French version of the Morningness-Eveningness Questionnaire (MEQ) of Horne and Östberg (1976). Twenty-four subjects completed the study: 12 morning types (MEQ scores 59 to 71, mean ± SEM: 65.9 ± 1.1) and 12 evening types (MEQ scores 27 to 40, mean ± SEM: 32.7 ± 1.2). There were 6 women in each group and age was similar in the two groups (morning types: 24.7 y ± 1.5; evening types: 23.4 y ± 0.7). All subjects, mostly students during summer vacation, were in good physical and psychological health and had no sleep or vigilance complaints, as assessed by questionnaires and interviews. Selected subjects had an habitual sleep duration between 7 and 9 h and a regular sleep schedule, as measured during screening by questionnaires and 7-day sleep diaries. A 24-h laboratory screening confirmed the absence of sleep or vigilance disorders by polysomnography and a multiple sleep latency test (MSLT; Carskadon *et al*., 1986). Exclusion criteria were: sleep efficiency lower than 85 %, sleep latency longer than 30 min, apneas/hypopneas index or periodic leg movements index higher than 5 per hour of sleep, and mean MSLT sleep latency shorter than 7 min. Subjects had no night work experience in the past year and no transmeridian travel in the past three months. They were all non-smokers, light users of alcohol (< 3/week) and caffeine (< 2/day) and reported not using drugs or medications, except contraceptives. Women not using hormonal contraception (3 morning types and 4 evening types) were studied during the follicular phase of their menstrual cycle. Each subject signed

an informed consent form following the principles of the Declaration of Helsinki and approved by the Sacré-Coeur Hospital ethics committee, and received a pecuniary compensation. Data presented in this paper come from of a larger study on sleep regulation in morningness-eveningness. Detailed results on circadian phase and sleep schedule can be found in Mongrain *et al*. (2004). As reported, circadian phases of melatonin secretion and core body temperature were respectively 2.5 h and 2 h earlier in morning types compared to evening types, whereas the averaged phases angles between circadian phases and wake time were the same in the two groups.

Procedures

For the study, individual sleep schedules were determined for a sleep duration of 8 hours according to each subject's preferred bedtime and wake time. Preferred sleep time was identified using screening sleep diaries during free days and preferred sleep schedule reported in the MEQ. Final decision for the study sleep schedule was made after discussion with the subject to ensure that it was the schedule that he/she would spontaneously adopt. Subjects were requested to follow their selected bedtime and wake time (± 30 min) for 7 days prior to laboratory admission. Compliance was verified by sleep diaries and by 24-h ambulatory measures of activity and light exposure (Actiwatch-L, Mini-Mitter Co, Bend, OR, USA).

Subjects were admitted to the laboratory 4 h before bedtime and slept two nights according to their individual sleep schedule. The first night was for adaptation and the second night was used for sleep analysis. During the study, subjects were asked to abstain from alcohol, drugs and medications. Sleep episodes were recorded with 25 EEG, 2 EOG and 3 chin EMG electrodes, using a referential montage with linked-ears. Signals were recorded using a polygraph Grass Model 15A54 amplifier system (Astro-Med Inc., West-Warwick, USA; gain 10000, bandpass 0.3-100 Hz) and digitized at a sampling rate of 256 Hz by a commercial software (Harmonie 5.1, Stellate Systems, Montreal, Canada). A 60-Hz notch filter was applied during signal acquisition.

Sleep Data Analysis

Sleep stages were visually scored from the C3 derivation on 20-sec epochs according to standard procedures (Rechtschaffen & Kales, 1968). NREM/REM sleep cycles were determined according to Feinberg & Floyd criteria (Feinberg & Floyd, 1979) except that no minimum duration was required for the last sleep cycle. Abnormally long first NREM episodes, where evidence of a ''skipped'' first REM period was observed, were divided into two separate NREM episodes (Feinberg & Campbell, 2003). This procedure was used in one morning-type women for whom the first REM episode was therefore missing in further analyses. Spectral analysis was performed on the Fz, Cz, Pz and Oz derivations of the sleep EEG with a commercial software package (Sensa, Stellate Systems, Montreal, Canada). Artifacts were automatically detected (Brunner et al., 1996) and further artifacts were identified by visual inspection. In REM sleep, there was a large amount of ocular artifacts and most of the EEG recordings kept for spectral analysis came from tonic REM sleep. Spectral power was obtained by fast Fourier transforms performed on 4-sec artifact-free sections using a cosine window tapering resulting in a 0.25 Hz spectral resolution. Spectral power was calculated separately over NREM sleep (excluding stage 1) and REM sleep epochs for 24 1-Hz frequency bins (identified by their lower boundary value). Spectral power was also averaged for SWA (1-5 Hz) and higher frequency bands: theta (4-8 Hz), alpha (8-12 Hz), low sigma (12-14 Hz), high sigma (14-16 Hz), and beta (16-24 Hz) for each sleep cycle.

Statistical Analysis

All analyses were done separately for NREM and REM sleep. Spectral power averaged for all-night EEG within the 24 1-Hz bins was compared using **a** Group-by-Derivation ANOVA for each 1-Hz bin (significance levels were adjusted for multiple testing according to Benjamini & Hochberg, 1995). EEG spectral activity in the 6 larger frequency bands was analyzed over the first 4 NREM/REM sleep cycles using **a** Group-by-Derivation-by-Cycle ANOVA on absolute data for each frequency band. The first 4 sleep cycles were used because it was the minimum number of sleep cycles observed in all of the subjects. Significance levels were adjusted for repeated measures by Huynh/Feldt corrections but original degrees of freedom are reported. Significant interactions were decomposed using simple effect analysis to evaluate: 1) differences between chronotypes at each sleep cycle

and, 2) differences between chronotypes in spectral activity changes between sleep cycle 1 and each of the other three sleep cycles. To further investigate the nighttime course of SWA in NREM sleep, a nonlinear regression analysis was calculated on all-night sleep EEG. The time of the cycle midpoint was determined for each cycle of each subject and used as the independent variable. The mean SWA value of all NREM sleep epochs within each cycle was expressed as the percentage of all-night NREM sleep SWA and used as the dependent variable. An exponential decay function was fitted to the data: $SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$, with t = time of the cycle midpoint, SWA_t = SWA averaged per cycle, $SWA\infty$ = horizontal asymptote for $t = \infty$, SWA_0 = intercept of the y axis minus asymptote, and r = slope of the decay (Dijk *et al*., 1989; Besset *et al*., 1998). Group estimates of $SWA\infty$, SWA_0 and r were compared with F tests (Motulsky & Christopoulos, 2003). All results are reported as mean ± SEM.

RESULTS

Sleep schedule and polysomnographic sleep parameters

The sleep schedule chosen for the laboratory study was more than 2.5 h earlier in morning types compared to evening types (bedtime: 23:08 ± 11' vs. 01:45 ± 17', $p < 0.0001$). Sleep architecture parameters, including number of minutes and percentages of all sleep stages, sleep latency, sleep efficiency, total sleep time, and duration of sleep cycles and NREM/REM sleep episodes all were similar for morning and evening types. These results are reported in details in Mongrain *et al*. (2005).

All night EEG power spectra in NREM sleep

NREM sleep spectral powers computed for all-night EEG in evening types relatively to morning types are shown in figure 1A. Visually, spectral activity seems lower in evening types compared to morning types in a majority of 1-Hz bins, but there was no significant group effect. The only Group-by-Derivation interaction found to be statistically significant was in the 13-14 Hz bin ($F_{3,66} = 5.8$, $p < 0.05$), where evening types showed lower activity values than morning types in Pz and Oz derivations ($p < 0.01$). Derivation effects were significant in all 1-Hz bins ($p < 0.05$).

Time course of EEG power spectra across the night in NREM sleep

Significant results from the Group-by-Derivation-by-Cycle ANOVAs in NREM sleep are summarized in Table 1. There was a significant Group-by-Derivation-by-Cycle interaction in SWA. As illustrated in figure 2, morning types tended to have more spectral power than evening types in the first and second cycles, in the Fz and Cz derivations ($p \leq 0.1$). Simple effect analysis revealed that both in Fz and Cz, the decrease of SWA between cycle 1 and cycle 3 was larger in morning types than in evening types ($p < 0.05$) but no difference was found for Pz and Oz. In the low sigma range, there was a significant Group-by-Derivation interaction and a trend for a Group-by-Derivation-by-Cycle interaction ($F_{9,198} = 2.1$, $p < 0.1$). As illustrated in figure 3, morning types had more spectral power than evening types in all derivations except Fz (Cz: $p < 0.05$; Pz: $p < 0.01$; Oz: $p < 0.001$). Since the Group-by-Derivation analysis on all-night EEG was significant for the 13-14 Hz bin, a Group-by-Derivation-by-Cycle ANOVA was also performed on data from that 1-Hz bin. The three-way interaction was significant ($F_{9,198} = 2.7$, $p < 0.05$) and the pattern closely matched the one illustrated for total low sigma in figure 3. Simple effect analysis revealed that the increase in 13-14 Hz activity between cycle 1 and cycle 3 was significantly larger in morning types compared to evening types in the Pz and Oz derivations ($p < 0.05$ and $p < 0.01$ respectively), but not in Fz and Cz. Finally, significant Derivation effects and Derivation-by-Cycle interactions were found for all frequency bands ($p < 0.001$) as well as significant Cycle effects in SWA, theta, alpha, low and high sigma frequency bands.

Nonlinear regression analysis of SWA during NREM sleep

Estimates of the parameters given by the exponential decay fit are shown in table 2. In the Fz derivation, morning types had a higher r value than evening types ($F_{1,126} = 4.4$, $p < 0.05$), but a similar SWA_0 value. The same tendency was found for the r value in the Cz derivation ($F_{1,126} = 3.6$, $p = 0.06$). In the Pz derivation, morning types tended to have both a higher r value ($F_{1,126} = 3.6$, $p < 0.06$) and a higher SWA_0 estimate ($F_{1,126} = 3.7$, $p < 0.06$) compared to evening types. Finally, in the Oz derivation, morning types showed a higher SWA_0 value ($F_{1,126} = 4.3$, $p < 0.05$) and a trend for a higher r estimate ($F_{1,126} = 3.7$, $p < 0.06$) compared to evening types. The $SWA\infty$ estimate did not differ between groups in any derivation. The goodness of fit was acceptable and appeared to be greatest at the Fz derivation. Regression curves are illustrated in figure 4.

All night EEG power spectra in REM sleep

Figure 1B presents spectral power for REM sleep EEG per 1-Hz bins for the entire night in evening types relatively to morning types. Visually, spectral power seems lower in evening types than in morning types in the low (2 to 5 Hz bins) and high (15 to 23 Hz bins) frequency ranges, whereas activity in the 7 to 11 Hz bins appears higher in evening types. However, Group-by-Derivation ANOVAs showed no significant Group effects or Group-by-Derivation interactions. Derivation effects were significant in all of the 1-Hz bins ($p < 0.05$).

Time course of EEG power spectra across the night in REM sleep

Significant results from Group-by-Derivation-by-Cycle ANOVAs in REM sleep are summarized in Table 3. We found significant Group-by-Derivation-by-Cycle interactions for high sigma and beta activities. For high sigma, morning types had more power than evening types in the first cycle of the Pz derivation ($p < 0.05$). A similar trend was found in the Cz derivation ($p = 0.07$). As illustrated in figure 5, the major difference observed in high sigma activity was the larger decrease of activity between cycles 1 and 2 in morning types compared to evening types in all derivations ($p < 0.05$) except Fz. In Cz and Pz, the activity decrease between cycle 1 and 3 and between cycle 1 and 4 was larger in morning types ($p \leq 0.05$); and in Oz, only the decrease between cycle 1 and 3 was larger in morning types compared to evening types ($p < 0.05$). Figure 6 illustrates the significant three-way interaction obtained for beta activity. Morning types showed more spectral power than evening types in the first cycle of the Pz derivation ($p = 0.05$), with a similar trend in the Cz derivation ($p = 0.09$). In Fz, morning types showed a larger decrease of beta activity between cycle 1 and 4 compared to evening types ($p < 0.05$). In the Cz and Pz derivations, morning types had a larger decrease of spectral activity between cycle 1 and 2 (Cz: $p = 0.01$; Pz: $p < 0.01$), a larger activity decrease between cycle 1 and 3 (Cz: $p < 0.01$; Pz: $p = 0.01$) and, a larger activity decrease between cycle 1 and 4 (Cz: $p < 0.01$; Pz: $p < 0.05$) compared to evening types. In the Oz site, no difference was found in the decrease of spectral activity between chronotypes. Derivation and Cycle main effects as well as Derivation-by-Cycle interactions were significant ($p < 0.05$) for all frequency bands.

DISCUSSION

Our results support the hypothesis of a difference in homeostatic sleep regulation between morning and evening types. As predicted, differences between morning types and evening types on absolute amplitude of SWA in NREM sleep varied according to brain topography with an apparent antero-posterior gradient. In anterior areas, morning types tended to begin their sleep episode with higher absolute SWA levels than evening types but the most noticeable difference was that SWA decreased more rapidly in morning types than in evening types. Accordingly, when the time course of relative SWA was examined on all-night NREM sleep EEG using an exponential decay function, morning types showed a faster dissipation rate of SWA compared to evening types, and this difference was statistically significant in the frontal derivation. These observations agree with the hypothesis of a faster dissipation rate of homeostatic sleep pressure in morning types compared to evening types.

In all but one studies that analyzed the dissipation rate of relative SWA, only central derivations were reported. The only study that compared regional estimates of the decay rate of low frequency EEG activity found no difference across antero-posterior derivations (Jenni et al., 2005). The authors concluded that the dynamics of the sleep homeostatic process are independent of derivation. In our data, the difference between morning and evening types was close to significance ($p < 0.06$) in the central, parietal and occipital derivations. Thus, our results seem to support this conclusion although we would add that group differences in those dynamics might be easier to observe in frontal derivations.

In addition to group differences in the rate of decay, we also found a significant difference between morning and evening types in the initial levels of relative SWA (SWA_0) in the occipital derivation. An increase in SWA_0 has been reported in normals during recovery nights following sleep deprivation (Dijk et al., 1990b; Besset et al., 1998). Although this observation was only reported for central derivations, it may suggest that our morningness-eveningness differences in SWA were related to a state of sleep deprivation in morning types compared to evening types. Our data, however, do not show any indication of sleep deprivation in morning types. During the week prior to laboratory admission, all subjects had to follow their preferred sleep schedule ($\pm$ 30 minutes), for a sleep duration between 7

and 9 hours as confirmed with ambulatory actigraphy and sleep diaries. As reported before, morning types slept on average 7:47 h (± 9') compared to 8:02 (± 12') in evening types (Mongrain *et al*., 2004). In addition, all parameters of sleep architecture recorded by polysomnography, including duration and percentage of slow-wave sleep, were very similar in the two groups of subjects (Mongrain *et al*., 2005). Therefore, even if this hypothesis cannot be totally excluded, we consider that the presence of sleep deprivation in our morning types is very unlikely and cannot explain the differences observed between our two groups of subjects.

An increased SWA_0 may also represent higher sleep pressure at the beginning of the sleep episode in morning types compared to evening types, due to a faster build-up during the daytime. This interpretation would be consistent with observations from Taillard et al. (2003) showing a faster build-up of sleep pressure in the waking EEG of morning types. For the same duration of time awake, morning types could then accumulate a higher sleep pressure at bedtime compared to evening types. However, it remains unclear why differences in initial relative values were statistically significant only in occipital regions when the trends observed for absolute SWA in the first sleep cycle seemed to be larger in the frontal and central areas. Future dose-response studies comparing SWA dynamics in different topography after various durations of time awake will be needed to clarify the nature of the differences in sleep homeostatic regulation between morning and evening types.

We have previously reported higher spectral power for NREM sleep low sigma activity in morning types compared to evening types (Mongrain *et al*., 2005). Here, we show that this difference depends on topographic area, being prevalent in the parieto-occipital derivations. This is consistent with previous observations showing that spindle frequency activity as well as sleep spindles of this frequency prevail in centro-parietal derivations (Jobert *et al*., 1992; Zeitlhofer *et al*., 1997; Zygierewicz *et al*., 1999; Finelli *et al*., 2001a, b; Knoblauch *et al*., 2002). We also found a trend for a different time course of low sigma activity between morning types and evening types, also derivation-dependent. As illustrated in figure 3, morning types showed indications of a steeper increase in low sigma spectral activity compared to evening types, mainly in the parietal derivation. This trend was statistically significant when examined specifically in the 13-14 Hz frequency bin. Even if sigma

activity is partly under circadian control, **s**pectral power in sigma frequencies has been shown several times to reflect dynamics of homeostatic sleep pressure, as it increases in the course of the sleep episode and decreases following sleep deprivation (Aeschbach *et al*., 1997; Dijk *et al*., 1997). These changes with homeostatic pressure were also found to be derivation-dependent with a centro-parietal dominance (Werth *et al*., 1997; Finelli *et al*., 2001b; Knoblauch *et al*., 2002). Moreover, a study reported that the maximum homeostatic effect in the sigma frequency was in the 13.25-13.5 Hz range (Dijk *et al*., 1997). Since averaged phase angles between circadian phase and wake time were the same in the two groups, it is unlikely that the difference in sigma activity reflects a difference in circadian regulation of the EEG. Our results on low sigma activity might therefore represent additional evidence of a difference in dynamics of homeostatic sleep regulation between morning-type and evening-type individuals.

As illustrated in figure 1, the pattern of differences between morning and evening types in all-night power spectra was visually different in REM sleep compared to NREM sleep EEG. Even if previous work suggest that time course of SWA and theta activity in REM sleep could reflect differences in homeostatic sleep pressure (Dijk *et al*., 1990a, b, 1997; Aeschbach & Borbély, 1993; Merica & Blois, 1997), these two frequency bands were not sensitive to the differences between our morning and evening types. The only group differences in REM sleep were restricted to the time courses of spectral power in the high sigma and beta frequency ranges. Beta is the major frequency band that distinguishes REM from NREM sleep mostly because its activity decreases in the course of the night in REM sleep but shows no change in NREM sleep (Aeschbach & Borbély, 1993; Dijk *et al*., 1997; Merica & Blois, 1997). High sigma activity in REM sleep has been shown to follow the same nighttime course as beta activity in REM sleep (Aeschbach & Borbély, 1993; Dijk *et al*., 1997; Merica & Blois, 1997), and a study found that both sigma and low beta activity (10.75-19.5 Hz) in REM sleep exhibit significant sleep-dependent effects (Dijk *et al*., 1997). **A**ccording to the one-stimulus model for NREM and REM sleep (Feinberg & March, 1995), the declining homeostatic sleep pressure in the course of the night is reflected in both sleep states by an increase in arousal level as the sleep episode progresses. In REM sleep, this increase in arousal level is associated with an increase in the density of eye movements (EM). Since epochs containing EM show lower beta activity compared to epochs without EM (Uchida *et al*., 1992), an increase in arousal level and in EM might

entrain a decrease in beta activity. Therefore, the decrease in REM sleep beta activity over the course of the night could be an additional, although indirect, sign of the dynamics of homeostatic sleep pressure, declining at a faster rate in morning types compared to evening types.

In conclusion, our data suggest that sleep regulation differs between morning type and evening type individuals. This conclusion derives essentially from the differences observed in the time course of SWA and is supported by consistent differences found in low sigma activity in NREM sleep and in the time course of high sigma and beta activity in REM sleep. Our data also show that the magnitude of the difference between chronotypes varies according to antero-posterior derivations. This is the first report of inter-individual differences observed in the rate of dissipation of homeostatic sleep pressure during normal sleep of healthy young humans. A genetic predisposition to the morningness-eveningness dimension is now well established (Archer *et al*., 2003; Johansson *et al*., 2003; Pereira *et al*., 2005). It is possible that homeostatic and circadian processes of sleep regulation co-vary according to the same genetic factors (Dijk & Franken, 2005; Gillette, 2004) to generate the morningness-eveningness orientation. Combination of circadian and genetic techniques in future studies will be crucial to gain a better comprehension of this individual trait.

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ABBREVIATIONS

ANOVA : analysis of variance

CTS : circadian timing system

EEG : electroencephalogram

EM : eye movements

EMG : electromyogram

EOG : electrooculogram

MEQ : morningness-eveningness questionnaire

MSLT : multiple sleep latency test

NREM : non-rapid-eye movement

REM : rapid-eye movement

SWA : slow-wave activity

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Table 1: Significant effects of Group-by-Derivation-by-Cycle ANOVAs of NREM sleep spectral activity in the six frequency bands. Adjusted probabilities are shown.

	Group effect (df=1,22)	Derivation effect (df=3,66)	Cycle effect (df=3,66)	Group-by-Derivation interaction (df=3,66)	Group-by-Cycle interaction (df=3,66)	Group-by-Derivation-by-Cycle Interaction (df=9,198)
SWA (1-5 Hz)	ns	F = 42.6 p < 0.001	F = 35.8 p < 0.001	ns	ns	F = 3.6 P < 0.05
Theta (4-8 Hz)	ns	F = 35.0 p < 0.001	F = 70.1 p < 0.001	ns	ns	ns
Alpha (8-12 Hz)	ns	F = 15.8 p < 0.001	F = 8.0 p < 0.01	ns	ns	ns
Low Sigma (12-14 Hz)	F = 6.0 p < 0.05	F = 33.3 p < 0.001	F = 6.4 p = 0.001	F = 5.3 p < 0.01	ns	ns
High Sigma (14-16 Hz)	ns	F = 43.6 p < 0.001	F = 9.8 p < 0.001	ns	ns	ns
Beta (16-24 Hz)	ns	F = 34.2 p < 0.001	ns	ns	ns	ns

ns: non significant

Table 2 : Parameters of the nonlinear regression analysis on the decay of SWA during NREM sleep for morning types and evening types in the different derivations. Values are mean and SEM in parentheses. ($SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$)

	M-types				**E-types**			
	Fz	**Cz**	**Pz**	**Oz**	**Fz**	**Cz**	**Pz**	**Oz**
SWA∞ (%)	38.6 (8.8)	49.7 (7.3)	50.1 (7.3)	59.1 (6.1)	23.3 (12.3)	37.2 (10.0)	37.4 (10.3)	48.4 (8.5)
SWA_0 (%)	206.4 (12.8)	179.5 (12.3)	194.1 (14.8)[1]	181.6 (16.3)*	174.1 (9.6)	154.8 (8.4)	161.3 (9.4)	146.1 (9.4)
R (min^{-1})	0.0079*	0.0085[1]	0.0094[1]	0.0109[1]	0.0046	0.0051	0.0055	0.0063
	(0.0014)	(0.0016)	(0.0018)	(0.0021)	(0.0009)	(0.0010)	(0.0012)	(0.0014)
R^2	0.83	0.81	0.80	0.78	0.87	0.85	0.82	0.78

[1] : $p \leq 0.06$ compared to evening types

***** : $p < 0.05$ compared to evening types

Table 3: Significant effects of Group-by-Derivation-by-Cycle ANOVAs of REM sleep spectral activity in the six frequency bands. Adjusted probabilities are shown.

	Group effect (df=1,21)	Derivation effect (df=3,63)	Cycle effect (df=3,63)	Group-by-Derivation interaction (df=3,63)	Group-by-Cycle interaction (df=3,63)	Group-by-Derivation-by-Cycle Interaction (df=9,189)
SWA (1-5 Hz)	ns	F = 54.5 p < 0.001	F = 22.3 p < 0.001	ns	ns	ns
Theta (4-8 Hz)	ns	F = 44.0 p < 0.001	F = 14.2 p < 0.001	ns	ns	ns
Alpha (8-12 Hz)	ns	F = 25.1 p < 0.001	F = 20.5 p < 0.001	ns	ns	ns
Low Sigma (12-14 Hz)	ns	F = 11.9 p < 0.001	F = 26.0 p < 0.001	ns	ns	ns
High Sigma (14-16 Hz)	ns	F = 18.5 p < 0.001	F = 21.2 p < 0.001	ns	F = 4.4 p < 0.05	F = 3.6 P < 0.05
Beta (16-24 Hz)	ns	F = 19.8 p < 0.001	F = 28.2 p < 0.001	ns	F = 5.3 p = 0.01	F = 3.0 P < 0.05

ns: non significant

LEGENDS

Figure 1 : All-night spectral EEG power of evening types in the Fz, Cz, Pz and Oz derivations expressed relatively to morning types values. Data are averaged within each 1-Hz frequency bin. Hz bins are identified by their lower boundary value. M-types refer to morning types. A: Spectral power calculated in NREM sleep (stages 2, 3 and 4 sleep). The circled bin indicate significant Group-by-Derivation interaction. B: Spectral power calculated in REM sleep.

Figure 2 : SWA (1-5 Hz) in NREM sleep in the first 4 sleep cycles for the 4 EEG derivations (mean and SEM). M-types are morning types, E-types are evening types. The SWA decrease between cycles 1 and 3 was significantly larger in morning types than in evening types, in the Fz and Cz derivations ($p < 0.05$).

Figure 3 : Low sigma activity (12-14 Hz) in NREM sleep in the first 4 sleep cycles for the 4 EEG derivations (mean and SEM). M-types are morning types, E-types; evening types. Simple effect analysis between morning types and evening types; *: $p \leq 0.05$, **: $p < 0.01$.

Figure 4 : Exponential decay function adjusted on relative SWA in NREM sleep for all-night EEG. Exponential decay fits: $SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$. Solid line: morning types, dashed line: evening types. Morning types data computed on 62 cycles (open triangles) and evening types data on 70 cycles (grey lozenges).

Figure 5 : High sigma activity (14-16 Hz) in REM sleep in the first 4 sleep cycles for the 4 EEG derivations (mean and SEM). M-types refer to morning types, E-types to evening types. The activity decrease between cycles 1 and 2 and between cycles 1 and 3 was significantly larger in morning types than in evening types, in the Cz, Pz and Oz derivations ($p \leq 0.05$). The activity decrease between cycles 1 and 4 was significantly larger in morning types than in evening types in the Cz and Pz derivations ($p \leq 0.05$). Simple effect analysis between morning types and evening types; *: $p \leq 0.05$.

Figure 6 : Beta activity (16-24 Hz) in REM sleep in the first 4 sleep cycles for the 4 EEG derivations (mean and SEM). M-types refer to morning types, E-types to evening types. The activity decrease between cycles 1 and 2 and between cycles 1 and 3 was significantly larger in morning types than in evening types in the Cz and Pz derivations ($p \leq 0.01$). The activity decrease between cycles 1 and 4 was significantly larger in morning types than in evening types in the Fz, Cz and Pz derivations ($p \leq 0.05$). Simple effect analysis between morning types and evening types; *: $p \leq 0.05$.

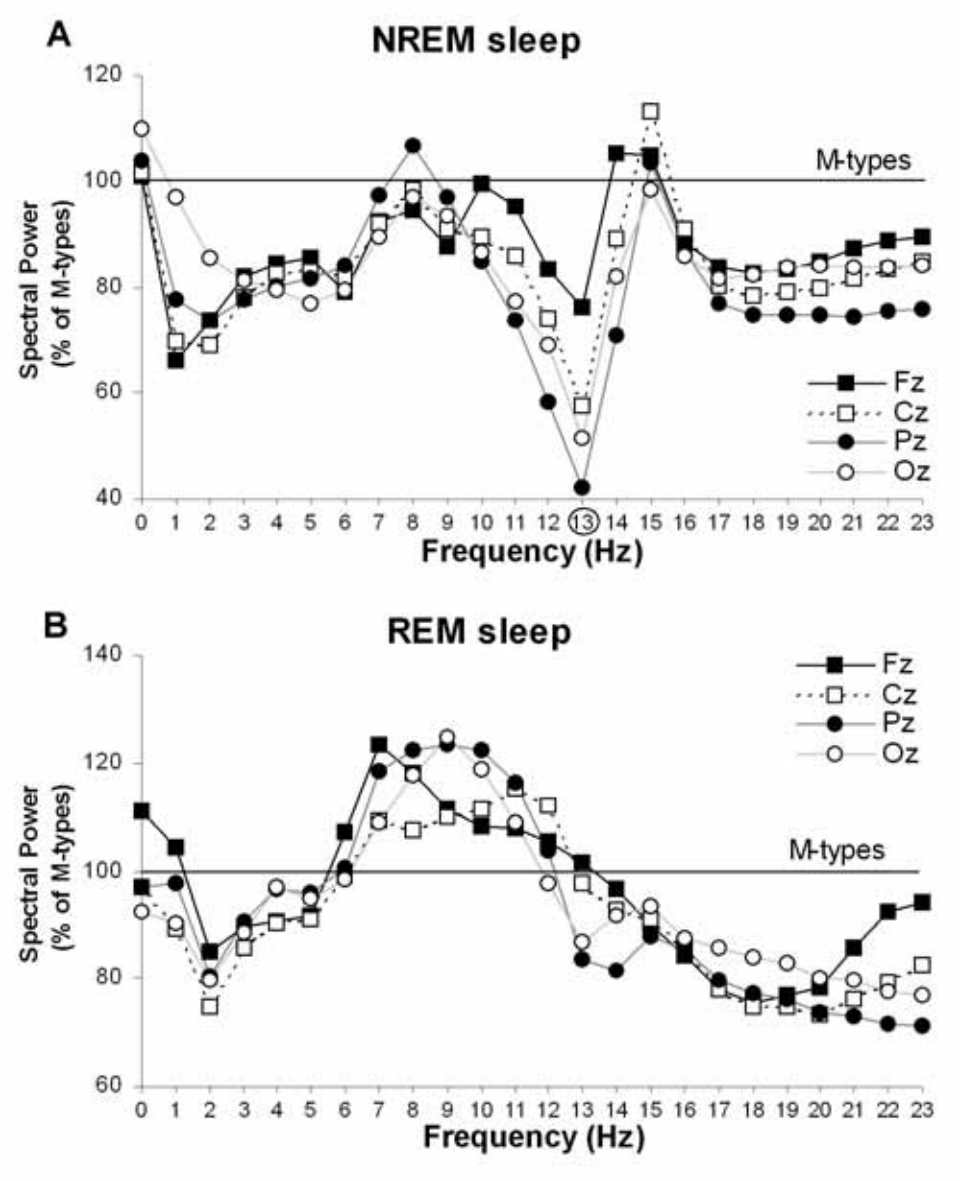
A
NREM sleep
Spectral Power (% of M-types)
M-types
Fz
Cz
Pz
Oz
Frequency (Hz)
B
REM sleep
Spectral Power (% of M-types)
M-types
Fz
Cz
Pz
Oz
Frequency (Hz)

Figure 1

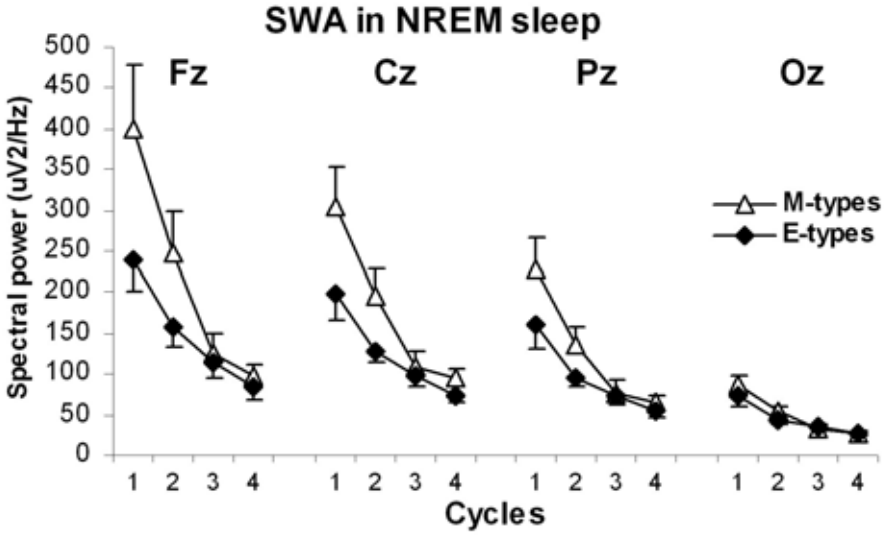

Figure 2

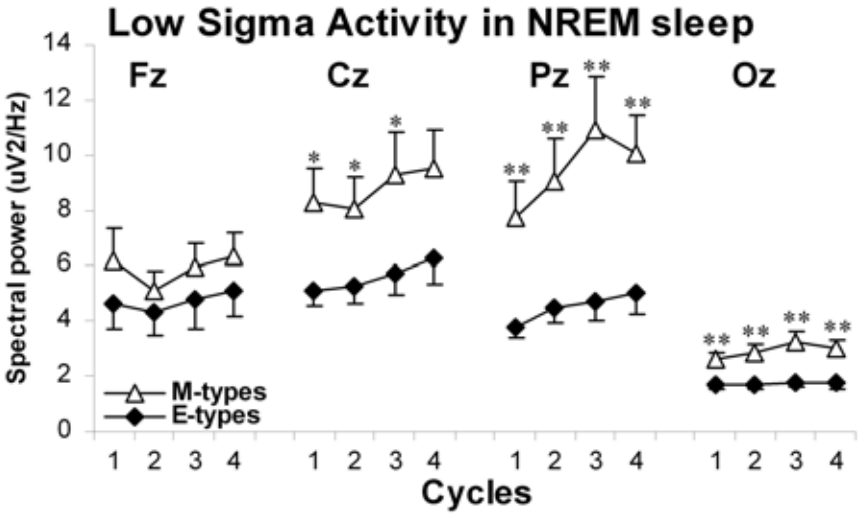

Figure 3

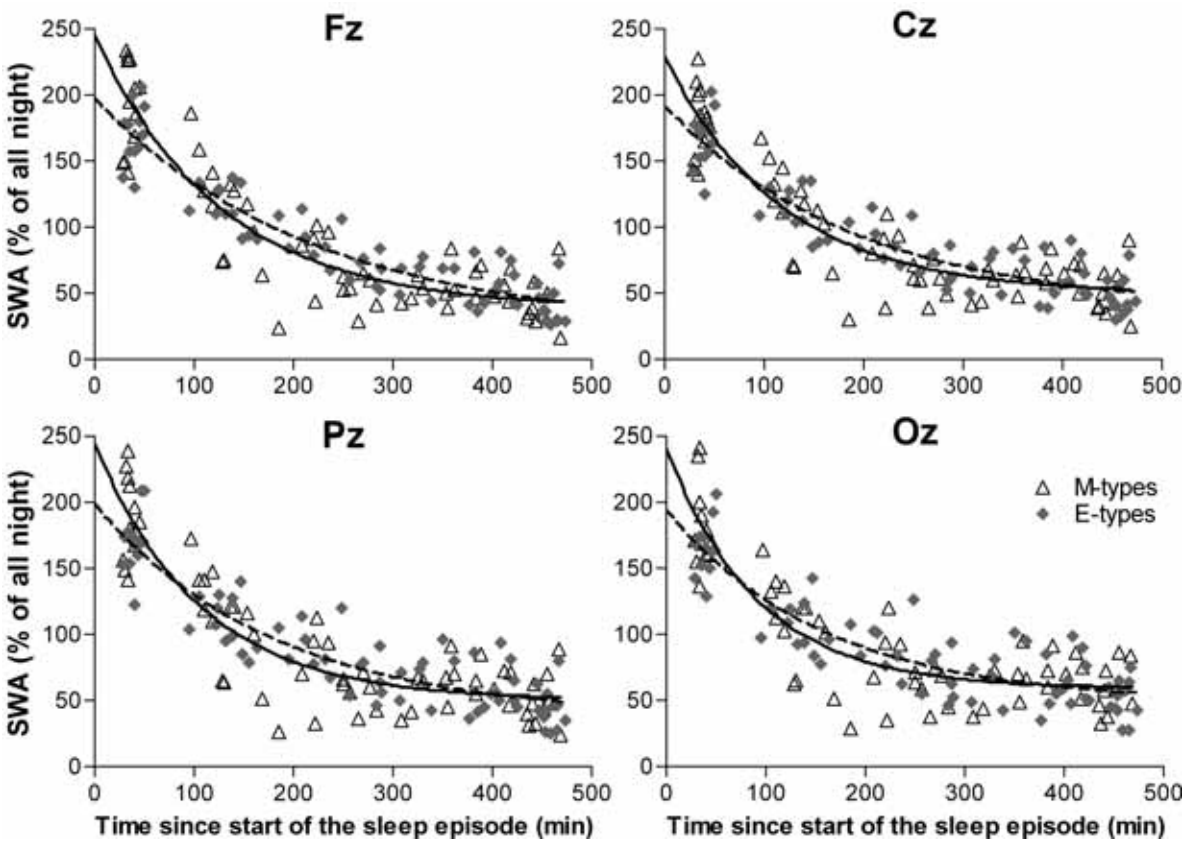
Fz
Cz
Pz
Oz
SWA (% of all night)
Time since start of the sleep episode (min)
M-types
E-types
0
50
100
150
200
250
300
400
500

Figure 4

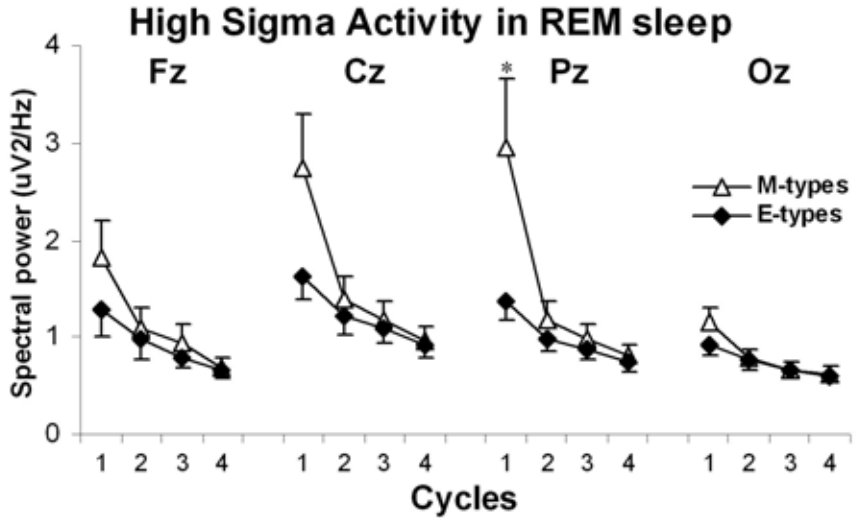

Figure 5

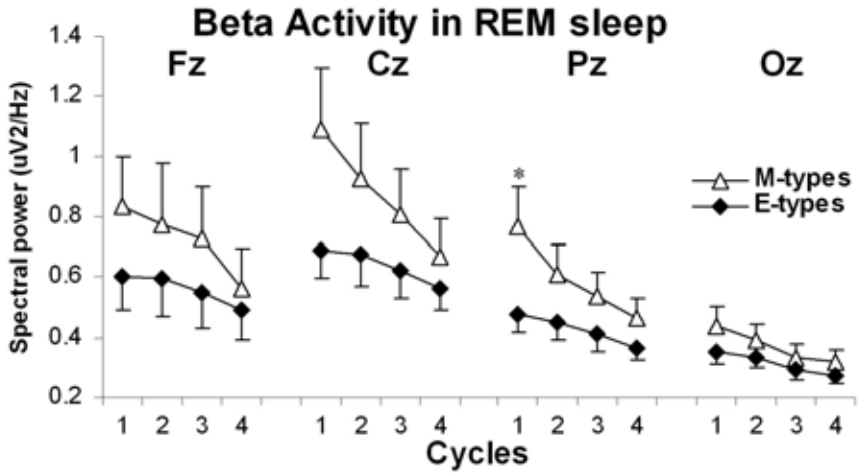

Figure 6

4.4 Quatrième article

Circadian and homeostatic sleep regulation in morningness-eveningness

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Circadian and Homeostatic Sleep Regulation in Morningness-Eveningness

Valérie MONGRAIN[1,2], Julie CARRIER[1,3] and Marie DUMONT[1,2,4]

[1]Chronobiology Laboratory, Sacré-Cœur Hospital of Montréal, Québec, Canada, H4J 1C5; Departments of [2]Neurosciences, [3]Psychology and [4]Psychiatry, University of Montréal, Québec, Canada, H3C 3J7.

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Running head: Sleep regulation in chronotypes

Corresponding author: Marie Dumont, Ph.D.
Chronobiology Laboratory
Sacré-Cœur Hospital
5400 blvd Gouin W.
Montréal (Québec)
Canada, H4J 1C5
Tel: (514) 338-2222 ext. 2246
Fax: (514) 338-2531
Email: marie.dumont@umontreal.ca

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ABSTRACT

Morningness-eveningness has been associated with the entrained circadian phase. However, we recently identified morning and evening types having similar circadian phases. In this paper, we compared parameters of slow-wave activity (SWA) decay in non-rapid-eye-movement (NREM) sleep between these two sub-groups to test the hypothesis that differences in the dynamics of nocturnal homeostatic sleep pressure could explain differences in sleep timing preference. Twelve morning types and twelve evening types (aged 19-34 y.) selected using the Morningness-Eveningness questionnaire were further classified according to the phase of their dim light melatonin onset (DLMO). The 6 morning types with the earliest DLMO were compared to the 6 evening types with the latest DLMO ("extreme" phases), and the 6 morning types with the latest DLMO were compared to the 6 evening types with the earliest DLMO ("intermediate" phases). Subjects slept according to their preferred sleep schedule. Spectral activity in 4 midline derivations (Fz, Cz, Pz, Oz) was calculated in NREM sleep and an exponential decay function was applied on SWA data averaged per sleep cycle. In the subjects with intermediate circadian phases, both initial level and decay rate of SWA in Fz were significantly higher in morning than in evening types. No difference appeared between chronotypes of extreme circadian phases. There was no correlation between individual estimates of SWA decay and DLMO. These results support the hypothesis that chronotype can originate from differences in the dissipation of sleep pressure and that homeostatic and circadian processes influence independently the sleep schedule preference.

Keywords: chronotype, circadian rhythms, sleep regulation, EEG spectral analysis, slow-wave activity, interindividual differences.

INTRODUCTION

Morningness-eveningness, also called "chronotype", is an individual characteristic that refers mostly to the preference in sleep timing, morning types choosing to sleep about 2 hours earlier than evening types (Mecacci and Zani, 1983). The main source of this individual difference is thought to be the internal circadian phase. This assumption is supported by numerous reports of an earlier circadian phase in morning than in evening types (Duffy *et al.*, 1999; Baehr *et al.*, 2000; Bailey and Heitkemper, 2001). However, we recently identified morning and evening types showing significant differences both in their morningness-eveningness scores and in their habitual sleep schedules, but having similar, intermediate circadian phases (Mongrain *et al*., 2004). Since a homeostatic process also contributes to the regulation of sleep-wake behavior (Dijk and Lockley, 2002), we suggested that morningness-eveningness could also result from individual differences in homeostatic sleep regulation. Our recent analyses supported that suggestion. They revealed a tendency for morning types to show a faster dissipation of homeostatic sleep pressure compared to evening types, as indexed by a faster rate of decline of slow-wave activity (SWA) in non-rapid-eye-movement (NREM) sleep (Mongrain *et al.*, 2005). This difference was statistically significant when measured in the frontal area of the brain (Mongrain *et al.*, 2006). The first goal of the present paper was to verify if differences in the dynamics of nocturnal homeostatic sleep pressure might explain differences in preferred sleep schedule, specifically in morning and evening types having similar, intermediate circadian phases.

It has been suggested recently that the two processes of sleep regulation might co-vary and might even come from common genetic sources (Dijk and Franken, 2005; Gillette, 2004). Since morningness-eveningness appears to involve variations in both processes, a second goal of this paper was to conduct exploratory analyses to identify possible co-variations between circadian phase and dissipation rate of homeostatic sleep pressure in morning and evening types.

METHODS

Subjects

Twenty-four subjects were recruited using a French version of the Morningness-Eveningness Questionnaire (MEQ) (Horne and Östberg, 1976): 12 morning types (MEQ scores 59 to 71; age 24.7 ± 1.5 y; 6 women) and 12 evening types (MEQ scores 27 to 40; age 23.4 ± 0.7 y; 6 women). All subjects, mostly students during summer vacation, were in good health and reported a regular sleep schedule. Subjects with habitual sleep duration shorter than 7 h or longer than 9 h were excluded. Sleep disorders were ruled out by questionnaires and by a screening night of polysomnography (PSG). Each subject signed an informed consent form approved by the hospital's ethics committee and received a pecuniary compensation. More details on subjects' selection can be found in Mongrain *et al.* (2004).

Procedures

For the study, individual sleep schedules were determined for a sleep duration of 8 h according to each subject's preferences. Subjects were requested to follow their selected bedtime and wake time (± 30 min) for 7 days prior to laboratory admission; this was verified by actigraphy and sleep diaries. In the laboratory, the subjects slept according to their individual sleep schedule. A first night was for adaptation and the second night was used for sleep analysis. Sleep EEG was recorded with a referential montage (linked-ears) and digitized at 256 Hz. Sleep stages were visually scored on 20-sec epochs from the C3 derivation (Rechtschaffen and Kales, 1968). NREM/REM sleep cycles were determined according to Feinberg and Floyd criteria (Feinberg and Floyd, 1979; Feinberg and Campbell, 2003). Spectral power, computed on Fz, Cz, Pz and Oz derivations, was obtained by fast Fourier transforms (cosine tapering) performed on 4-sec artifact-free sections giving a 0.25 Hz spectral resolution. Spectral power calculated in NREM sleep epochs (excluding stage 1) was averaged for SWA (1-5 Hz). More details on EEG recording and analyses are available in Mongrain *et al.* (2005; 2006).

Circadian phase was determined with the salivary melatonin rhythm. Saliva samples were collected every half-hour in dim light (< 15 lux), starting 5 h before bedtime. Melatonin

concentration was determined by radioimmunoassay. Circadian phase was estimated with the Dim Light Melatonin Onset (DLMO), interpolated at a threshold of 1.3 pg/ml (Mongrain *et al.*, 2004).

Statistical Analysis

The nighttime course of SWA in NREM sleep was investigated with a nonlinear regression analysis on all-night sleep EEG. The time of the cycle midpoint was determined for each cycle of each subject and used as the independent variable (t). The mean SWA of NREM sleep epochs within each cycle was expressed as the percentage of all-night NREM sleep SWA and used as the dependent variable (SWA_t). An exponential decay function was fitted to the data: $SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$, giving the estimates of $SWA\infty$ (lower asymptote for $t = \infty$), SWA_0 (intercept of the y axis minus asymptote), and r (slope of the decay). Group estimates of $SWA\infty$, SWA_0 and r were compared between morning types and evening types of each subgroup using F tests (Motulsky and Christopoulos, 2003). Correlations were computed with Spearman tests. Wake times and sleep durations were averaged over the 7 days preceding admission to the laboratory. Results are reported as mean ± SEM.

RESULTS

SWA during NREM sleep in subjects with extreme or intermediate circadian phases

Nonlinear regression analysis was calculated on group data, separately for morning and evening types with extreme or intermediate circadian phases. Subjects with extreme circadian phases included 6 morning types with a DLMO between 17:25 and 20:59 h (mean: 19:33 h ± 33') and 6 evening types with a DLMO between 23:57 and 01:35 h (mean: 24:36 h ± 16'). As reported in Table 1, these two subgroups did not differ in any estimates of SWA decay. Subjects with intermediate circadian phases included 6 morning types with a DLMO between 21:07 and 23:03 h (mean: 21:48 h ± 17') and 6 evening types with a DLMO between 21:18 and 23:04 h (mean: 22:10 h ± 17'). As shown in Table 1, the r estimate (decay slope) of SWA was significantly higher in morning types than evening types for the frontal derivation ($F_{1,60} = 4.2$, $p < 0.05$). A similar trend was found for the Cz

and Pz derivations ($F_{1,60} \geq 3.3$, $p \leq 0.07$). SWA_0 value was also higher in those morning types than in evening types for the Fz derivation ($F_{1,60} = 3.9$, $p = 0.05$). Nonlinear regression curves computed in each subgroup from the data recorded in the Fz derivation are illustrated in Figure 1.

Correlations with habitual sleep schedule

To explore further the relationship between the sleep schedule and parameters of SWA decay computed in the Fz derivation, correlations were calculated between individual estimates of SWA decay and averaged wake times and sleep durations. Results computed with data from the Fz derivation are shown in Table 2. A steeper slope (higher r estimate) tended to be associated with an earlier wake time and a shorter sleep duration, only in the 12 subjects with intermediate circadian phases. In addition, a higher initial value of SWA (SWA_0) was significantly correlated with an earlier wake time and a shorter sleep duration, also specifically in the 12 subjects with intermediate circadian phases. In the 12 subjects with extreme circadian phases, there was no correlation between r estimates and sleep schedule, but a higher SWA_0 tended to correlate with a longer sleep duration. Relationships between SWA estimates and preferred wake time are illustrated in Figure 2 (left panels). As expected, a later circadian phase was significantly associated with a later wake time in the 12 subjects with extreme circadian phases (Table 2) but this correlation was not significant in the 12 subjects with intermediate phases. No correlation was found between DLMO and habitual sleep duration.

Correlations between individual estimates of SWA decay and DLMO

Individual estimates of r and SWA_0 were not correlated with DLMO in any of the 4 midline derivations, either when computed for the entire group of 24 subjects (r_s between -0.24 and 0.11), or in the subgroups of 12 subjects with extreme (r_s between -0.38 and 0.27) or intermediate (r_s between -0.41 and 0.06) circadian phases. Relationships between SWA estimates measured in the Fz derivation and DLMO for the 24 subjects are illustrated in Figure 2 (right panels).

DISCUSSION

Our results show that morning types and evening types with similar circadian phases differ in the dynamics of homeostatic sleep pressure dissipation. In this subgroup of subjects, morning types showed both higher initial levels (higher SWA_0) and faster dissipation rates (larger r estimates) of homeostatic sleep pressure, when compared to evening types. None of the parameters of SWA decay differed between morning types and evening types with extremely early or late circadian phases. In addition, the preferred wake time was correlated with SWA parameters only in subjects with intermediate circadian phases, whereas it was correlated with circadian phase only in subjects with extremely early or late DLMO. Those results support the hypothesis that individual variations in homeostatic or circadian processes of sleep regulation can both result in morningness-eveningness preference.

We previously reported that morning types with intermediate circadian phases had a shorter phase angle between DLMO and wake time compared to evening types with similar phases, and that they also tended to show a shorter sleep duration (Mongrain *et al.*, 2004). A faster dissipation of sleep pressure may favor an earlier sleep satiation and a shorter sleep duration. It can therefore allow for a spontaneous awakening at an earlier phase of the circadian rhythm of wake propensity. The finding of a higher initial level of relative SWA in morning types with intermediate circadian phases was less expected since a higher sleep pressure should take a longer time to dissipate and therefore, be associated with a later wake time. In fact, in the subgroup of subjects with intermediate circadian phases, high initial levels of SWA were positively correlated with faster rates of decay (r_s between 0.70 and 0.47 in the 4 derivations; p between 0.05 and 0.15). This specific shape of SWA decay is well illustrated by the regression curve computed on group data in Figure 1. A higher initial level of SWA seems therefore to be associated with a very fast decay rate in morning types with intermediate phases and thereafter be linked to shorter sleep duration. These characteristics of SWA dynamics may result in an earlier wake time and therefore in greater morningness than in subjects with a slower decay rate.

In chronotypes, the rate of dissipation of SWA could also be associated with the buildup rate of homeostatic sleep pressure. In support to this hypothesis, a previous study found indications of a faster buildup of sleep pressure during wakefulness in morning types than in evening types (Taillard 2003). A faster buildup of sleep pressure in morning types could result in an earlier increase of sleep propensity and thus in an earlier preferred bedtime than in evening types. Such a faster buildup would also be consistent with a higher level of sleep pressure at sleep onset, after a given duration of wakefulness.

By comparison, morning types and evening types with very early or late circadian phases did not differ on any of the parameters of SWA decay. In this subgroup of subjects, morningness-eveningness preference seems therefore to depend more directly on the internal circadian phase. As reported by others (Duffy *et al.*, 1999; Baehr *et al.*, 2000), we found a longer phase angle in morning types than in evening types in this subgroup of subjects (Mongrain *et al.*, 2004). This is in agreement with the report of a shorter circadian period in morning types (Duffy *et al.*, 2001), which is predicted to result in an earlier circadian phase within the sleep-wake / light-dark cycle (Pittendrigh and Daan, 1976). In subjects with extreme circadian phases, there was no correlation between initial levels and dissipation rates of sleep pressure (r_s between –0.12 et +0.07 in the 4 derivations). High initial sleep pressure should then take a longer time to dissipate, which is consistent with our observation that subjects with higher initial values tended to show longer sleep durations. Since sleep timing and sleep duration are both affected by sleep homeostasis, it might be of interest in future studies to select morning and evening types without exclusion criteria for habitual sleep duration.

The results of our exploratory analyses do not support the hypothesis that circadian and homeostatic processes are co-regulated since we found no correlation between variations in circadian phase and parameters of SWA decay. In addition, the fact that circadian phase or SWA estimates correlated with preferred wake time only in subjects with extreme or intermediate circadian phases respectively, also suggests that the two processes were having independent influences on the sleep-wake cycle. Therefore, even if a co-regulation of circadian and homeostatic processes cannot be totally excluded on the basis of our data, our observations rather suggest that the two regulatory processes vary in an independent manner.

In conclusion, results of this study show that there are at least two sources for individual variability in morningness-eveningness preference. First, there are variations in endogenous circadian phase, probably related to variations in the length of endogenous period, which result in chronotypes with early or late circadian phases. Second, in subjects having intermediate circadian phases, variations in parameters of homeostatic dissipation of sleep pressure may result in preferences for an early or late sleep schedule. The nature of the relationship between homeostatic regulation of sleep pressure and sleep-wake behavior will have to be determined in future studies that would include morning, evening and "neither" types, all having intermediate circadian phases.

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Table 1 : Parameters of the nonlinear regression analysis of SWA decay calculated on group data for morning types and evening types having extreme or intermediate circadian phases (see text). Results are shown separately for the 4 midline EEG derivations. Values are mean (SEM). ($SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$)

	Morning types				Evening types			
	Fz	**Cz**	**Pz**	**Oz**	**Fz**	**Cz**	**Pz**	**Oz**
SWA∞ (%)								
Extreme	28.8 (16.0)	41.6 (13.2)	41.5 (12.8)	51.7 (9.5)	8.9 (31.5)	28.1 (23.4)	24.6 (25.7)	38.9 (20.6)
Intermediate	47.9 (9.9)	57.4 (8.6)	58.4 (8.6)	66.6 (8.0)	32.8 (10.0)	43.5 (9.1)	45.8 (9.3)	54.3 (8.0)
SWA_0 (%)								
Extreme	199.8 (16.4)	175.1 (15.2)	188.7 (17.6)	180.0 (17.5)	177.0 (25.1)	153.9 (18.2)	162.5 (20.0)	140.9 (16.6)
Intermediate	222.6 (23.2)*	192.0 (22.5)	210.5 (28.6)	195.2 (35.8)	177.0 (9.0)	159.1 (9.3)	165.8 (11.7)	156.7 (13.4)
r (min^{-1})								
Extreme	.0063 (.0018)	.0068 (.0019)	.0075 (.0021)	.0088 (.0022)	.0036 (.0015)	.0043 (.0017)	.0043 (.0018)	.0049 (.0021)
Intermediate	.0105 (.0025)*	.0111 (.0029)[1]	.0126 (.0034)[1]	.0148 (.0048)	.0055 (.0010)	.0060 (.0012)	.0067 (.0015)	.0077 (.0018)
R^2								
Extreme	0.84	0.82	0.81	0.82	0.83	0.80	0.78	0.74
Intermediate	0.84	0.82	0.80	0.75	0.92	0.92	0.87	0.84

[1] : $p \leq 0.07$ compared to evening types;

* : $p < 0.05$ compared to evening types.

Table 2 : Spearman correlations coefficients computed between sleep schedule parameters and individual estimates of SWA decay and circadian phase, in subgroups of subjects having extreme (n= 12) or intermediate (n= 12) circadian phases.

	Wake time	**Sleep Duration**
r values - Fz		
Extreme phases	-0.29	0.31
Intermediate phases	-0.54^{+}	-0.52^{+}
SWA_0 - Fz		
Extreme phases	0.25	0.57^{+}
Intermediate phases	-0.59*	-0.66*
DLMO		
Extreme phases	0.73**	0.13
Intermediate phases	0.42	0.02

$^{+}$: $p < 0.1$; * : $p < 0.05$; ** : $p < 0.01$.

LEGEND

Figure 1. Exponential decay functions adjusted on relative SWA in NREM sleep for all-night EEG in the Fz derivation. Exponential decay fits: $SWA_t = SWA\infty + SWA_0 * e^{(-r*t)}$. Solid line: morning types; dashed line: evening types. In subjects with extreme circadian phases (left panel), regressions were computed on 32 cycles for morning types (open triangles) and 34 cycles for evening types (grey lozenges). In subjects with intermediate circadian phases (right panel), regressions were computed on 30 cycles for morning types (open triangles) and 36 cycles for evening types (grey lozenges).

Figure 2. Left panels: SWA estimates in relation to preferred wake time in subjects with extreme or intermediate circadian phases. The lines represent the linear fits computed in each subgroup of 12 subjects. Right panels: SWA estimates measured in the Fz derivation in relation to circadian phase estimated with the salivary dim light melatonin onset (DLMO). The lines represent linear fits computed for the entire group of 24 subjects.

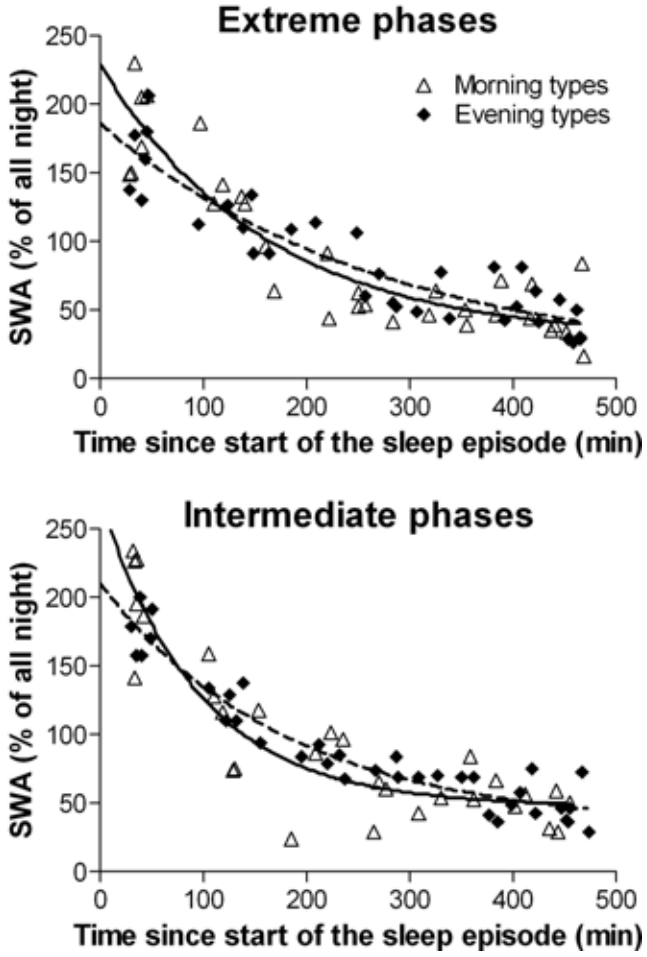
Extreme phases
Morning types
Evening types
SWA (% of all night)
250
200
150
100
50
0
0
100
200
300
400
500
Time since start of the sleep episode (min)
Intermediate phases
SWA (% of all night)
250
200
150
100
50
0
0
100
200
300
400
500
Time since start of the sleep episode (min)

Figure 1

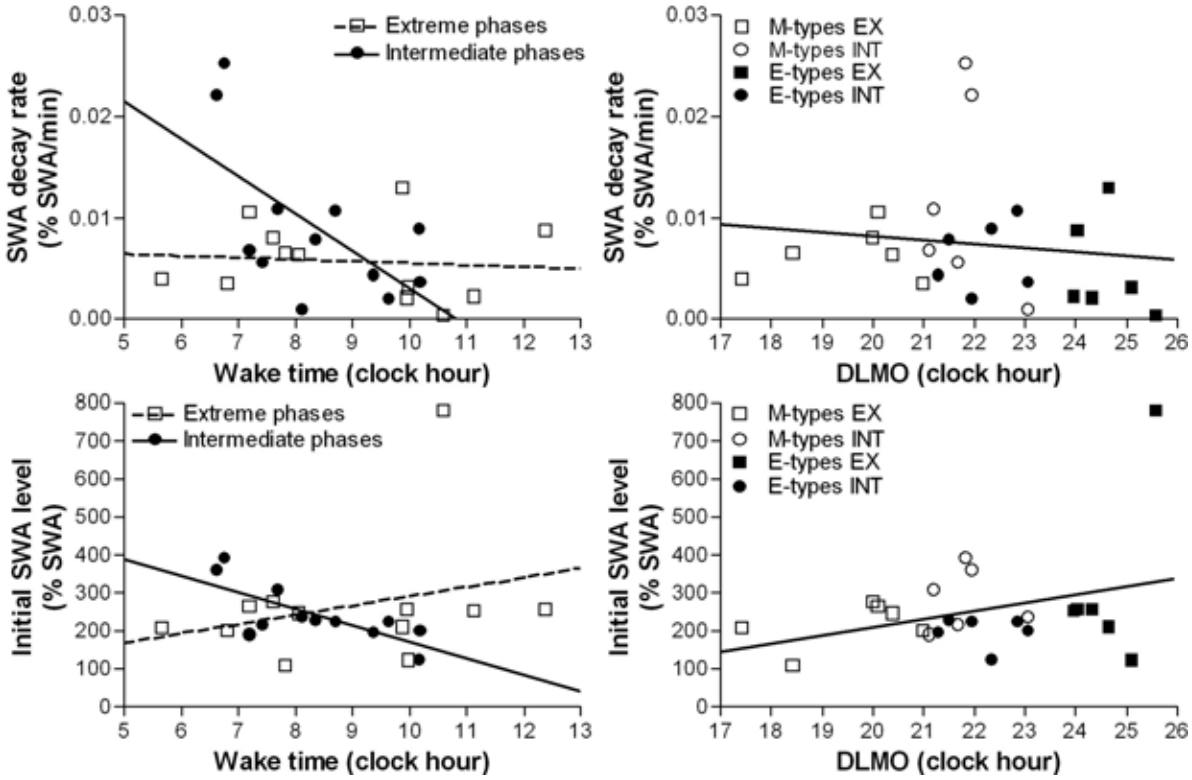
Extreme phases
Intermediate phases
SWA decay rate (% SWA/min)
Wake time (clock hour)
M-types EX
M-types INT
E-types EX
E-types INT
DLMO (clock hour)
Initial SWA level (% SWA)

Figure 2

4.5 Cinquième article

Increased homeostatic response to behavioral sleep fragmentation in morning compared to evening types

Article soumis dans « *Sleep* »

Increased Homeostatic Response to Behavioral Sleep Fragmentation in Morning compared to Evening Types

Valérie MONGRAIN M.Sc.[1,2] and Marie DUMONT Ph.D.[1,2,3]

[1]Chronobiology Laboratory, Sacré-Cœur Hospital of Montréal, Québec, Canada, H4J 1C5; [2]Department of Neurosciences, [3]Department of Psychiatry, Université de Montréal, Québec, Canada, H3C 3J7.

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Corresponding author: Marie Dumont, Ph.D.

Chronobiology Laboratory

Sacré-Cœur Hospital

5400 blvd Gouin W.

Montréal (Québec)

Canada, H4J 1C5

Tel: (514) 338-2222 ext. 2246

Fax: (514) 338-2531

Email: marie.dumont@umontreal.ca

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ABSTRACT

Study Objectives: To evaluate the influence of chronotype on sleep stages and quantitative sleep EEG when sleep pressure is increased without changing the sleep schedule.

Design: A 5-day session in the laboratory consisting in an adaptation night, a baseline night, 2 nights of sleep fragmentation and a recovery night.

Setting: Chronobiology laboratory.

Participants: Twenty-four normal young subjects aged 19-34 years: 12 morning types and 12 evening types selected by questionnaire. Each group included 6 men and 6 women free of sleep, vigilance, psychiatric and neurologic disorders.

Interventions: Two nights of behavioral sleep fragmentation including a forced awakening of 5 minutes every half-hour.

Measurements and Results: Each night of polysomnography recording was of 8 hours in duration and followed each subject's preferred sleep schedule. The induced sleep fragmentation increased stage 1 sleep and decreased total sleep time, sleep efficiency and slow-wave sleep. No difference appeared between morning types and evening types in sleep architecture during or after sleep fragmentation. Spectral analysis of all-night NREM sleep EEG showed that sleep fragmentation induced a larger fronto-central increase in low frequency activities and a larger centro-parietal decrease in 14-15 Hz activity in morning types than in evening types. The difference between chronotypes was predominant in the first part of the sleep episode for slow-wave activity in the fronto-central area.

Conclusions: These results further suggest a difference in homeostatic sleep regulation between morning types and evening types, morning types showing indications of a higher response to sleep disruption.

Key Words: morningness-eveningness, sleep fragmentation, sleep, EEG spectral analysis, circadian rhythms, human, sleep regulation.

INTRODUCTION

Circadian typology refers to preferred timing for activity and sleep. This preference, identified by various questionnaires, results in the classification of people into chronotypes: morning types, neither types and evening types. This interindividual difference has been associated with a difference in endogenous circadian phase as measured using core body temperature and melatonin secretion rhythms.[1-4] In general, morning-type individuals have their sleep episode as well as their circadian phase position about 2 hours earlier than evening types.

The homeostatic process of sleep regulation adjusts sleep intensity as a function of the duration of prior waking and sleep.[5,6] During sleep, dynamics of homeostatic sleep pressure is reflected by the decreasing activity in low EEG frequencies (< 10 Hz) and by the increasing trend of spindle frequency activity (12-16 Hz) in non-rapid-eye-movement (NREM) sleep.[7-9] In wakefulness, the increase in sleep pressure is associated with increased theta-alpha activity (5-9 Hz).[10-12] We recently reported that morning-type individuals have a faster decay of slow-wave activity (SWA; 1-5 Hz) in the frontal brain area, and a steeper increase in 13-14 Hz activity in the parieto-occipital area during normal sleep.[13] Another group found that morning types may have a faster increase of theta-alpha activity during wakefulness compared to evening types.[14] These results suggest that chronotypes differ not only in the position of their circadian phase but also in the dynamics of homeostatic sleep regulation, morning types showing indications of faster buildup and dissipation of sleep pressure than evening types.

Since the homeostatic process depends on duration of wakefulness, the response to the manipulation of duration of time awake can be used to evaluate homeostatic sleep regulation.[15,16] Effectively, an increase in the duration of time awake produces a proportional increase in NREM sleep low frequency activities, especially at the beginning of the sleep episode, and a decrease in spindle frequency activities during subsequent sleep.[7-9,17-21] Therefore, for a given augmentation of wakefulness, a different response in those sleep EEG activities could reveal a difference in the dynamics of sleep homeostasis.

In chronotypes, differences in day sleep have been evaluated following a night of sleep deprivation, such as in night workers. Some studies found that both sleep and post-sleep alertness were more affected in morning types than in evening types when the sleep episode was delayed,[22,23] whereas another found larger changes in sleep cycles and time course of low EEG activities in evening types than in morning types.[24] In all of those studies, the relationship between circadian phase and the timing of the sleep episode was modified, which is known to alter sleep structure.[25-27] To study the specific effects of an increased sleep pressure, it is therefore necessary to modulate the duration of time awake while controlling for circadian phase. One way to increase the time awake without changing the timing of the sleep episode is to increase the time awake within the sleep episode.

The goal of this paper was to evaluate the difference between morning and evening circadian types in their response to an increase in sleep pressure caused by behavioral sleep fragmentation. This response was expected to represent solely the response of sleep homeostasis since the relationship between sleep episode and circadian phase was kept constant. Given that morning types have been found to show faster dynamics of buildup and dissipation of sleep pressure, we predicted that they were going to show an increased response to sleep fragmentation compared to evening types, as assessed by EEG markers of homeostatic sleep pressure during sleep.

METHODS

Subjects

Morning types (M-types) and evening types (E-types) aged 19 to 34 y. were recruited using a French version of the Morningness-Eveningness Questionnaire (MEQ) of Horne and Östberg (1976).[28] Twenty-four subjects completed the study: 12 M-types (MEQ scores 59 to 71) and 12 E-types (MEQ scores 27 to 40). There were 6 women and 6 men in each group. Age was similar in the two groups (M-types: 24.7 y ± 1.5; E-types: 23.4 y ± 0.7). All subjects were in good physical and psychological health, and had no sleep complaints. Selected subjects had a regular sleep schedule with an habitual sleep duration between 7 and 9 h. A 24-h laboratory screening confirmed the absence of sleep and vigilance disorders by a night of polysomnography and a multiple sleep latency test.[29] Inclusion

criteria were: sleep efficiency higher than 85 %, sleep latency lower than 30 min, apneas/hypopneas index and periodic leg movements index lower than 5, and averaged day sleep latency higher than 7 min. Subjects had no night work experience in the past year and no transmeridian travel in the past three months. They were all non-smokers and reported not using drugs or medications, except oral contraceptives. Women not using hormonal contraception (3 M-types and 4 E-types) were studied during the follicular phase of their menstrual cycle. Each subject signed an informed consent form approved by the hospital ethics committee and received a financial compensation. Results presented in this paper come from a larger study on sleep regulation in morningness-eveningness conducted with the same subjects. Detailed information on sleep-wake cycle and circadian phase assessments can be found in Mongrain et al. (2004).[4] Briefly, circadian phases of salivary melatonin and rectal temperature rhythms were respectively 2.5 and 2 h earlier in M-types than in E-types. Averaged phase angles between circadian phases and sleep schedule were similar in the two groups of subjects.

Procedures

For the study, individual sleep schedules were determined according to each subject's preferred bedtime and wake time using information from screening sleep diaries during free days and preferred wake time and bedtime reported in the MEQ. The final decision for the study sleep schedule was made after discussion with the subject to ensure that it was close to the schedule that he/she would spontaneously adopt. Bedtime and wake time were determined for a sleep duration of 8 hours. Subjects were requested to follow their selected sleep schedule (± 30 min) for 7 days prior to laboratory admission. Compliance was verified by sleep diaries and by ambulatory measures of activity and light exposure (Actiwatch-L, Mini-Mitter Co, Bend, OR).

Subjects were admitted to the laboratory 4 h before their scheduled bedtime and slept five consecutive nights according to their individual sleep schedule: an adaptation night (AD), a baseline night (BL), two nights of behavioral sleep fragmentation (FR1 and FR2) and a recovery night (REC). During the nights of sleep fragmentation, subjects were awakened for a duration of 5 minutes every half-hour for a total of 15 awakenings per night.[30] For each awakening, a technician knocked on the door and entered the room with a small

flashlight. Subjects had to interact verbally with the technician for the entire 5 minutes. Room light was not turned on and subjects were not required to open their eyes. Another technician stayed in the control room to keep track of the time and to confirm wakefulness according to on-line EEG recordings. Subjective sleep quality was assessed on each morning using a five-point scale. Sleep episodes were recorded with 25 EEG, 2 EOG and 3 chin EMG electrodes, using a referential montage with linked-ears. Signals were recorded using a polygraph Grass Model 15A54 amplifier system (Astro-Med Inc., West-Warwick, USA; gain 10000, bandpass 0.3-100 Hz) and digitized at a sampling rate of 256 Hz with a commercial software (Harmonie 5.1, Stellate Systems, Montreal, Canada).

Sleep Data Analysis

Sleep stages were visually scored from the C3 derivation, on 20-sec epochs, according to standard procedures.[31] Sleep latency was defined as the time from lights off to the first min of stage 1 or to the first epoch of any other sleep stage. Sleep period was the time from sleep onset to final awakening. Sleep efficiency was the time spent asleep divided by the sleep period, multiplied by 100. EEG spectral analysis was performed on Fz, Cz and Pz derivations with a commercial software package (Sensa, Stellate Systems, Montreal, Canada). Artifacts were automatically detected,[32] and further artifacts were identified by visual inspection. Spectral power was obtained by fast Fourier transforms (FFT) performed on 4-sec artifact-free sections using a cosine window tapering resulting in a 0.25 Hz spectral resolution. All-night spectral power was calculated over NREM sleep stages (excluding stage 1) for 24 one-Hz frequency bins identified by their lower boundary value. Spectral power was averaged for six frequency bands (SWA (1-5 Hz), theta (4-8 Hz), alpha (8-12 Hz), low sigma (12-14 Hz), high sigma (14-16 Hz), and beta (16-24 Hz)) and then averaged in two-hour periods for the sleep episode of each night. Two-hour periods were calculated according to real time (clock time) and are also identified by their lower boundary value. Spectral analysis data for the Pz derivation for a M-type woman have been excluded because of technical difficulties during recordings of FR2 and REC nights.

Statistical Analysis

Group-by-Night ANOVAs were used to assess differences in sleep architecture variables and in all-night EEG power spectra. Because between-group differences were expected to

prevail at the beginning of the nights, changes in power spectra in each EEG frequency band were evaluated over the four 2-hours periods of the nights using Group-by-Night-by-Period ANOVAs. Huynh/Feldt corrections were used for repeated measures, but the original degrees of freedom are reported. Significant interactions were decomposed using simple effect analysis. Statistical significance was set to 0.05 and results are reported as mean ± SEM.

RESULTS

Sleep schedule and architecture

Laboratory 8-h sleep schedules, selected according to each subject's preference, were more than 2.5 h earlier in M-types than in E-types (bedtime: 23:08 ± 11' vs. 01:45 ± 17'). Subjective sleep quality and parameters of sleep architecture for nights of recordings are shown in Table 1. Total sleep time decreased by more than 2 h between BL and FR1 and increased by approximately 0.75 and 1.5 h between FR1 and FR2 and between FR2 and REC respectively ($F_{3,66} = 154.7$, $p < 0.001$). Significant Night effects were also observed for subjective sleep quality, REM sleep latency, sleep efficiency, and both duration and percentage of each sleep stage ($F_{3,66} \geq 17.1$, $p < 0.001$). Subjective sleep quality, sleep efficiency, duration of stage 2 sleep and duration and percent of slow-wave sleep and REM sleep decreased in FR1 compared to BL but increased thereafter from FR1 to FR2 and from FR2 to REC. Duration and percent of slow-wave sleep also showed a significant increase between BL and REC. REM sleep latency, duration and percent of stage 1 sleep and percent of stage 2 sleep showed a reverse pattern: these increased in FR1 compared to BL, and decreased subsequently from FR1 to FR2 and from FR2 to REC. Percent of stage 2 sleep also showed a significant decrease between BL and REC. There was no significant difference between M-types and E-types for self-reported sleep quality or for sleep architecture parameters on any of the four nights. Moreover, there was no significant Group-by-Night interaction.

Effects of sleep fragmentation on quantitative EEG

A main Night effect was significant in all frequency bands for the Fz, Cz and Pz derivations. SWA decreased significantly between BL and FR1, and increased significantly

afterward (between FR1 and FR2, and FR2 and REC) in the 3 derivations. Theta and alpha activities showed similar patterns although changes between nights were not all significant. SWA and theta activity also showed a significant increase between BL and REC. For low sigma activity, an increase was found between BL and FR1 in Fz and between FR1 and FR2 in Cz and Pz, and a decrease was found between FR2 and REC (not significant in Cz). Finally, high sigma and beta activities increased in FR1 compared to BL and decreased thereafter from FR1 to FR2 (not significantly for high sigma in Cz and Pz) and from FR2 to REC in all derivations.

Between-chronotype differences in quantitative EEG

All night EEG power spectra in NREM sleep

All-night power spectra computed by one-Hz bins for M-types relatively to E-types are shown in Figure 1. In the Fz derivation (Figure 1A), no main Group effect was found. Significant Group-by-Night interactions were found in Hz bins 2 to 5 ($F_{3,66} \geq 3.2$, $p < 0.05$). Morning individuals showed a higher activity increase between FR1 and FR2 (simple effect analysis, $p < 0.05$), between the FR1 and REC ($p < 0.05$ except 5 Hz) and between BL and REC ($p < 0.01$) than E-types.

In the Cz derivation (Figure 1B), a main Group effect was found in the 13-Hz bin ($F_{1,22} = 5.7$, $p < 0.05$) with M-types having higher activity level compared to E-types. Significant Group-by-Night interactions were found in Hz bins 2, 3, 5, 6, 7 and 14 ($F_{3,66} > 3.1$, $p < 0.05$). In frequencies between 2 and 8 Hz, M-types showed a higher activity increase between BL and FR2 ($p < 0.05$ except 3 Hz), between FR1 and FR2 ($p < 0.05$ except 6 and 7 Hz), and between BL and REC ($p < 0.05$) than E-types. For the 14-Hz bin, M-types showed a higher activity decrease between FR1 and FR2 ($p < 0.05$), between FR1 and REC ($p < 0.01$), and between BL and REC ($p < 0.05$) than E-types.

In the Pz derivation (Figure 1C), main Group effects were found in the 12 and 13 Hz bins ($F_{1,21} \geq 4.4$, $p < 0.05$) with M-types having higher activity level than E-types. A significant Group-by-Night interaction was found in the 14-Hz bin ($F_{3,63} = 4.8$, $p < 0.01$). M-types showed a higher activity decrease between FR1 and FR2 ($p = 0.05$), between FR1 and REC ($p < 0.001$), and between BL and REC ($p < 0.05$) than E-types.

Time course of NREM sleep power spectra

NREM spectral power averaged within the four 2-hour periods were compared using Group-by-Night-by-Period ANOVAs for each EEG frequency band. Significant results are summarized in Table 2. In the Fz derivation, significant Group-by-Night-by-Period interactions were found for SWA, theta and high sigma and are illustrated in Figure 2. During BL, the SWA decrease between periods 0 and 2 and between periods 0 and 4 was steeper in M-types than E-types ($p \leq 0.05$). During FR1, the dynamics of SWA was similar in both diurnal types. During FR2, the SWA decrease between periods 0 and 6, between periods 2 and 6 and between periods 4 and 6 was steeper in M-types than E-types ($p < 0.05$). During REC, SWA was higher in M-types than E-types in the first 2-h period ($p < 0.05$). Also, the SWA decrease between periods 0 and 2, 0 and 4, and 0 and 6 was larger in M-types than E-types ($p < 0.05$). During BL, the theta activity decrease between periods 0 and 2 and between periods 0 and 4 was steeper in M-types than E-types whereas the reverse was found between periods 4 and 6 ($p \leq 0.05$). During FR1 and FR2, the dynamics of theta activity was similar in both diurnal types. During REC, the theta activity decrease between periods 0 and 2 and between periods 0 and 4 was larger in M-types than E-types ($p < 0.05$). High sigma activity during BL decreased between periods 0 and 4 in M-types while it increased in E-types ($p \leq 0.05$). During FR1, the dynamics of high sigma activity was similar in both chronotypes. During FR2 and REC, high sigma activity decreased between periods 0 and 4 in M-types while it slightly increased in E-types ($p < 0.05$), and the activity increase between periods 0 and 6 was larger in E-types than M-types ($p < 0.05$) in both FR2 and REC. The main Period effect in Fz was significant for all frequency bands except for low sigma.

For the Cz derivation, a main Group effect was found for low sigma with M-types having higher activity than E-types. As for the Fz derivation, significant Group-by-Night-by-Period interactions were found for SWA, theta and high sigma. They are illustrated in Figure 3. During BL, the SWA decrease between periods 0 and 4 was larger in M-types than in E-types ($p < 0.05$). During FR1, the dynamics of SWA was similar in both diurnal types. During FR2, the SWA decrease between periods 0 and 6, and between periods 2 and 6 was larger in M-types than E-types ($p < 0.05$). During REC, the results were the same as

for the Fz derivation: SWA was higher in M-types than E-types in the first 2-h period ($p < 0.05$), and the SWA decrease between periods 0 and 2, 0 and 4, and 0 and 6 was larger in M-types than in E-types ($p < 0.05$). For theta activity, the decrease between periods 0 and 2 and periods 0 and 4 was steeper in M-types than E-types ($p < 0.05$), for both BL and REC nights. During FR1 and FR2, the dynamics of theta activity was similar in both diurnal types. During BL, high sigma activity decreased between periods 0 and 4 in M-types while it increased in E-types ($p = 0.05$). A similar tendency was observed during REC ($p < 0.07$), whereas the dynamics of high sigma activity was similar in both chronotypes in the two fragmentation nights. The main Period effect was significant for all frequency bands in Cz.

In the Pz derivation, a significant Group-by-Night-by-Period interaction was found for alpha activity which is illustrated in Figure 4A. Changes in alpha activity between 2-h periods did not differ between diurnal types in any of the nights. However, the decreasing trend of alpha activity between 2-h periods was significant in M-types for BL and FR2 ($p < 0.05$) whereas it was significant only during FR1 for E-types ($p < 0.01$). A significant Group-by-Period interaction was found in low sigma and is illustrated in Figure 4B. The increase in low sigma activity between periods 0 and 2 and between periods 0 and 4 was larger in M-types than E-types ($p < 0.05$). In addition, the activity decrease between periods 2 and 6 and between periods 4 and 6 was also larger in M-types than E-types ($p < 0.05$). The main Period effect was significant for all frequency bands.

DISCUSSION

These results add further support to the hypothesis of a difference in homeostatic sleep regulation between circadian types. The procedure of behavioral sleep fragmentation increased the duration of time awake by more than 2 h on the first night (FR1) and by about 1.3 h on the second night (FR2) in both groups of subjects. This procedure was successful in increasing sleep pressure, as shown by increased activity in low EEG frequencies during FR2 as well as during REC. As predicted, the increase in homeostatic sleep pressure produced a larger increase in SWA in M-type individuals than in E-types. This difference

was observed in all-night activities and was more pronounced in the first 2 hours of the sleep episode, especially during REC. The larger response of M-types to the increased duration of time awake covered a wide range of slow EEG frequencies, including the theta band. Moreover, it was not only observed in the frontal derivation, considered to be the most sensitive brain area for sleep homeostasis,[9,13,20,21] but also in the central site.

Behavioral sleep fragmentation was efficient in increasing the time awake (decreasing total sleep time and sleep efficiency). The procedure also induced perturbations in the sleep architecture as shown by increased percentages of stages 1 and 2 sleep, and decreased percentages of SWS and REM sleep (Table 1). Moreover, sleep fragmentation modified the activity in all frequency bands in NREM sleep, decreasing low frequency EEG activity during the nights of fragmentation. Therefore, the changes observed in sleep EEG in response to the sleep perturbation cannot be attributed solely to the increase in time awake, which is relatively modest compared to sleep deprivation protocols. Repeated short awakenings or increased stage 1 sleep occurrence during a sleep episode have been shown to have a large impact on the recuperative value of sleep,[33,34] and the present increase in low frequency EEG activity during the recuperation from sleep fragmentation is consistent with a homeostatic response. Of primary importance in the context of this study is that the changes induced in sleep architecture by the procedure of sleep fragmentation, including the increase of time awake, were similar in the two groups of chronotypes.

We have shown previously that, in accordance with some other studies,[14,23] there was no difference in sleep architecture between our M-type and E-type subjects during a normal sleep episode.[35] The present result show that sleep architecture remained similar in the two groups even during and after experimental disruption of the sleep episode. When quantitative EEG of the baseline night was analyzed, the only significant difference in spectral power found between the 2 groups was in the low sigma frequency band (12-14 Hz), with more activity in M-types than in E-types, in all derivations except Fz.[13,35] A similar difference was also observed in the nights of sleep fragmentation and in the night of recuperation. However, there was no Group-by-Night interaction, showing that the response of this frequency band to the increase in sleep pressure was similar in the two groups of subjects. Therefore, even if this frequency range has been associated with the

homeostatic response to sleep deprivation,[9,19] the difference between the two groups does not seem to depend on the previous duration of time awake, at least in the context of this protocol.

Sleep fragmentation increased high sigma activity, which decreased thereafter during recovery. These effects are consistent with the known repercussion of increased sleep pressure on NREM sleep EEG activity.[8,9] In all-night analysis, M-types showed a larger decrease in centro-parietal 14-Hz activity after sleep fragmentation than E-types. This observation is also consistent with an increased homeostatic response in M-types. As observed in our data, activity in high spindle frequencies, as well as its diminution following sleep deprivation, generally dominates in centro-parietal area.[9,36,37] Conversely, in the present study, E-types showed a fronto-central increase in high sigma activity in FR2 and REC compared to BL in the last half of the nights. A frontal increase in high sigma activity following sleep deprivation has also been observed previously.[21] Sleep spindles have been hypothesized to preserve sleep as inhibitors of information processing,[38,39] and sigma activity has been associated with thalamus inhibition.[40] Since E-types do not show a considerable increase in SWA in response to sleep fragmentation, an increase in sleep spindles may help to consolidate their sleep and explain how they could show comparable sleep architecture to M-types. More direct measures of sleep spindles would be necessary in order to confirm this hypothesis.

In conclusion, our results show that M-type individuals have a higher response than E-types to an increase in homeostatic sleep pressure, when the influence of the circadian phase is kept constant. Following sleep fragmentation, low frequency activities (< 8 Hz) showed a higher increase in M-types than in E-types in both frontal and central brain areas, and this response was predominant in the first part of the sleep episode particularly for SWA. This result points toward a higher homeostatic response in M-types for a similar increase in time awake. A higher homeostatic response could be the result of a faster rate of accumulation of sleep pressure during wakefulness, as proposed by Taillard et al. (2003),[14] or either by a higher capacity to generate slow waves as observed in older adolescents.[16] Dose-response curves with varying amount of time awake will be needed to determine more precisely the nature of the homeostatic differences associated with morningness-eveningness.

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FIGURE LEGENDS

Figure 1: All-night spectral EEG power of morning types, expressed relatively to evening types (E-types), during the baseline night (BL), the first and second nights of sleep fragmentation (FR1 and FR2) and the recovery night (REC). Hz-bins are identified by their lower boundary value. Spectral power was calculated in NREM sleep (stages 2, 3 and 4 sleep) for the Fz (A), Cz (B), and Pz (C) derivations. Stars indicate Hz-bins showing significant Group-by-Night interactions.

Figure 2: NREM sleep spectral activity in the Fz derivation, averaged by 2-hour periods during baseline night (BL), first and second nights of sleep fragmentation (FR1 and FR2) and recovery night (REC), for morning types (M-types) and evening types (E-types). The 2-hour periods are identified by their lower boundary value. A. Time course of SWA (1-5 Hz). The star indicates a significant between-group difference. B. Time course of theta activity (4-8 Hz). C. Time course of high sigma activity (14-16 Hz).

Figure 3: NREM sleep spectral activity in the Cz derivation, averaged by 2-hour periods during baseline night (BL), first and second nights of sleep fragmentation (FR1 and FR2) and recovery night (REC), for morning types (M-types) and evening types (E-types). The 2-hour periods are identified by their lower boundary value. A. Time course of SWA (1-5 Hz). The star indicates a significant between-group difference. B. Time course of theta activity (4-8 Hz). C. Time course of high sigma activity (14-16 Hz).

Figure 4: NREM sleep spectral activity in the Pz derivation, averaged by 2-hour periods during baseline night (BL), first and second nights of sleep fragmentation (FR1 and FR2) and recovery night (REC), for morning types (M-types) and evening types (E-types). The 2-hour periods are identified by their lower boundary value. A. Time course of alpha activity (8-12 Hz). B. Time course of low sigma activity (12-14 Hz).

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Table 1 : Sleep architecture parameters (mean ± SEM) for M-type and E-type individuals in baseline night, first and second night of sleep fragmentation and during recovery night.

		Baseline		**Fragmentation 1**		**Fragmentation 2**		**Recovery**	
		M-types	**E-types**	**M-types**	**E-types**	**M-types**	**E-types**	**M-types**	**E-types**
Subjective sleep quality		4.8 ± 0.1	4.2 ± 0.2	2.7 ± 0.3	2.0 ± 0.2	2.9 ± 0.3	3.2 ± 0.3	4.5 ± 0.3	4.4 ± 0.1
Sleep latency (min)		6.7 ± 0.8	7.4 ± 1.5	14.7 ± 6.2	10.3 ± 1.2	6.9 ± 1.5	6.2 ± 1.2	9.3 ± 1.9	11.9 ± 2.5
REM latency (min)		71 ± 10	67 ± 3	179 ± 20	164 ± 21	106 ± 13	86 ± 9	58 ± 5	61 ± 5
Total sleep time (min)		450 ± 4	452 ± 3	318 ± 18	330 ± 11	369 ± 7	369 ± 7	460 ± 2	455 ± 3
Sleep efficiency (%)		95.9 ± 0.6	96.3 ± 0.6	68.3 ± 3.6	70.2 ± 2.3	78.2 ± 1.4	77.8 ± 1.5	97.6 ± 0.2	97.2 ± 0.5
Stage 1	min	27.1 ± 4.8	22.5 ± 2.4	38.4 ± 3.4	32.3 ± 2.5	31.9 ± 2.1	27.4 ± 1.4	21.1 ± 3.7	17.2 ± 2.2
	%	6.1 ± 1.1	5.0 ± 0.6	12.8 ± 1.5	10.1 ± 1.0	8.7 ± 0.6	7.5 ± 0.4	4.6 ± 0.8	3.8 ± 0.5
Stage 2	min	264 ± 7	273 ± 8	216 ± 15	227 ± 6	241 ± 10	248 ± 5	256 ± 8	262 ± 8
	%	58.7 ± 1.4	60.4 ± 1.8	67.6 ± 1.6	69.1 ± 1.3	65.2 ± 2.1	67.3 ± 1.5	55.6 ± 1.6	57.7 ± 2.0
Slow-wave sleep	min	42.4 ± 9.8	38.2 ± 8.0	18.4 ± 5.7	16.2 ± 4.5	32.8 ± 6.7	25.6 ± 5.4	60.8 ± 10.5	52.6 ± 9.3
(stage 3 and 4)	%	9.4 ± 2.1	8.4 ± 1.7	5.8 ± 1.8	4.7 ± 1.2	9.0 ± 1.8	6.8 ± 1.3	13.2 ± 2.3	11.4 ± 2.0
REM sleep	min	116.1 ± 4.7	118.1 ± 5.8	45.2 ± 5.8	54.3 ± 5.8	63.5 ± 3.2	68.5 ± 5.4	122.1 ± 3.5	123.1 ± 8.0
	%	25.9 ± 1.1	26.1 ± 1.3	13.8 ± 1.2	16.1 ± 1.4	17.2 ± 0.7	18.4 ± 1.2	26.6 ± 0.8	27.1 ± 1.7

Table 2: Significant effects of Group-by-Night-by-Period ANOVAs of NREM sleep spectral activity in 3 derivations for the 6 EEG frequency bands.

		Group effect (df=1,22)	Night effect (df=3,66)	Period effect (df=3,66)	Group x Night interaction (df=3,66)	Group x Period interaction (df=3,66)	GroupxNight x Period interaction (df=9,198)
SWA	Fz	ns	30.4***	39.1***	4.5*	3.7*	4.4**
(1-5 Hz)	Cz	ns	33.0***	51.1***	3.5*	3.8*	3.1*
	Pz	ns	33.6***	47.5***	ns	ns	ns
Theta	Fz	ns	18.9***	58.2***	4.2**	ns	2.9*
(4-8 Hz)	Cz	ns	12.4***	55.2***	5.7**	ns	2.9*
	Pz	ns	11.5***	46.3***	ns	ns	ns
Alpha	Fz	ns	7.5**	11.8***	ns	ns	ns
(8-12 Hz)	Cz	ns	3.8*	9.3**	ns	ns	ns
	Pz	ns	5.7**	9.7**	ns	ns	2.3*
Sigma1	Fz	ns	10.0***	ns	ns	ns	ns
(12-14 Hz)	Cz	4.4*	9.8***	6.8***	ns	ns	ns
	Pz	8.0**	9.8***	19.6***	ns	5.3**	ns
Sigma2	Fz	ns	24.3***	21.0***	ns	ns	3.2*
(14-16 Hz)	Cz	ns	29.0***	31.3***	ns	ns	2.4*
	Pz	ns	22.8***	14.4***	ns	ns	ns
Beta	Fz	ns	13.9***	10.0***	ns	ns	ns
(16-24 Hz)	Cz	ns	18.0***	12.5***	ns	ns	ns
	Pz	ns	14.6***	10.5***	ns	ns	ns

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$. Adjusted probabilities are shown.

Degrees of freedom are indicated for Fz and Cz (see text).

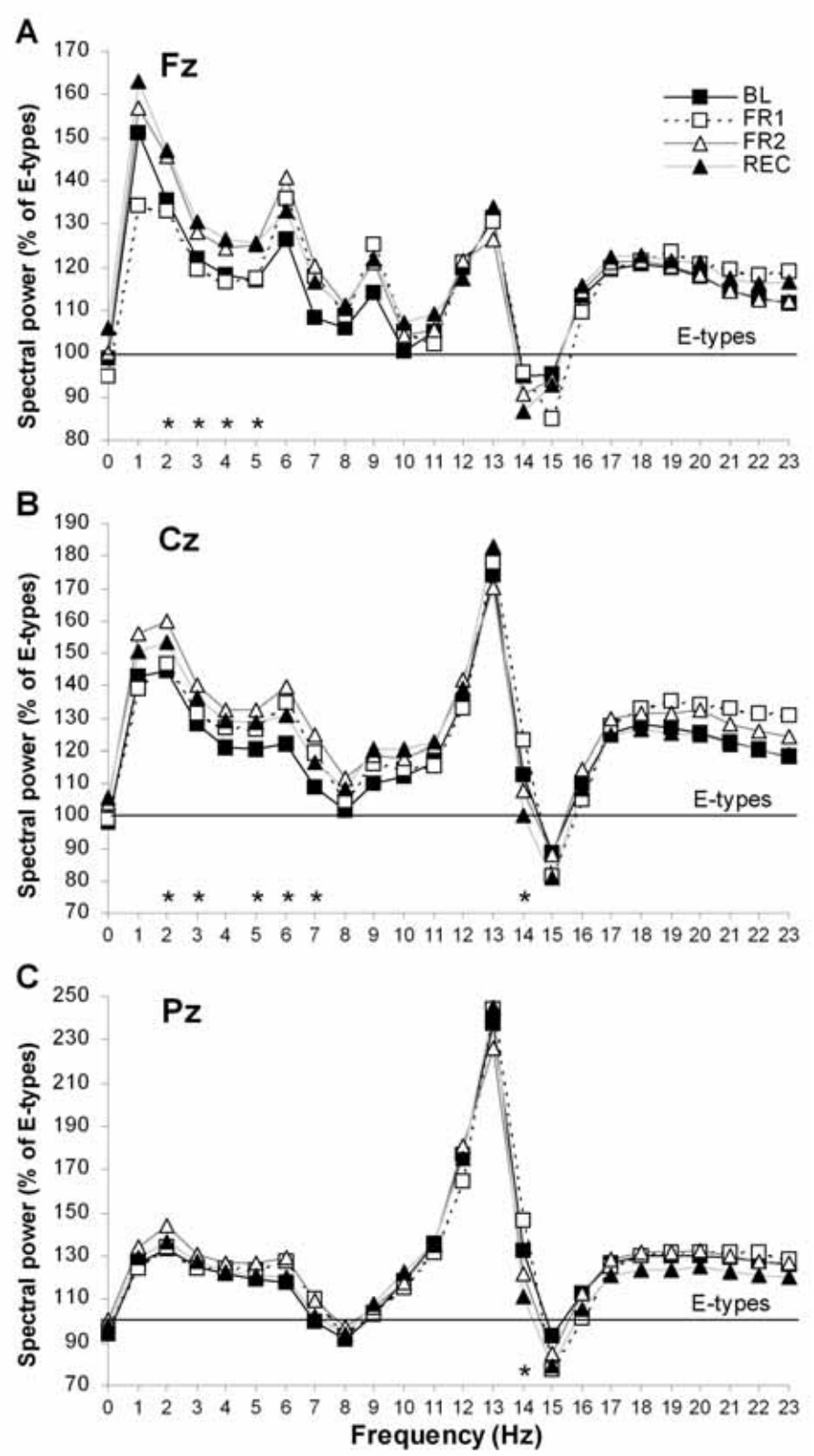
A
Fz
BL
FR1
FR2
REC
E-types
Spectral power (% of E-types)
B
Cz
E-types
Spectral power (% of E-types)
C
Pz
E-types
Spectral power (% of E-types)
Frequency (Hz)

Figure 1

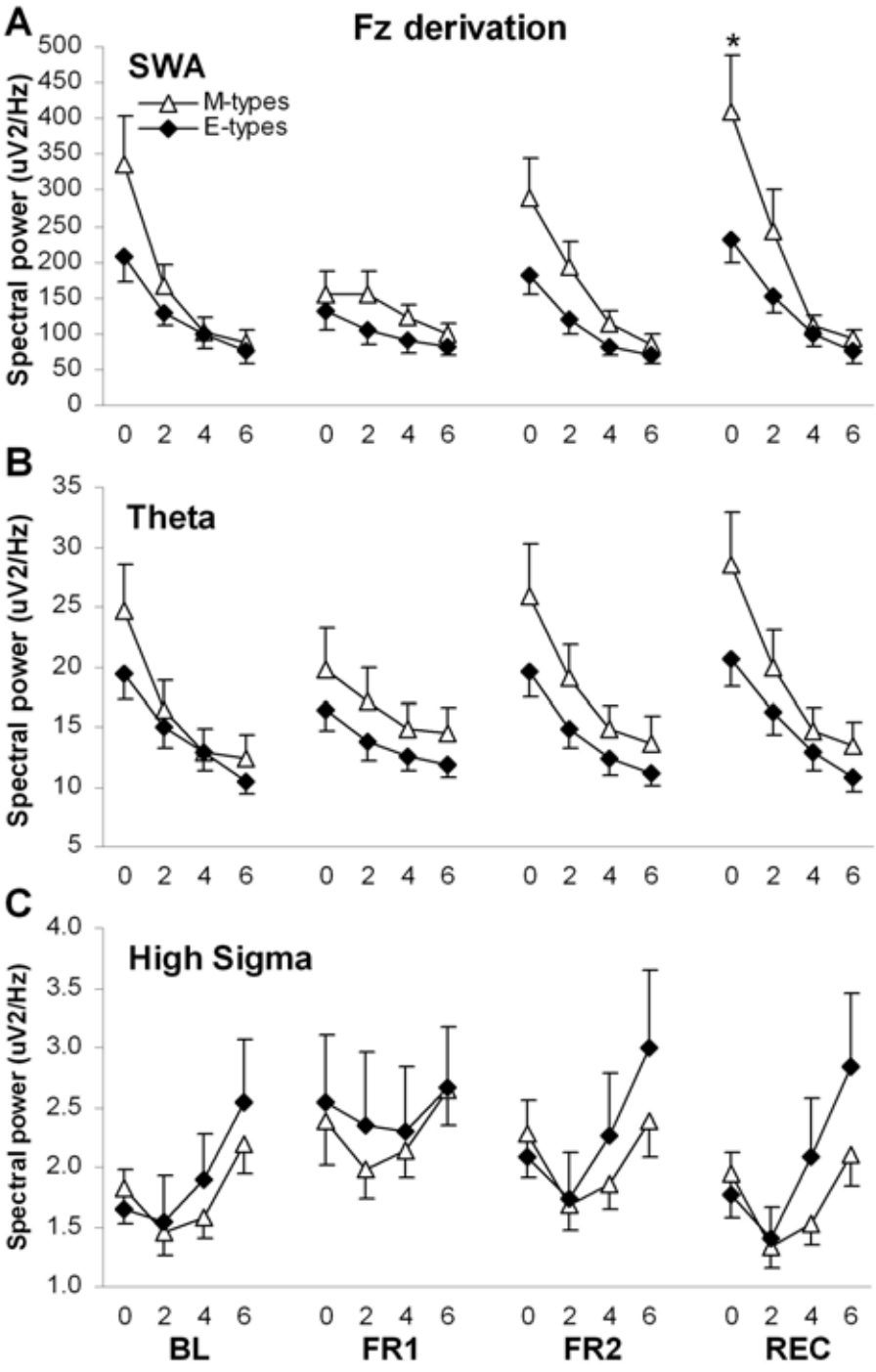
A
Fz derivation
SWA
M-types
E-types
Spectral power (uV2/Hz)
500
450
400
350
300
250
200
150
100
50
0
*
0 2 4 6 0 2 4 6 0 2 4 6 0 2 4 6
B
Theta
Spectral power (uV2/Hz)
35
30
25
20
15
10
5
0 2 4 6 0 2 4 6 0 2 4 6 0 2 4 6
C
High Sigma
Spectral power (uV2/Hz)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0 2 4 6 0 2 4 6 0 2 4 6 0 2 4 6
BL
FR1
FR2
REC

Figure 2

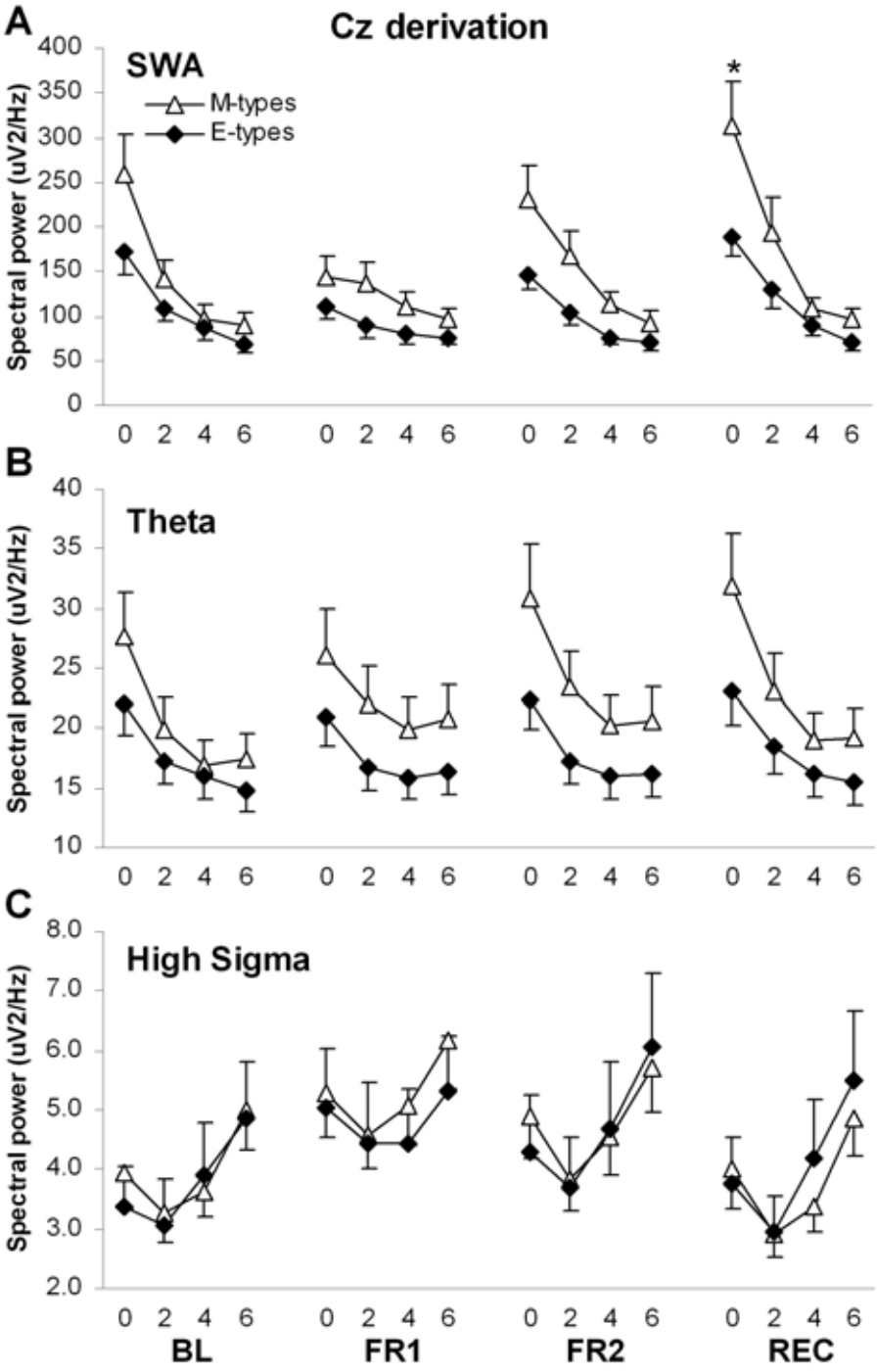
A
Cz derivation
SWA
M-types
E-types
Spectral power (uV2/Hz)
400
350
300
250
200
150
100
50
0
*
0 2 4 6
B
Theta
40
35
30
25
20
15
10
C
High Sigma
8.0
7.0
6.0
5.0
4.0
3.0
2.0
BL
FR1
FR2
REC

Figure 3

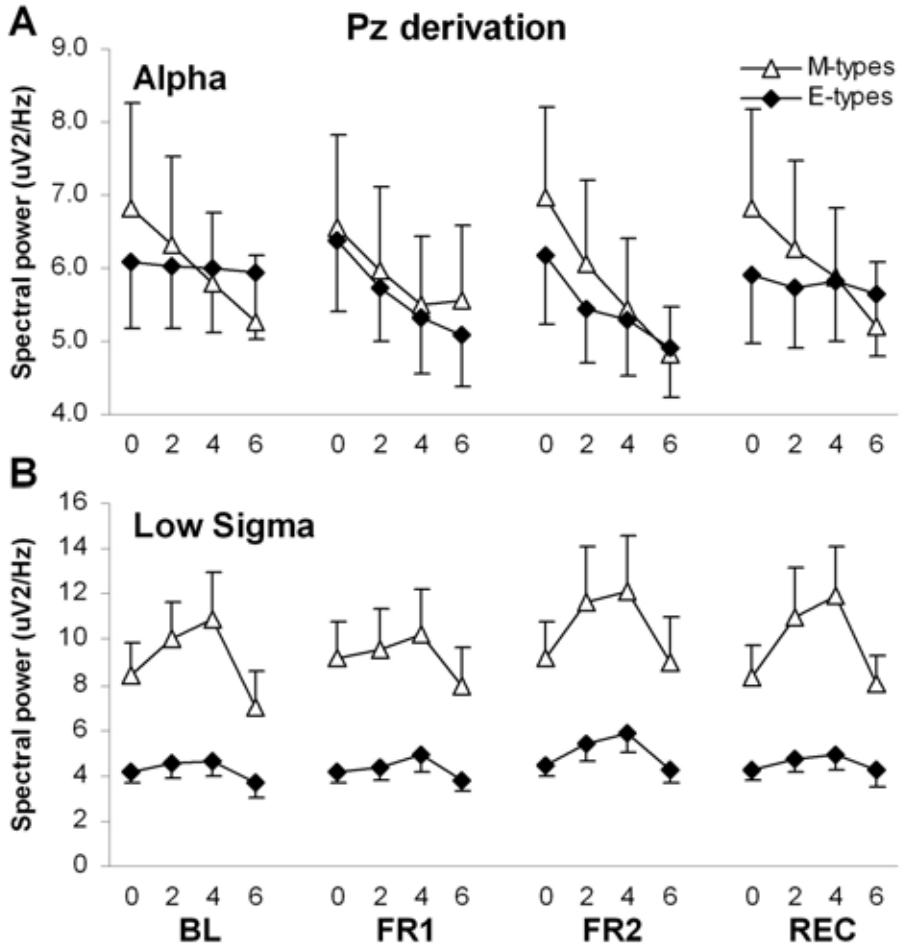
A
Pz derivation
Alpha
M-types
E-types
Spectral power (uV2/Hz)
9.0
8.0
7.0
6.0
5.0
4.0
0 2 4 6 0 2 4 6 0 2 4 6 0 2 4 6
B
Low Sigma
Spectral power (uV2/Hz)
16
14
12
10
8
6
4
2
0
0 2 4 6 0 2 4 6 0 2 4 6 0 2 4 6
BL
FR1
FR2
REC

Figure 4

4.6 Sixième article

Daytime alertness in chronotypes: diurnal variations and effects of behavioral sleep fragmentation

Article en préparation pour soumission dans « *Behavioural Brain Research* »

Daytime Alertness in Chronotypes: Diurnal Variations and Effects of Behavioral Sleep Fragmentation

Valérie MONGRAIN B.Sc.[1,2], Jonathan NOUJAIM[1], Hélène BLAIS[1] and Marie DUMONT Ph.D.[1,2,3]

[1]Chronobiology Laboratory, Sacré-Cœur Hospital of Montréal, Québec, Canada, H4J 1C5; [2]Department of Neurosciences, [3]Department of Psychiatry, Université de Montréal, Québec, Canada, H3C 3J7.

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Corresponding author: Marie Dumont, Ph.D.
Chronobiology Laboratory
Sacré-Cœur Hospital
5400 blvd Gouin W.
Montréal (Québec)
Canada, H4J 1C5
Tel: (514) 338-2222 ext. 2246
Fax: (514) 338-2531
Email: marie.dumont@umontreal.ca

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ABSTRACT

Diurnal variations of subjective and objective measures of alertness were investigated in 12 morning-type and 12 evening-type individuals when their preferred sleep schedule was respected. Levels of alertness were also assessed in the two groups following an increase in homeostatic sleep pressure with behavioral sleep fragmentation. Daytime alertness was measured with a visual analog scale, a profile of mood states, a psychomotor vigilance task, a waking EEG and a multiple sleep latency test. Test series were administered every 4 hours, beginning 1.5 hour after preferred wake time. Alertness levels were assessed after a baseline night, each of two nights of induced sleep fragmentation (5 minutes of forced awakening every half-hour), and after a recovery night. Spectral analysis of waking EEG was performed in Fz and Cz derivations for 5 frequency bands (delta, theta, alpha, sigma, beta). Diurnal variations of alertness were equivalent between morning types and evening types, except for delta EEG activity in Fz and Cz that showed a larger increase between 1.5 and 5.5 h after wake time and a larger decrease between 5.5 and 13.5 h after wake time in evening types than in morning types. Sleep fragmentation induced an increase in beta activity in Cz only in morning types, and a decrease in fastest reaction times only in evening types. These results highlight the similarities in diurnal variations of alertness in chronotypes when studied at their preferred sleep schedule. Nevertheless, our results agree with the hypothesis of a difference in homeostatic sleep regulation between morning and evening types.

Key Words: morningness-eveningness, vigilance, EEG spectral analysis, circadian rhythms, sleep regulation, performance, human.

INTRODUCTION

A major source of interindividual variability in human circadian rhythms resides in morningness-eveningness: some individuals prefer to go to bed early and wake up early (morning-types; M-types) whereas others go to bed late and wake up late (evening-types; E-types). Chronotypes have been reported to differ by approximately 2 hours in both their sleep timing and circadian phase (Kerkhof, 1991; Natale and Cicogna, 2002; Duffy et al., 1999; Baehr et al., 2000; Liu et al., 2000; Bailey and Heitkemper, 2001; Mongrain et al., 2004). Current models of sleep and alertness regulation have defined the interaction of circadian and homeostatic regulatory mechanisms as essential to consolidate sleep and wakefulness (Borbély, 1982; Akerstedt and Folkard, 1995), and morningness-eveningness has also been linked to differences in the dynamics of homeostatic sleep pressure (Taillard et al., 2003; Mongrain et al., 2006). Perturbations of the circadian or homeostastic processes, or of their relationship, affect sleep quality and sleep structure (Carskadon and Dement, 1980; Borbély et al., 1981; Dijk et al., 1990; Dijk and Czeisler, 1995; Dijk et al., 1997), as well as alertness, performance and other neurobehavioral functions (Monk et al., 1989, 1992; Dijk et al., 1992; Jewett et al., 1999; Wyatt et al., 1999; Cajochen et al., 2001; Taillard et al., 2003). It can therefore be expected that M-types and E-types might also differ in the regulation of their levels of alertness.

Alertness and performance during wakefulness have been assessed many times in chronotypes. In general, M-types present earlier diurnal peaks of alertness and performance than E-types (Horne et al., 1980; Foret, 1982; Lack and Bailey, 1994; Kerkhof and Van Dongen, 1996; Natale and Cicogna, 1996; Taillard et al., 2003). Also, M-types have been reported to have high levels of alertness, performance and vigilance in the morning, along with low levels later during the day, when compared to E-types (Horne et al., 1980; Kerkhof et al., 1980; Clodoré et al., 1986, 1990; Petros et al., 1990; Volk et al., 1994; Natale et Cicogna, 1996, 2002). However, these results come from studies that have imposed the same clock time of testing to all subjects, thereby modifying the spontaneous sleep-wake schedule of morning and evening types. Effectively, a testing time around 8 or 9 AM forces a majority of E-types to wake up earlier than usual, while a testing time in the late evening does the reverse for M-types, changing the habitual duration of sleep and

wakefulness in those subjects. In addition, with such a design, M-types and E-types are not tested at their normal circadian time given their circadian phase difference: E-types being tested in the morning closer to their phase of lowest circadian wake propensity, and M-types being tested in the evening also closer to their phase of lowest circadian wake propensity. Therefore, studies that respect the spontaneous, or preferred, sleep schedule of chronotypes are needed to understand the normal diurnal variations of alertness associated to morningness-eveningness.

We recently reported that M-types were having a higher increased than E-types in low frequency EEG activities during recovery sleep following an increase in homeostatic pressure using sleep fragmentation (Mongrain et Dumont, submitted). Therefore, it is possible that levels of alertness also respond differently to homeostatic pressure in M-types and E-types. This hypothesis is supported by a previous study that found a steeper increase in subjective sleepiness and in EEG markers of sleepiness (6-9 Hz spectral activity) in M-types compared to E-types (Taillard et al., 2003). Others studies that have increased homeostatic sleep pressure with a delay of the sleep episode have found that alertness levels were more affected in M-types than in E-types (Breithaupt et al., 1978; Foret et al., 1985), but their design modified both the homeostatic sleep pressure and the relationship between circadian and homeostatic processes of regulation at the same time. To verify the presence of a difference in the dynamics of homeostatic sleep pressure during wakefulness between chronotypes, a method that modifies sleep pressure without changing the normal relationship between the sleep episode and the circadian phase is required.

In this study, we first examined diurnal variations in various measures of alertness in M-type and E-type individuals assessed when sleeping according to their preferred sleep-wake schedule. In this condition, alertness variations are expected to reflect the spontaneous levels of wakefulness associated to morningness-eveningness without interference from an imposed sleep schedule. The second and third objectives of this study were to assess vigilance levels and indexes of homeostatic regulation during wakefulness in chronotypes in response to an increase in homeostatic sleep pressure and after a night of recovery. To achieve these goals, we used a procedure of behavioral sleep fragmentation that increases the levels of homeostatic sleep pressure without changing the timing of the sleep episode.

Because M-types have been reported to have a faster dynamics of build-up and decay of sleep pressure (Taillard et al., 1999; Mongrain et al., 2006, Mongrain et Dumont, submitted), we predicted that M-types will show a larger increase in markers of homeostatic sleep pressure during wakefulness after sleep fragmentation and a better vigilance recuperation following recovery.

METHODS

Subjects

M-type and E-type participants (19 to 34 y) were recruited using a French version of the Morningness-Eveningness Questionnaire (MEQ; Horne and Östberg, 1976). Twenty-four subjects completed the study: 12 M-types (MEQ scores 59 to 71, mean 65.9 ± 1.1) and 12 E-types (MEQ scores 27 to 40, mean 32.7 ± 1.2). There were 6 women and 6 men in each group. Age was similar in the two groups (M-types: 24.7 y ± 1.5; E-types: 23.4 y ± 0.7). All subjects were in good physical and psychological health, and had no sleep complaints. Selected subjects had a regular sleep schedule with an habitual sleep duration between 7 and 9 h. A 24-h laboratory screening confirmed the absence of sleep and vigilance disorder by polysomnography and a multiple sleep latency test (MSLT; Carskadon et al., 1986). Inclusion criteria were: sleep efficiency higher than 85 %, sleep latency lower than 30 min, apneas/hypopneas index and periodic leg movements index lower than 5, and average diurnal sleep latency higher than 7 min. Subjects had no night work experience in the past year and no transmeridian travel in the past three months. They were all non-smokers and reported not using drugs or medications, except oral contraceptives. Women not using hormonal contraception (3 M-types and 4 E-types) were studied during the follicular phase of their menstrual cycle. Each subject signed an informed consent form approved by the hospital ethics committee and received a financial compensation. As reported previously (Mongrain et al., 2004), circadian phase of salivary melatonin and core body temperature were 2.7 h and 2 h earlier, respectively, in M-types than in E-types (melatonin onset: 20:41 ± 27’ vs. 23:23 ± 25’ and temperature minimum: 04:17 ± 23’ vs. 06:17 ± 29’).

Procedures

For the study, individual sleep schedules were determined according to each subject's preferred bedtime and wake time using information from screening sleep diaries during free days and preferred wake time and bedtime reported in the MEQ. The final decision for the study sleep schedule was made after discussion with the subject to ensure that it was similar to the schedule that he/she would spontaneously adopt. Bedtime and wake time where determined for a sleep duration of 8 hours. Subjects were requested to follow their selected sleep schedule (± 30 min) for 7 days prior to laboratory admission. Compliance was verified by sleep diaries and by 24-h ambulatory measures of activity and light exposure (Actiwatch-L, Mini-Mitter Co, Bend, OR). Selected sleep schedules were more than 2.5 h earlier in M-types than in E-types (wake time: 07:08 ± 11 vs. 09:45 ± 17; Mongrain et al., 2005).

Subjects were admitted to the laboratory 4 h before their scheduled bedtime and slept five nights according to their individual sleep schedule: an adaptation night, a baseline night (BL), two nights of behavioral sleep fragmentation (FR1 and FR2) and a recovery night (REC). The last four nights were followed by days of alertness assessments (dBL, dFR1, dFR2 and dREC). During the nights of sleep fragmentation, subjects were awakened for a duration of 5 minutes every half-hour for a total of 15 awakenings per night (Dumont et al., 2000). For each awakening, a technician knocked on the door and entered the room with a small flashlight. Subjects had to interact verbally with the technician for the entire 5 minutes. The room light was not turned on and subjects were not required to open their eyes. Another technician stayed in the control room to keep track of the time and to confirm wakefulness according to on-line EEG recordings. Sleep architecture, including the increase in wake time due to the induced sleep fragmentation, was similar in M-types and E-types for BL, FR1, FR2 and REC nights (Mongrain and Dumont, submitted).

Daytime alertness was assessed using a sequence of five different tests repeated every four hours, starting 1.5 h after wake time. Therefore, the sequence was administered at 1.5, 5.5, 9.5 and 13.5 h after wake time for dBL, dFR1 and dFR2. The sequence scheduled at 13.5 h after wake time was not administered on dREC because subjects were leaving the laboratory at that time. Each of the 15 sequences lasted approximately 1.5 h, and the tests

were administered in the following order: a 10-cm visual analog scale of alertness (VAS; McCormack et al., 1988); a profile of mood states (POMS; McNair et al., 1971); a 10-min visual psychomotor vigilance task (PVT; Dinges et al., 1997); a 2-min waking EEG recording (wEEG) with eyes open fixing a black cross on a wall approximately 3 meters away; and finally a multiple sleep latency test of a maximum duration of 25 minutes (MSLT; Carskadon et al., 1986).

EEGs recordings were done with 25 EEG, 2 EOG and 3 chin EMG electrodes, using a referential montage with linked-ears. Signals were recorded using a polygraph Grass Model 15A54 amplifier system (Astro-Med Inc., West-Warwick, USA; gain 10000, bandpass 0.3-100 Hz) and digitized at a sampling rate of 256 Hz with a commercial software (Harmonie 5.1, Stellate Systems, Montreal, Canada). For wEEGs, spectral analysis was performed with a commercial software package (Sensa, Stellate Systems, Montreal, Canada) on EEG selections free of artifact for Fz and Cz derivations. Spectral power was obtained by fast Fourier transforms (FFT) computed on 2-sec sections using a Hanning window tapering resulting in a 0.5 Hz spectral resolution. Spectral power was averaged for each EEG recording in 5 frequency bands: delta (0-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (12-18 Hz) and beta (18-25 Hz). For MSLT, sleep latencies were determined using the C3 derivation and defined as the time from lights off to the first min of stage 1 or to the first epoch of any other sleep stage, or as 25 minutes if no sleep occurred.

Statistical Analysis

Each measure was transformed in percent of the mean of the four results obtained during BL to normalize the data and to reduce variability. For the POMS, results are presented only for the Vigor scale because the other scales did not show enough variations during the protocol. For the PVT, mean reaction time (mean RT) and mean of the 10% fastest reaction times (10% fastest RT) were analyzed. PVT data of a M-type woman were missing due to technical problems during dBL. To examine diurnal variations in alertness in M-type and E-type individuals, Group-by-Moment (2x4) ANOVAs were used to assess between-group differences in diurnal variations of alertness during dBL. To assess vigilance levels and indexes of homeostatic regulation during wakefulness in chronotypes in response to an increase in homeostatic sleep pressure, a second set of analyses were conducted. Group-by-

Day-by-Moment (2x3x4) ANOVAs were used to assess between-group differences in variations of alertness in dBL, dFR1 and dFR2 for the 4 moments of the 3 days. For this set of analyses, wEEGs data of one E-type man and Vigor data of one M-type man were not used because of an artifactual wEEG during dFR1 and a missing Vigor scale during dFR2. Finally, to assess vigilance levels and indexes of homeostatic regulation after a night of recovery, Group-by-Day-by-Moment (2x2x3) ANOVAs were this time used to assess between-group differences in changes between dBL and dREC for the first 3 moments of the days. For this third set of analyses, Vigor data of one M-type woman were not used because of a missing value during REC. Huynh/Feldt corrections were used for repeated measures but the original degrees of freedom are reported. Significant interactions were decomposed using simple effect analysis. Statistical significance was set to 0.05.

RESULTS

Diurnal variations

Results of alertness measures are presented in figure 1 for subjective alertness, POMS, PVT and MSLT data, and in figure 2 for wEEG frequency bands. A trend for a Group-by-Moment interaction was found for subjective sleepiness (VAS; $F_{3,66} = 2.7$, $p = 0.07$). As illustrated in figure 1 (upper panel), alertness tended to be lower in E-types than in M-types 1.5 h after awakening, but higher than in M-types 13.5 h after awakening. There was no Group-by-Moment interaction for the Vigor scale, PVT or MSLT results, but for the wEEG, a significant interaction was found for delta activity in both Fz ($F_{3,66} = 3.2$, $p = 0.03$) and Cz ($F_{3,66} = 3.4$, $p = 0.02$) derivations. As illustrated in figure 2 (upper panels) for dBL, delta activity was higher in E-types than in M-types 5.5 h after awakening ($p < 0.01$). Also, for both derivations the increase in delta activity between 1.5 and 5.5 h after awakening was larger in E-types than in M-types ($p < 0.01$). Finally, delta activity decreased between 5.5 and 13.5 h after awakening in E-types while it slightly increased in M-types ($p < 0.05$). Significant Moment effects were found for VAS, sleep latency (MSLT) and spectral activity in all frequency bands of the wEEG for both Fz and Cz derivations ($F_{3,66} \geq 3.7$, $p \leq 0.03$). However, no significant diurnal variation was observed for the results of the Vigor scale and of the PVT test during dBL.

Insert Figure 1 about here (3.x inches wide, xx inches high)

Insert Figure 2 about here (6.x inches wide, xx inches high)

Effects of sleep fragmentation

Two measures of alertness showed a different effect of sleep fragmentation in the two chronotypes: the 10% fastest RT on the PVT and beta activity in the Cz derivation of the wEEG. A significant Group-by-Day interaction was found for the 10 % fastest RT ($F_{2,42}$ = 4.1, $p < 0.05$), indicating a decrease in performance only in E-types after the first night of sleep fragmentation ($p < 0.05$; Figure 1, fourth panel from the top). The significant Group-by-Day interaction found for beta activity in the Cz derivation of the EEG ($F_{2,42} = 3.3$, $p < 0.05$) showed that beta activity increased between dBL and dFR1 only in M-types ($p = 0.01$).

Significant main Day effects were found for VAS, Vigor, RT and 10% fastest RT (PVT) and sleep latency (MSLT) ($F_{2,42\text{-}2,44} \geq 3.8$, $p < 0.05$), indicating a deterioration of alertness in the two groups for dFR1 (significant between dBL and dFR2 only for Vigor scale). Except for beta activity in Cz ($F_{2,42} = 2.9$, $p = 0.06$), sleep fragmentation increased wEEG activity in the two groups, for all frequency bands in both derivations ($F_{2,42} \geq 3.7$, $p < 0.05$).

Recovery from sleep fragmentation

Subjective alertness was the only measure to show a significant difference between the two groups for the results on dREC compared to dBL. The significant Group-by-Day interaction found for VAS ($F_{1,22} = 6.5$, $p < 0.05$) showed that M-types tended to report lower alertness levels during dREC than during dBL ($p = 0.1$), while E-types reported the reverse ($p = 0.05$; Figure 1, top panel). A significant Group effect was found for the 10% fastest RT of the PVT ($F_{1,22} = 4.5$, $p < 0.05$), with M-types having faster RT than E-types ($p < 0.05$; mainly on REC; Figure 1, fourth panel from the top). For wEEG, a significant Group-by-Moment interaction was found for delta activity in Cz ($F_{2,44} = 3.3$, $p < 0.05$). As detailed in the first results section, the activity increased between 1.5 and 5.5 h after awakening was larger in E-types than in M-types. Also, while delta activity increases from 1.5 to 9.5 h after awakening in M-types; activity peaks at 5.5 h after awakening in E-types and decreases thereafter. In the Fz derivation, no significant effect including the Group factor was found in any of the frequency bands for the analyses including REC. Significant

main Day effects were found for Vigor and MSLT ($F_{1,22} \geq 9.9$, $p \leq 0.01$), indicating a deterioration of Vigor and a lengthening of sleep latencies in dREC compared to dBL. For wEEG, a significant Day effect was observed for alpha activity in Fz and Cz ($F_{1,22} \geq 5.0$, $p \leq 0.05$), with an increase of alpha activity in dREC compared to dBL ($p < 0.05$).

DISCUSSION

Our results indicate that when chronotypes are studied during their preferred waking phase, differences in diurnal variations of alertness are greatly reduced. Effectively, only one of our alertness measures (wEEGs) showed significant Group-by-Moment interactions. Therefore, diurnal time course of subjective sleepiness, mood, performance and sleep tendency appears to be the similar in M-type and E-type individuals with respect to their habitual sleep-wake schedule. These results highlight the importance, in studies interested in the regulation of alertness, of studying populations according to their own rhythms in the assessment of sleep and wakefulness quality. Our results also show that the increase in homeostatic sleep pressure due to sleep fragmentation produced a different response in the two groups of chronotypes regarding optimal psychomotor performance and beta EEG activity. These results suggest that, when the relationship between the sleep schedule and circadian phase is kept constant, sleep pressure affects specific aspects of alertness in different ways in the two circadian types.

Regarding diurnal variations, our results are contrasting with most of previous studies on quality of wakefulness in chronotypes (Horne et al., 1980; Foret, 1982; Lack and Bailey, 1994; Kerkhof and Van Dongen, 1996; Natale and Cicogna, 1996; Taillard et al., 2003). Effectively, our M-types and E-types were having similar time courses of mood, performance and sleep latencies during wakefulness. These between-study differences originate from the fact that sleep schedule of subjects was respected in our study. We have reported previously that when these subjects were studied according to their preferred sleep schedule, the position of their circadian phase in relation to their sleep episode (the "phase angle") was equivalent (Mongrain et al., 2004). It is therefore not surprising that those

alertness variables having a strong circadian modulation, as for example sleep propensity (Dijk and Czeisler, 1994; Lavie, 2001), showed identical variations in our different chronotypes. In the present study, if alertness had been evaluated according to clock time, we would have probably also observed earlier diurnal peaks of vigilance in M-types than in E-types.

However, even when studied in optimal conditions, E-types tend to report being sleepier upon awakening than before sleep and M-types sleepier before going to bed than after awakening. This is in agreement with previous observations on subjective alertness (Kerkhof et al., 1980; Ishihara et al., 1987; Clodoré et al., 1986; Volk et al., 1994; Natale et Cicogna, 1996, 2002). It would be possible that E-types had stronger sleep inertia and/or a less recuperative sleep. In fact, even if these M- and E-types have similar sleep architecture, E-types tended to show less slow-wave activity (Mongrain et al., 2005) which could be an indication of less efficient sleep. As proposed before, M-types may have a faster buildup of sleep pressure during wakefulness (Taillard et al., 2003), what could confer them a lower feeling of alertness before sleep.

Our results regarding EEG activity do not agree with Taillard et al. (2003) work. Our data failed to show difference in diurnal variations of theta activity between chronotypes. Moreover, diurnal variations in delta activity differ between M-types and E-types. E-types showed a higher increase in delta during the first part of the day and a decrease after whereas M-types showed an increase from waketime to 9.5 h later and a slight decrease before bedtime. Delta activity has been reported to be under the influence of both the circadian system and the sleep homeostat (Aeschbach et al., 1997; Dumont et al., 1999; Cajochen et al., 2002). Since chronotypes in this study were having the same position of their circadian phase in relation to their sleep-wake schedule, differences in delta time course should be expected to reflect differences in homeostatic sleep regulation. Therefore, the stronger increase in delta activity in E-types in the first half of the day could indicate a faster buildup in E-types compared to M-types. Conversely, while delta activity decreases in E-types from 5.5 h after wake time to the end of the day, it increases in M-types. The circadian wake propensity increases during the day, which should produce a decrease in delta activity during wakefulness (delta activity being negatively associated with alertness:

Cajochen et al., 1999). Therefore, the time course of delta activity in M-types may point toward a higher accumulation of sleep pressure. Definitely, more studies evaluating the dynamics of homeostatic sleep pressure during prolonged wakefulness when the habitual relationship between sleep and circadian phase is taken into account would be needed in chronotypes to clarify these interpretations.

Regarding the effects of an increase in sleep pressure using behavioral sleep fragmentation, we observed different consequences in chronotypes. M-types showed an increase in beta activity after the first night of sleep fragmentation, but E-types showed a decrease in optimal RT following the first night of sleep fragmentation. Vis-à-vis the recovery from behavioral sleep fragmentation, M-types maintained a better optimal performance than E-types, which agree with our hypothesis. However, M-types tended to have a lower subjective alertness than E-types. Dissociation in the variations in different alertness measures have been reported several times (Broughton, 1982; Clodoré et al., 1986; Danker-Hopfe et al., 2001; Leproult et al., 2003), therefore, it is not unexpected that M-types and E-types differ on distinct ways to qualify vigilance. Moreover, vigilance is not only regulated by circadian and homeostatic processes but also by the level of stimulation in the environment and the level of motivation (Mavjee and Horne, 1994; Hull et al., 2003), the last variable having hardly been controlled in our experiment.

From one point of view, the diminished performance observed in E-types following sleep disruption and recovery may be associated with differences in sleep quality. One study have shown that fastest RT where associated with an increase amount of slow-wave sleep (SWS; Jurado et al., 1989) whereas another reported that increase performance was associated with increase SWA (Huber et al., 2004). In the present sample of subjects, M-types and E-types had similar amount of SWS but different amount of SWA in the recovery night (Mongrain et Dumont, submitted). The higher quantity of slow EEG activity during sleep might have conferred to M-types the capacity to remain quite fast in the performance task. However, the slowdown of fastest RT appears after the first night of sleep fragmentation while the difference in SWA is restricted to recovery night. The link between these two variables will have to be further examined before any conclusion could be traced.

Beta activity has also been reported to be under the influence of homeostatic sleep pressure and most of the reports have shown that this frequency band was free of circadian influences (Corsi-Cabrera et al., 1992; Aeschbach et al., 1997, 1999; Dumont et al., 1999 except Cajochen et al., 2002). Moreover, variations in waking EEG spectral activities have been reported to emerge when the amount of sleepiness becomes considerable, as during a sleep deprivation for example (Åkerstedt et Gillberg, 1990; Aeschbach et al., 1997). Therefore, a higher beta increases following an increase sleep pressure could be interpreted as a higher homeostatic response in M-types. If M-types don't have a faster increase of sleep pressure during wakefulness, they could alternatively have a higher capacity for accumulation of sleep pressure: a higher maximum level, as also suggested by our observations in diurnal variations of delta activity. The reason why between-chronotypes difference is only observed for the beta frequency band and not for delta and theta frequency remains unknown. However, differences might emerge in beta in the context of sleep fragmentation because of the association between this frequency band and the effort to stay awake (Lorenzo et al., 1995; Dumont et al., 1999; Strijkstra et al., 2003).

These results underline the importance of studying characteristics of alertness when the habitual/preferred sleep schedule of individuals is respected in order to understand its regulation. In morningness-eveningness, the diurnal variations in different measures of alertness appear to be surprisingly similar with respect to spontaneous wake position. However, when the level of sleep pressure is increased and the position of circadian phase is kept constant, M-type and E-type individuals are affected on specific parameters of vigilance. These results agree with the hypothesis of difference in homeostatic sleep regulation between chronotypes and add up that effects of an increase in sleep pressure could affect different arousal system in different chronotypes.

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FIGURE LEGENDS

Figure 1: The 15 assessments of alertness shown for subjective sleepiness (VAS, upper panel), Vigor of the POMS (second panel from the top), mean PVT reaction times (third panel from the top), mean 10% fastest PVT reaction time (fourth panel from the top) and daytime sleep latencies (MSLT, lower panel). Alertness assessments are separated by days of measurements: baseline day (dBL), fragmentation 1 day (dFR1), fragmentation 2 day (dFR2) and recovery day (dREC). All measures are expressed in percent of the mean of the baseline day. Y scales have been reversed for Vigor, mean PVT reaction times and mean 10% fastest PVT reaction times in order for upper values to represent higher alertness in every measures.

Figure 2: Spectral activity during the 15 waking EEG recordings in the Fz derivation (left panels) and Cz (right panels) shown for delta (0-4 Hz, upper panels), Theta (4-8 Hz, second panels from the top), Alpha (8-12 Hz, third panels from the top), Sigma (12-18 Hz, fourth panels from the top) and Beta (18-25 Hz, lower panels). Activities are separated by days of measurements: baseline day (dBL), fragmentation 1 day (dFR1), fragmentation 2 day (dFR2) and recovery day (dREC). All activities are expressed in percent of the mean of the baseline day.

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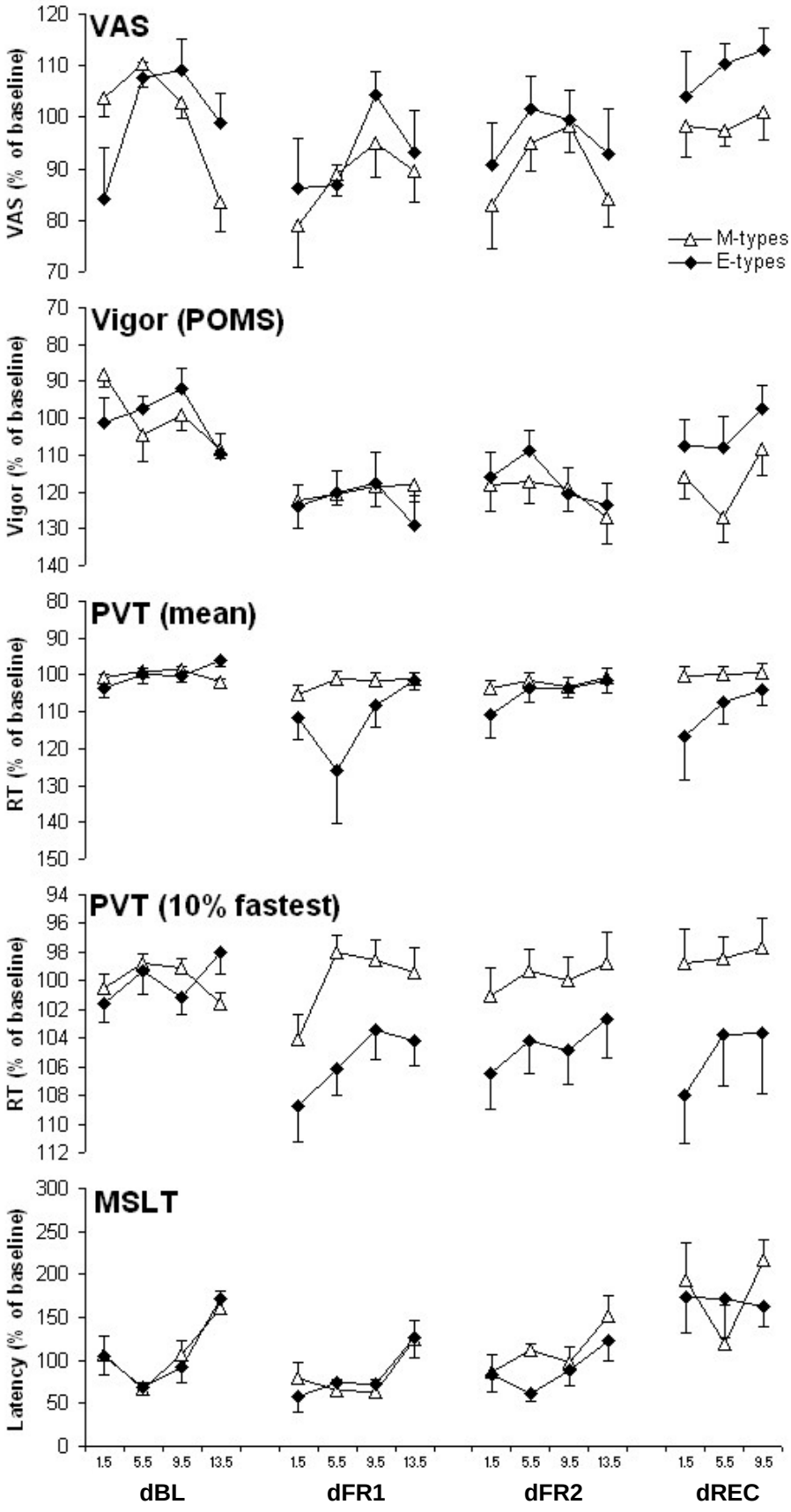
VAS
VAS (% of baseline)
M-types
E-types
Vigor (POMS)
Vigor (% of baseline)
PVT (mean)
RT (% of baseline)
PVT (10% fastest)
RT (% of baseline)
MSLT
Latency (% of baseline)
1.5
5.5
9.5
13.5
dBL
dFR1
dFR2
dREC

Figure 1

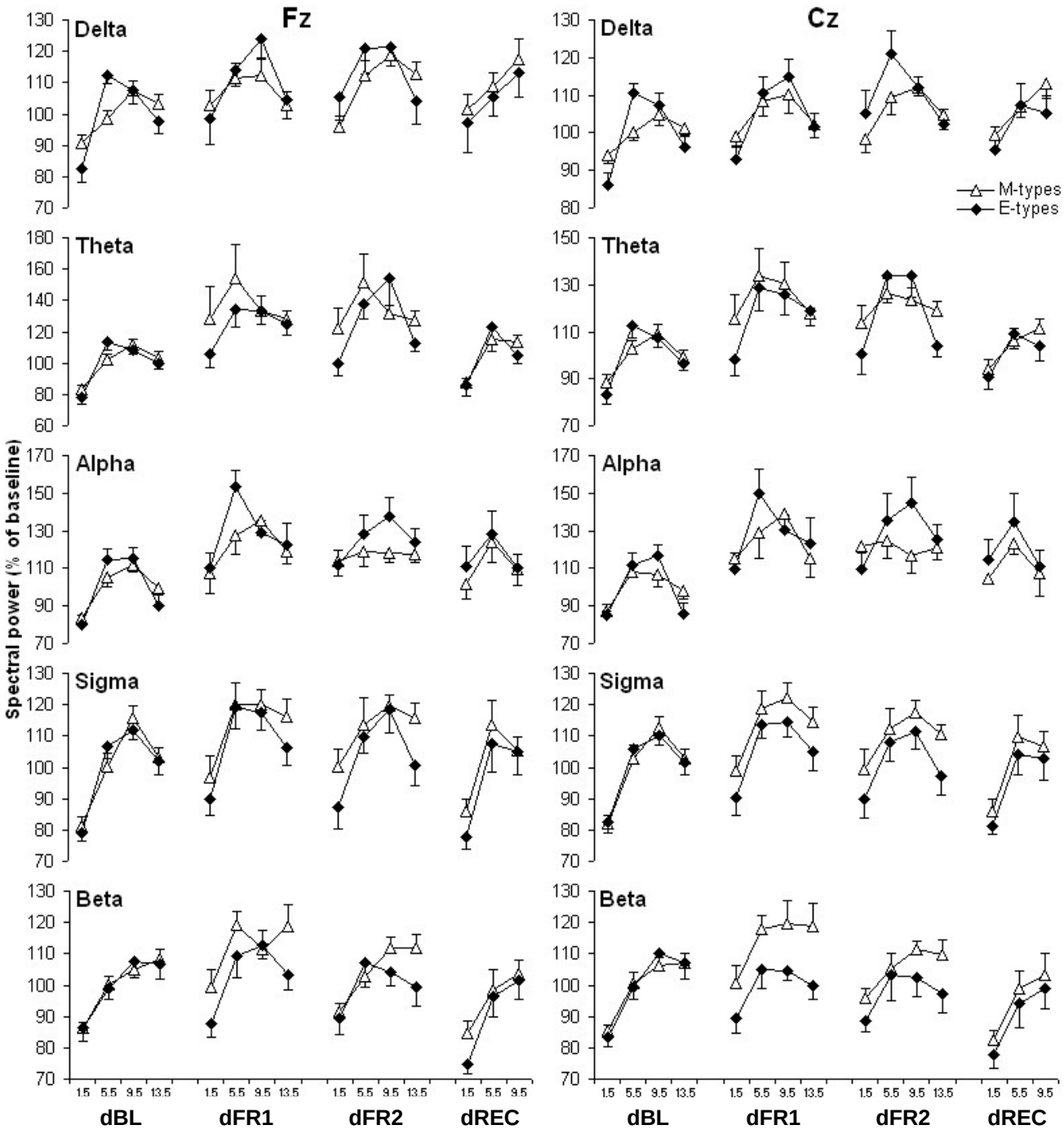
Fz
Cz
Delta
Theta
Alpha
Sigma
Beta
M-types
E-types
Spectral power (% of baseline)
1.5 5.5 9.5 13.5
dBL
dFR1
dFR2
dREC

Figure 2

5. Discussion

Dans le cadre de ce programme de recherche, le premier objectif général était d'examiner le processus S chez des individus synchronisés de façon normale à des angles de phase différents, c'est-à-dire chez qui la relation entre l'épisode de sommeil et la phase circadienne diffère. La première étape était donc de vérifier si nos Types-M et nos Types-S différaient bel et bien dans leur angle de phase. Or, contrairement à ce qui avait été rapporté dans la littérature, aucune différence d'angle de phase n'a été trouvée entre les chronotypes. Par le fait même, les trois objectifs généraux du présent programme de recherche ne pouvaient plus être atteints : il devenait impossible de vérifier la force du processus S, l'effet d'une augmentation de pression homéostatique et la récupération suite à celle-ci chez des personnes qui dorment à des phases circadiennes différentes. Cependant, les données accumulées lors de ce projet nous permettaient, en alternative, d'étudier la régulation du sommeil normal chez les chronotypes. De plus, tel que détaillé plus loin (section 5.4), certaines observations inattendues allaient tout de même nous permettre d'étudier l'interaction entre les processus C et S de régulation du sommeil avec le modèle des chronotypes, but premier de ce projet de recherche.

5.1 Absence de différence d'angle de phase

Dans un premier temps, nous avons observé que les Types-M avaient un horaire de sommeil ainsi qu'une phase circadienne de sécrétion de mélatonine et de température corporelle plus hâtive que les Types-S. Malencontreusement, nos résultats ont montré une absence de différence d'angle de phase entre chronotypes. Après vérification, nous n'étions pas les seuls à ne pas avoir observé de différence d'angles de phase entre les différents chronotypes (Ishihara et al., 1987; Bailey et Heitkemper, 2001; Crowley et al., 2006). Cependant, lorsque la relation entre la position de la phase et l'angle de phase a été vérifiée dans l'ensemble de l'échantillon, Types-M et Types-S regroupés, une phase tardive était associée à un angle de phase long, exactement comme ce qui avait été observé auparavant (Martin et Eastman, 2002) et comme le prédisent les mécanismes d'entraînement en

fonction de la longueur de la période endogène (Pittendrigh et Daan, 1976; Aschoff, 1981; Lewy et al., 2001). Ce dernier résultat était fort surprenant puisqu'il soulevait une contradiction importante dans nos résultats. Comment était-il possible que deux groupes de sujets ayant des phases circadiennes différentes ne montrent pas de différence d'angle de phase si la position de la phase etait associée à l'angle de phase dans cet échantillon ? (voir raisonnement illustré à la figure 4 ci-dessous)

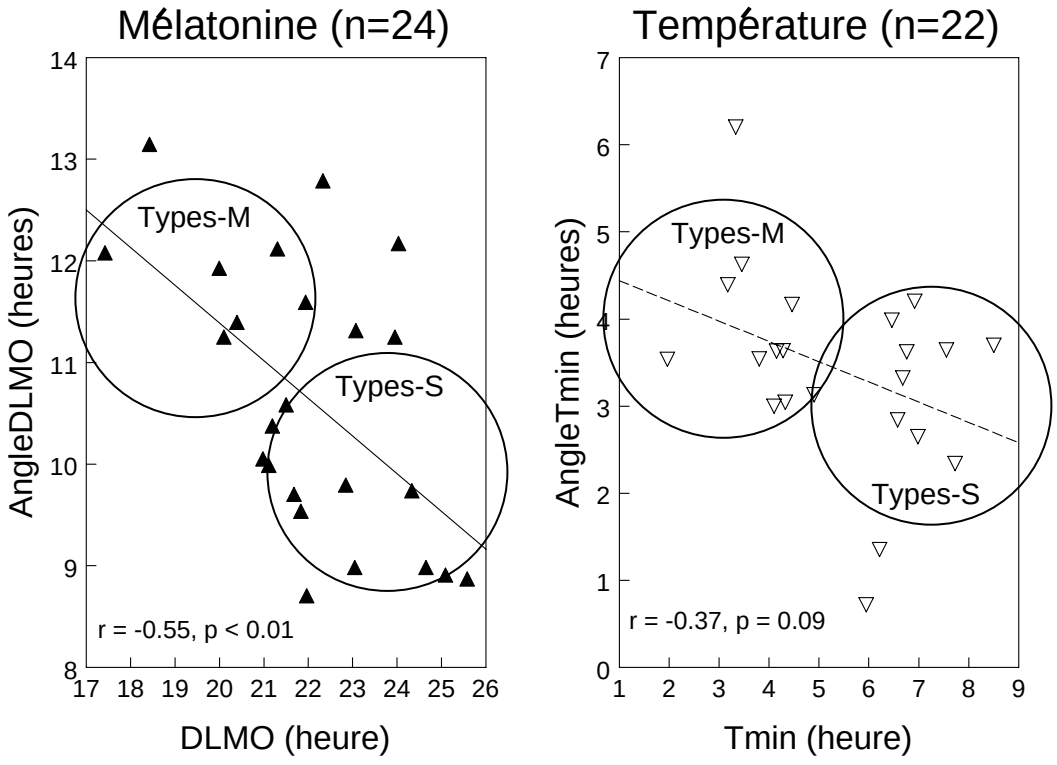

Figure 4 : Relation entre la position de la phase et l'angle de phase observée chez tous les sujets pour la mélatonine et la température. Pour la mélatonine, la relation significative sous-tend que phase tôt = angle long et phase tard = angle court (même tendance pour les données de température). Les cercles représentent la position attendue de chaque groupe de chronotype étant donné les différences de phase entre les groupes.

L'explication provenait du fait que la relation entre phase et angle de phase était significative à l'intérieur même des 2 groupes de chronotypes et non seulement avec la combinaison des 2 groupes ensemble (figure 1 du premier article de cette thèse, Mongrain

et al., 2004; page 52). Ceci signifiait que peu importe le chronotype, les mécanismes d'entraînement selon la période semblaient à l'oeuvre pour une variété étendue de périodes endogènes. Cependant, cette observation n'a pu être faite que parce que les 2 groupes de chronotypes avaient un éventail plutôt large de positions de phase circadienne. Effectivement, une grande partie des Types-M et des Types-S avaient des positions de phase assez intermédiaires. La suite des réflexions conduisit à l'identification de 2 sous-groupes de chronotypes : des individus avec une phase circadienne très avancée ou très retardée, ayant différents pointages au QMV et différents horaires de sommeil, chez lesquels la différence d'angle de phase était dans le sens attendu; et des individus avec une phase circadienne similaire, ayant aussi différents pointages au QMV et différents horaires de sommeil, mais chez qui la différence d'angle de phase allait dans le sens inverse. Les angles de phases opposés dans les 2 sous-groupes expliquaient que dans l'ensemble, les Types-M et les Types-S avaient en moyenne des angles de phase similaires. Mais quel était donc le mécanisme produisant des horaires de sommeil différents chez les Types-M et les Types-S du second sous-groupe, s'ils possèdaient des phases circadiennes semblables ? Puisque le sommeil et l'éveil sont régulés par deux grands processus, la solution se trouvait peut-être au niveau de l'autre mécanisme de régulation du sommeil. Tel que présenté dans le premier article cette thèse, nous avons proposé l'hypothèse d'une différence dans le processus homéostatique de régulation du sommeil comme source de différences d'horaire de sommeil chez les chronotypes.

5.2 Différences homéostatiques entre chronotypes

Les résultats subséquents de nos travaux ont fortement appuyé l'hypothèse d'une différence dans la régulation homéostatique du sommeil entre les chronotypes. Les personnes à typologie circadienne matinale ont une dynamique homéostatique plus rapide que les personnes à typologie circadienne vespérale.

5.2.1 En sommeil

L'étude des marqueurs du processus S en sommeil a été d'un grand intérêt dans ce projet de recherche. En premier lieu, les marqueurs de S ont été étudiés au cours d'un épisode de

sommeil normal, plus particulièrement en sommeil lent au niveau de l'aire centrale du cortex cérébral à l'aide de la dérivation classique C3 (Mongrain et al., 2005). Nous avons observé que les Types-M et les Types-S semblaient différer dans la dissipation de l'AOL et de l'activité thêta entre cycles sommeil lent/sommeil paradoxal au cours de la nuit. Les Types-M apparaissaient donc avoir une dynamique plus rapide de dissipation homéostatique, ce qui concordait avec certaines données antérieures (Kerkhof, 1991; Lancel et Kerkhof, 1991) mais avec l'avantage d'avoir respecté rigoureusement l'horaire libre et spontané des individus. Au cours de cette étude, l'interaction avec le genre a également été étudiée et nous a révélé que les chronotypes diffèrent davantage chez les hommes que chez les femmes sur certains paramètres reliés à la qualité du sommeil mais pas au niveau des marqueurs du processus S. Ces derniers résultats cadrent avec une plus grande différence de phase entre chronotypes chez les hommes comparativement aux femmes (Griefahn, 2002).

Dans un deuxième temps, les marqueurs de S ont été étudiés encore une fois en sommeil normal mais cette fois-ci au niveau des dérivations de la ligne médiane du cortex cérébral et au cours des sommeil lent et paradoxal (Mongrain et al., 2006a). Lors ces analyses, une dynamique plus rapide du décours de l'AOL en sommeil lent a été observée chez les Types-M comparativement aux Types-S, spécifiquement à la position frontale du cortex. Puisque les études antérieures ont montré que la réponse homéostatique prédomine en position corticale frontale (Cajochen et al., 1999; Finelli et al., 2000, 2001a, b; Knoblauch et al., 2002; Tinguely et al., 2006), cette observation représente une évidence assez forte en faveur d'une dissipation homéostatique plus rapide chez les types matinaux. En troisième lieu, puisque la dynamique du processus S a été déterminée à l'aide d'études variant la durée de l'éveil (Webb et Agnew, 1977; Borbély et al., 1981; Åkerstedt et Gillberg, 1986a, b; Dijk et al., 1987, 1990a, b, 1993), il était nécessaire de vérifier si l'augmentation de l'AOL en sommeil lent suite à une augmentation de la durée de l'éveil à l'aide de la fragmentation du sommeil serait aussi différente entre les chronotypes (Mongrain et Dumont, soumis). Nous avons de ce fait observé que, pour une même augmentation d'éveil, les Types-M ont une plus grande augmentation d'AOL et de thêta que les Types-S confirmant ainsi la dynamique homéostatique plus rapide chez les matinaux.

5.2.2 À l'éveil

Nos premières analyses de la qualité de l'éveil n'ont pas été très concluantes surtout en ce qui concerne la journée de référence (J2). En poussant un peu plus loin les analyses des marqueurs du processus S pendant l'éveil, des différences dans les variations diurnes de l'activité delta ont été observées entre les chronotypes (Mongrain et al., en préparation). Les Types-S montraient une plus grande augmentation dans la première moitié de la journée et une plus grande diminution par la suite comparativement aux Types-M. Puisque l'angle de phase est équivalent entre ces chronotypes, ceci suggère des différences dans la régulation homéostatique diurne du delta entre types circadiens. Cependant, nous n'avons trouvé aucune différence dans l'activité thêta, contrairement aux résultats rapportés récemment par des collègues français (Taillard et al., 2003). Malheureusement, les travaux de ces collègues ont, comme une majorité d'autres avant eux, imposé un même horaire fixe à tous leurs chronotypes. D'où leur recommandation très appropriée en dernière phrase de leur manuscrit :

> ''To determine if this is trait-dependent or state-dependent (because of imposed sleep times), further studies under self-selected schedules are now required.''[2]

Or, la qualité de l'éveil au cours d'une journée normale est habituellement très bien maintenue chez des sujets vivant sur un cycle éveil-sommeil régulier d'environ 24 heures et des différences ne peuvent souvent être perçues qu'en conditions de pression homéostatique élevée, par exemple lors de privations de sommeil (Åkerstedt et Gillberg, 1990; Aeschbach et al., 1997; Wyatt et al., 1997; Graw et al., 2004). Puisque nous avons observé une plus grande réponse homéostatique à la fragmentation du sommeil chez les Types-M comparativement aux Types-S lors des analyses du sommeil, il était attendu que les chronotypes différeraient également dans les indices du processus S au cours de l'éveil. Effectivement, si on augmente la quantité d'éveil de manière équivalente chez deux groupes de sujets et qu'un des groupes montre une plus grande augmentation d'AOL en sommeil lent suite à cette perturbation, on interprète les différences observées pendant le sommeil comme découlant de différences dans la dynamique de la pression homéostatique pendant l'éveil. Les résultats d'EEG à l'éveil suite à la fragmentation du sommeil ont

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[2] Taillard J, Philip P, Coste O, Sagaspe P, Bioulac B (2003) The circadian and homeostatic modulation of sleep pressure during wakefulness differs between morning and evening chronotypes. J Sleep Res 12:275-282.

montré des différences selon le chronotype dans l'activité bêta, une bande de fréquence étant sous influence homéostatique au cours de l'éveil. L'augmentation du bêta suite à l'augmentation de la pression homéostatique était plus grande chez les types matinaux que vespéraux, ceci appuie l'hypothèse d'une réponse homéostatique amplifiée chez les personnes matinales.

5.3 Deux lignes de variabilité

Nos travaux de recherche, en combinaison avec ceux de plusieurs autres groupes précédemment mentionnés, démontrent donc que la typologie circadienne provient d'au moins deux lignes de variabilités interindividuelles : des différences au niveau du processus C de régulation du sommeil et de la vigilance ainsi que des différences au niveau du processus S. D'une importance considérable, nos recherches démontrent que les deux lignes de variabilités sont indépendantes dans la détermination du chronotype. Effectivement, grâce aux deux sous-groupes de chronotypes observés dans notre échantillon, il peut être conclu que les différences circadiennes engendrent des différences dans les préférences concernant l'horaire de sommeil uniquement chez les individus à phase extrême tandis que les différences homéostatiques engendrent des différences dans l'horaire de sommeil seulement chez les sujets à phase intermédiaire (Mongrain et al., 2006b; Mongrain et Dumont, en préparation). Les différences dans l'un et l'autre des mécanismes de régulation du sommeil seront discutées ici avec les possibles sources de ces différences dans les deux grands processus de régulation du sommeil.

5.3.1 Différences circadiennes

Nous avons observé que chez les individus ayant une position de phase circadienne extrême, c'est-à-dire très avancée ou très retardée, les différences dans l'angle de phase allaient dans la direction initialement attendue (Mongrain et al., 2004) : les Types-M avaient un angle de phase long tandis que les Types-S avaient un angle de phase court, comme ce qui avait été rapporté dans plusieurs travaux précédents (Kerkhof, 1991; Duffy et al., 1999; Baehr et al., 2000; Liu et al., 2000). Cette observation concorde parfaitement avec l'hypothèse d'une période endogène courte de l'oscillateur circadien chez les

individus matinaux et d'une période endogène longue chez les individus vespéraux. De plus, nous avons rapporté récemment que ces chronotypes "extrêmes" diffèraient également au niveau de leur patron d'exposition journalier à la lumière relativement à la position de leur phase (Goulet et al., accepté). Effectivement, les Types-M montrent une plus grande exposition à la lumière avant leur phase de mélatonine et de température (le soir): un patron d'exposition favorisant un délai circadien. Un tel délai quotidien est nécessaire pour qu'un individu avec une période endogène courte se synchronise avec le cycle environnemental de 24 heures. Inversement, les Types-S montrent un patron d'exposition avec plus de lumière suivant leur phase de mélatonine et de température (le matin), favorisant ainsi une avance de l'horloge circadienne ce qui est nécessaire à la synchronisation d'une longue période endogène. Somme toute, l'observation que la position de l'horloge circadienne est associée à la position de l'horaire de sommeil seulement chez ces chronotypes "extrêmes" (Mongrain et al., 2006b), appuie fortement le fait que la préférence dans l'horaire de sommeil puisse être déterminée par des différences dans le processus C.

Les différences dans le processus C pourraient provenir de différences dans la fine régulation moléculaire de l'horloge biologique principale chez les mammifères. Comme mentionné à la section 1.2 de l'introduction, la rythmicité endogène provient de boucles de rétroaction moléculaire des gènes de l'horloge. De plus, il a été démontré que des modifications ou éliminations de plusieurs gènes de l'horloge peuvent modifier la longueur de la rythmicité circadienne endogène chez l'animal. Par exemple, chez les rongueurs, des mutations dans les gènes *mPer1*, *mPer3* et *mCry1* ont été associées à une période endogène courte en obscurité constante (van der Horst et al., 1999; Shearman et al., 2000; Cermakian et al., 2001) tandis que des mutations dans les gènes *mClock* et *mCry2* ont généré des périodes longues (Vitaterna et al., 1994; van der Horst et al., 1999). Il est donc tout à fait probable que la variation de la longueur de la période endogène chez les chronotypes provienne de différences dans un ou l'autre des gènes de l'horloge, d'autant plus que l'hérédité du chronotype a déjà été confirmée (Hur et al., 1998; Vink et al., 2001). De manière générale, une modification génique qui engendrerait une accélération de la boucle de rétroaction moléculaire donnerait lieu à une période endogène plus courte, alors qu'une modification causant un ralentissement de la boucle de rétroaction donnerait lieu à une période plus longue. De plus, puisque l'héritabilité du chronotype est complexe et n'obéit pas aux lois de transmission classique Mendélienne (Vink et al., 2001), l'implication d'une

combinaison de variations dans plusieurs gènes est hautement probable. Comme mentionné à la section 1.3, plusieurs polymorphismes dans les gènes de l'horloge ont été mis en relation avec la typologie circadienne (Katzenberg et al., 1998; Archer et al., 2003; Johansson et al., 2003; Mishima et al., 2005; Carpen et al., 2005; Pereira et al., 2005). Cependant, l'implication de ces variations génétiques dans une modification de la période endogène demeure sujet à recherche.

Parallèlement, il est possible que les différences circadiennes ait été engendrées au cours du développement du système nerveux central, plus particulièrement des noyaux suprachiasmatiques de l'hypothalamus. Le développement de la circuiterie du système nerveux central humain dépend en bonne partie de l'environnement (Changeux, 1983), et les noyaux suprachiasmatiques sont encore en développement après la naissance (Swaab et al., 1990). Chez l'animal, il a été démontré que l'expérience lumineuse suivant la naissance pouvait modifier la longueur de la période endogène (Yamazaki et al., 2002). Effectivement, l'exposition à un cycle lumière-obscurité allonge la période comparativement aux animaux gardés dans l'obscurité. Comme nous avons observé un lien entre la photopériode à la naissance et la typologie circadienne (voir article complémentaire à l'annexe 8.4 : Mongrain et al., 2006c), il est possible que l'aspect circadien du chronotype origine, du moins en partie, de l'expérience lumineuse de l'horloge biologique en développement.

De concert, les différences circadiennes pourraient provenir non pas de différences endogènes dans la longueur de la période mais bien de différences dans la sensibilité du système circadien à la lumière. Une plus grande sensibilité à la lumière correspond à une plus grande force du synchroniseur ce qui affecte la phase d'entraînement au cycle lumière-obscurité (Roenneberg et al, 2003a). Chez l'homme, en tenant compte du fait que la période endogène moyenne est supérieure à 24 heures (Carskadon et al., 1999; Czeisler et al., 1999), une personne ayant une sensibilité plus grande à la lumière, aura une phase d'entraînement plus hâtive (comme un Type-M). Chez l'animal, la sensibilité du système circadien à la lumière semble aussi être modifiée par l'expérience lumineuse post-natale (Canal-Corretger et al., 2001; Prichard et al., 2004). Outre des différences dans la sensibilité globale à la lumière, les chronotypes pourraient montrer des différences de sensibilité à des moments circadiens spécifiques. Effectivement, le système circadien ne

répond pas à la lumière de la même manière à tous les moments de la phase circadienne. Selon la courbe de réponse de phase à la lumière : la lumière le matin, après le minimum circadien de la température corporelle ou le maximum de la mélatonine, produit une avance circadienne tandis que la lumière le soir, avant le minimum de la température ou le maximum de la mélatonine, produit un délai de phase (Minors et al., 1991; Khalsa et al., 2003). Les Types-M pourraient être plus sensibles à la lumière le matin ou moins sensible à celle du soir, ou les Types-S pourraient être plus sensibles à la lumière le soir ou moins le matin. Il est possible d'observer dans la figure 2 d'une étude antérieure de notre laboratoire que les Types-S semblent avoir une plus grande suppression de la mélatonine que les Types-M lorsqu'exposés à la lumière le soir (Rufiange et al., 2002). Ceci pourrait indiquer une plus grande sensibilité circadienne à la lumière le soir chez les Types-S, et donc une plus grande facilité à retarder leur phase circadienne. Notamment, une plus grande suppression de la mélatonine a été rapportée chez des patients souffrant du syndrome de sommeil en délai de phase, les extrêmes vespéraux du continuum de matinalité-vespéralité (Aoki et al., 2001).

Les possibilités de variabilité circadienne en lien avec le chronotype mentionnées précédemment sont loin d'être mutuellement exclusives. Le plus probable est que la typologie circadienne soit l'oeuvre d'une combinaison de différences dans les gènes de l'horloge et de différentes expériences lumineuses post-natales, elle-même à la source de différences dans la longueur de la période endogène et dans la sensibilité circadienne à la lumière.

5.3.2 Différences homéostatiques

Les résultats de nos travaux de recherche appuient fortement l'hypothèse que des différences dans le processus S de régulation du sommeil soient également déterminantes dans la génération de la typologie circadienne. Cependant, nos travaux indiquent aussi que cette seconde ligne de variabilité se retrouve spécifiquement chez les individus ayant des phases circadiennes semblables. Effectivement, les différences dans la dissipation de l'AOL dans la zone corticale frontale ne se retrouvent que chez les sujets intermédiaires (Mongrain et al., 2006b). De plus, au cours de la nuit de récupération suite à une augmentation de la pression homéostatique par la fragmentation du sommeil, le niveau initial d'AOL relative à la journée de base est plus élevé chez les Types-M que chez les Types-S seulement dans le

sous-groupe d'individus intermédiaires (Mongrain et Dumont, en préparation). Des plus intéressants, les paramètres homéostatiques de la modélisation de l'AOL sont associés à des différences d'horaire de sommeil uniquement chez les sujets à phase circadienne semblable (Mongrain et al., 2006b; Mongrain et Dumont, en préparation). Nos résultats montrent donc que des variations dans la dynamique du mécanisme homéostatique de régulation du sommeil peuvent à elles seules engendrer un continuum de typologie circadienne. Plusieurs autres groupes ont aussi tenté de trouver des différences dans cet aspect de la régulation du sommeil pour expliquer des différences dans l'horaire de sommeil de sujets normaux, mais sans succès (Aeschbach et al., 1996; Jenni et Carskadon, 2004). Nos observations sont les premières à montrer des différences dans la régulation homéostatique du sommeil chez des individus normaux et en santé.

La source précise du processus S est actuellement inconnue tout autant que sa localisation neuroanatomique. Cependant, plusieurs joueurs moléculaires et réseaux neuronaux sont maintenant reconnus comme ayant un rôle dans les mécanismes homéostatiques de récupération. Des différences au niveau de ces candidats homéostatiques potentiels pourraient avoir un rôle dans la genèse du chronotype. Chez l'animal, la dynamique de la régulation homéostatique semble aussi être sous contrôle génétique (Franken et al., 2001). De plus, chez l'humain, la quantité de SLP paraît être déterminée génétiquement (Linkowski, 1999). Donc, il est hautement vraisemblable que, comme en ce qui a trait à l'aspect circadien, les différences homéostatiques soient sous contrôle génique.

Très récemment, un groupe travaillant sur les gènes de l'horloge a trouvé, en étudiant de manière plus spécifique les individus selon leur génotype, qu'un polymorphisme d'une répétition en tandem du gène *hPer3*, associé à la typologie circadienne (Archer et al., 2003; Pereira et al., 2005), ne semble produire aucune différence dans la position de la phase circadienne (Viola et al., 2006). Or, ils ont rapporté que les individus génétiquement différents montrent des différences dans l'amplitude des ondes lentes de l'EEG. Il apparaît donc que certains gènes connus comme étant "de l'horloge" pour leur rôle dans le maintien des rythmes circadiens ont aussi un rôle dans la force du marqueur de récupération de l'EEG chez l'humain et donc possiblement dans l'homéostasie du sommeil (Wisor et al., 2002; Dudley et al., 2003). Il est possible que ce soit pertinemment ce dernier rôle qui influence la préférence pour l'horaire de sommeil liée au chronotype.

L'adénosine est une candidate ayant acquis beaucoup de renommée dans le domaine de recherche du processus S et d'un facteur de sommeil. Il semble que cette molécule soit particulièrement importante dans la régulation négative des systèmes d'éveil, plus particulièrement des systèmes cholinergiques du tronc cérébral et du prosencéphal basal (Porkka-Heiskanen et al., 1997, 2002; Thakkar et al., 2003). Aussi, l'adénosine semble avoir un rôle direct au niveau de l'interrupteur du sommeil : l'aire préoptique ventrolatérale (Gallopin et al., 2005). En fait, le contrôle génétique de la régulation homéostatique a été associé à une région chromosomique contenant des gènes du métabolisme de l'adénosine (Franken et al., 2001). Des différences dans le métabolisme, le transport ou la régulation de l'adénosine pourraient donc être à la source de différences homéostatiques. En ce sens, une variation génétique fonctionnelle du gène de l'adénosine désaminase (enzyme de dégradation de l'adénosine en inosine) a été récemment liée à une augmentation du SLP et de l'AOL, confirmant ainsi un rôle de l'adénosine dans la régulation homéostatique chez l'humain (Rétey et al., 2005).

Le SLP et l'AOL sont le fruit intégré d'oscillations neuronales cortico-thalamiques, thalamo-corticales et cortico-corticales (Amzica et Steriade, 1998, 2000). Chez l'humain, étant donné la complexité du système nerveux, des différences dans la migration des cellules nerveuses ainsi que dans leur connectivité engendrent des différences dans la micro-morphologie du système nerveux central de manière à produire des patrons aussi uniques que les empreintes digitales (Changeux, 1983). Cependant, ces différences conduisent la majorité du temps à un résultat comportemental similaire. Or, il est possible que certains patrons de migration ou de connectivité synaptique puissent favoriser l'établissement d'un phénotype homéostatique rapide ou lent menant à la matinalité ou la vespéralité. De plus en plus d'études relient les états de vigilance et la régulation homéostatique à la plasticité synaptique (Steriade et Timofeev, 2003; Cirelli et al., 2004; Basheer et al., 2005; Tononi et Cirelli, 2006). Ce type de variabilité pourrait avoir une source génique (gène de structure, de migration, de développement, etc.) ou environnementale (épigenèse : Changeux, 1983). Bien que cette proposition demeure purement spéculative, il n'en demeure pas moins que le signal EEG est le reflet fidèle du mécanisme S et que la source neuroanatomique du signal ne doit pas être négligée en tant que source de variabilité interindividuelle.

Pour terminer, les différences dans la mécanique homéostatique ne proviennent pas nécessairement des cellules nerveuses elles-même mais peut-être bien de d'autres types cellulaires tout aussi importants, en l'occurence la glie. Effectivement, les cellules gliales ont un rôle important dans le signal EEG en tant que médiateurs de la transition entre les stades de sommeil, de contributeurs au potentiel de champ et même comme régulateurs métaboliques lors de la privation de sommeil (Amzica et Steriade, 1998; Amzica et Massimini, 2002; Kong et al., 2002; Magistretti, 2006). De plus, les cellules gliales sont une source importante d'adénosine (Porkka-Heiskanen et al., 2002). Actuellement, peu d'évidences claires sont disponibles pour tracer un portrait fiable de la source des différences homéostatiques entre chronotypes. Cependant, il est indéniable que la porte est ouverte sur d'innombrables molécules et structures du système nerveux central.

5.4 Interaction C et S

Puisque nos travaux suggèrent fortement que le chronotype est déterminé par des différences circadiennes ou par des différences homéostatiques, les chronotypes demeurent alors un modèle hors-pair pour l'étude de l'interaction entre les processus C et S de régulation du sommeil et de l'éveil. Effectivement, chez cette population particulière, les deux lignes de variabilité peuvent être soit corrélées, soit utilisées pour vérifier l'effet de variations de l'une sur la seconde. Donc, les chronotypes restent une population de choix pour étudier l'interaction circadienne et homéostatique, but premier du projet, et plusieurs informations à ce sujet pourront finalement être extraites du présent programme de recherche.

5.4.1 Variabilités indépendantes

Nous avons pu observer dans nos travaux que les deux lignes de variabilité reliées au chronotype ne sont pas associées. Premièrement, il n'y a pas de corrélation entre la phase circadienne et les paramètres homéostatiques du décours de l'AOL (Mongrain et al., 2006b; Mongrain et Dumont, en préparation). De plus, à l'intérieur des sous-groupes de chronotypes, seules des différences circadiennes ou homéostatiques sont liées avec l'horaire de sommeil (Mongrain et al., 2006b; Mongrain et Dumont, en préparation). Ces

observations sont particulièrement importantes puisqu'un grand nombre de travaux contemporains en chronobiologie ont observé de forts liens moléculaires et structuraux entre les deux processus de régulation du sommeil (Naylor et al., 2000; Wisor et al., 2002; Dudley et al., 2003; Mendelson et al., 2003; Easton et al., 2004). Nos résultats signifient que les mécanismes circadiens et homéostatiques fonctionnent bel et bien de manière distincte chez l'humain, en particulier à ce qui à trait à leur implication dans la préférence pour l'horaire de sommeil. Ce fonctionnement distinct des mécanismes de régulation provient probablement de distinctions anatomiques plutôt que de différences dans les joueurs moléculaires. Par exemple, le rôle circadien des protéines de l'horloge apparaît dans l'oscillateur central, les noyaux suprachiasmatiques de l'hypothalamus; tandis que leur rôle homéostatique est décelé dans le prosencéphale basal et le cortex cérébral (Wisor et al., 2002; Dudley et al., 2003; Cirelli et al., 2004).

Ensuite, dans le groupe d'individus avec des phases circadiennes extrêmes, incluant les Types-M avec des phases très hâtives et les Types-S avec des phases très tardives, les différences dans l'angle de phase vont dans le sens attendu au départ (Mongrain et al., 2004). Ce sous-groupe permet donc de répondre en partie aux objectifs généraux du présent projet de recherche. Effectivement, bien que constituant une comparaison de groupes restreints (seulement 6 sujets par groupe), il est possible d'évaluer la force du processus S chez des individus qui dorment à une position de phase différente puisque ces sujets sont effectivement entraînés avec des angles de phase différents. Les résultats présentés dans le quatrième article de cette thèse ainsi que ceux dans un autre article présentement en préparation montrent que cette différence d'entraînement n'affecte pas la force de la régulation homéostatique en conditions normales ou suite à un challenge homéostatique, du moins au niveau de la dissipation de l'AOL qui est identique chez les chronotypes pour lesquels on suppose des périodes endogènes différentes. Ces observations ajoutent également un support à l'hypothèse de l'indépendance des grands mécanismes de régulation du sommeil car une différence de C n'influence aucunement les paramètres de S.

Subséquemment, chez les sujets ayant une phase intermédiaire, les Types-M et les Types-S ont une phase circadienne semblable mais des différences dans la dynamique du mécanisme homéostatique (Mongrain et al., 2006b; Mongrain et Dumont, en préparation). Chez ce groupe, les différences homéostatiques ne semblent pas avoir affecté la position de la phase

circadienne par rapport au cycle du synchroniseur, le cycle lumière-obscurité, car ces chronotypes ont une phase équivalente. Alors, ces données s'additionnent encore une fois pour supporter l'indépendance des processus de régulation du sommeil puisqu'une variation dans S n'influence pas la position de C. (Ajout p. 189b)

5.4.2 Entraînement stable

Les chronotypes à phases intermédaires sont aussi entraînés avec des angles de phase différents. Toutefois, ces individus sont entraînés à des phases circadiennes différentes de manière inverse aux chronotypes extrêmes, c'est-à-dire que les types matinaux ont un angle de phase court et les types vespéraux un angle de phase long (Mongrain et al., 2004). Cette observation est particulièrement intéressante puisqu'elle nous renseigne sur la relation entre les processus C et S nécessaire à un entraînement stable chez des individus normaux avec une phase circadienne semblable mais avec des différences dans le processus S. Il semble donc que les personnes ayant une dynamique plus rapide du mécanisme homéostatique sont entraînées avec un angle de phase court : étant donné leur forte et rapide accumulation de pression S pendant l'éveil, il leur est possible d'initier le sommeil relativement tôt, dès que le système circadien diminue sa propension pour l'éveil; aussi, étant donné leur forte et rapide dissipation de S pendant le sommeil, il leur est possible d'initier l'éveil dès que le signal circadien pour l'éveil commence à augmenter (figure 5, panneau du haut). Les personnes avec une dynamique plus lente du processus S sont entraînés avec un angle de phase long : ils semblent être capables d'initier le sommeil uniquement lorsque le système circadien diminue considérablement sa pression pour l'éveil étant donné leur faible accumulation de pression S pendant l'éveil; aussi, il leur est possible d'initier spontanément l'éveil uniquement lorsque le signal circadien pour l'éveil a augmenté significativement, étant donnée leur lente dissipation de S pendant le sommeil, (figure 5, panneau du bas). Il apparaît donc qu'une modification de S change la force nécessaire de C pour initier les périodes de sommeil et d'éveil en changeant sa position par rapport au sommeil, dans le but de maintenir une consolidation du sommeil et de l'éveil. Cette observation, d'une importance fondamentale, n'a jamais été rapportée auparavant car nos résultats sont des précurseurs pour ce qui est de différences homéostatiques normales.

Une particularité intéressante de nos sous-groupes extrêmes et intermédiaires de chronotypes provient de l'inégalité de la répartition des sexes à l'intérieur des groupes. Ces différences pourraient avoir contribué à certains effets du chronotype observés dans le cadre des travaux de recherche du premier article de la présente thèse. En effet, puisque les femmes ont une phase circadienne plus hâtive que les hommes (Gibertini et al., 1999; Baehr et al., 2000; Baker et al., 2001b; Mongrain et al., 2004) et un horaire de sommeil semblables, ceci leur confère un angle de phase plus long que les hommes (Baehr et al., 2000; Mongrain et al., 2004). Dans les sous-groupes extrêmes, le nombre élevé de femmes chez les Types-M (4) et le nombre réduit de femmes chez les Types-S (1) pourrait avoir contribué à la phase plus avancée et à l'angle de phase plus long des Types-M comparativement aux Types-S. Dans les sous-groupes intermédiaires, le nombre réduit de femmes chez les Types-M (2) et le nombre élevé de femmes chez les Types-S (5) pourraient avoir contribué à l'angle de phase plus long des Types-S. Cependant, il semble improbable que les nombres inégaux de femmes et d'hommes aient participé à la différence observée dans la dissipation de l'AOL entre les chronotypes intermédiaires tel que rapporté dans le quatrième article de la thèse. Effectivement, comme nous avons observé dans le second article de cette thèse que le niveau initial et la dissipation de l'AOL étaient semblables entre les hommes et les femmes, il nous est possible d'interpréter les différences obtenues dans le marqueur du processus homéostatique en fonction de la préférence pour l'horaire de sommeil des sujets.

On pourrait penser que la différence d'angle de phase présente entre les Types-M et les Types-S intermédiaires aurait pu permettre au processus S de s'exprimer plus ou moins intensément. En effet, un effet circadien a été rapporté sur la dynamique de l'AOL (Dijk et Czeilser, 1995; Putilov, 1995). Cependant, puisque la différence d'angle de phase présente entre les chronotypes extrêmes n'engendre aucune différence dans le marqueur homéostatique, il est de ce fait peu probable que la différence d'angle de phase du même ordre de grandeur entre les chronotypes intermédiaires puisse expliquer les différences obtenues dans la dissipation de l'AOL.

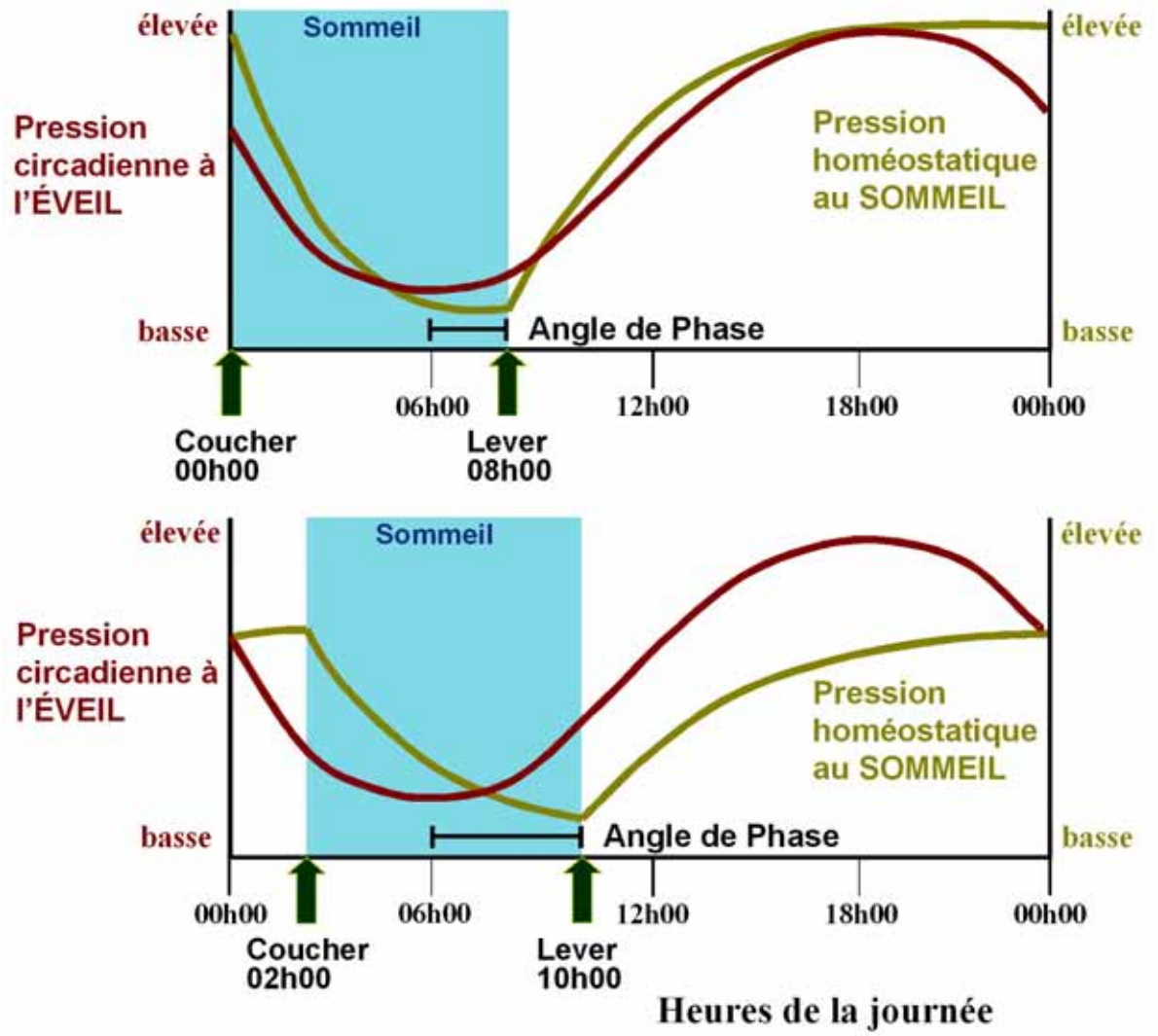

Figure 5 : Angles de phase chez des individus avec régulation homéostatique différente. Dans le panneau du haut, une personne ayant une dynamique rapide de la pression homéostatique s'endormira plus tôt et dès que la pression circadienne à l'éveil commence à descendre. Une telle dynamique engendrera un éveil dès l'augmentation de la pression circadienne à l'éveil générant donc un angle de phase court. Dans le panneau du bas, une personne ayant une dynamique homéostatique plus lente s'endormira plus tardivement et lorsque le signal circadien pour l'éveil sera beaucoup plus diminué. Aussi, l'éveil ne pourra être initié qu'avec une plus grande pression circadienne à l'éveil générant un angle de phase plus long.

La relation de phase observée chez les Types-M et les Types-S ayant des phases circadiennes semblables pourrait expliquer certaines hypothèses antérieures quant à la source des chronotypes. En particulier l'hypothèse avancée par le groupe italien de Natale, qui postule une plus grande force de S chez les types matinaux et une plus grande force de C chez les types vespéraux (Natale et Cicogna, 1996; Natale et al., 2005). La dynamique

homéostatique plus rapide des Types-M accompagnée de l'angle de phase court feraient en sorte que la régulation homéostatique semblerait dominante au cours des variations diurnes de la vigilance chez ces sujets. Tandis que la dynamique homéostatique modérée des Types-S combinée à l'angle de phase long révèleraient une forte composante circadienne dans la régulation de la vigilance. Cette interprétation pourrait indiquer que d'autres études auraient aussi étudié des chronotypes à phase circadienne moyenne dont la préférence pour l'horaire de sommeil et d'activité proviendrait plutôt de différences homéostatiques.

L'observation que des différences homéostatiques peuvent engendrer une modification de la relation de phase entre l'épisode de sommeil et la phase circadienne peut contribuer à la compréhension de la physiopathologie de certains désordres circadiens, en particulier le syndrome de sommeil en délai de phase (DSPS), une condition extrême du continuum de matinalité-vespéralité où les patients dorment beaucoup plus tard que désiré (Weitzman et al., 1981; ASDA, 1990). Les patients DSPS ont un angle de phase très long (Ozaki et al., 1996; Shibui et al., 1999; Uchiyama et al., 2000a; Oh et al., 2004). Ils se lèvent donc très longtemps après leur minimum circadien de température corporelle, ce qui contraste avec l'hypothèse que leur condition provient d'une période endogène anormalement longue. En fait, certains travaux ont montré quelques indices selon lesquels les patients DSPS seraient aussi déficients au niveau de leur mécanisme S : ils démontreraient une dynamique très lente pendant le sommeil et une faible compensation suite à une augmentation de la durée d'éveil (Uchiyama et al., 2000b; Watanabe et al., 2003). Les résultats de nos travaux suggèrent que les modifications dans la régulation homéostatique chez les patients DSPS conduisent au changement d'angle de phase et à l'exacerbation des symptômes vespéraux.

Les résultats de ce projet de recherche démontrent donc que l'importante relation temporelle entre les 2 mécanismes de régulation dépend de différences retrouvées dans l'un ou l'autre des processus. En conséquence, nos résultats soutiennent l'hypothèse que les effets circadiens et homéostatiques sur le sommeil et l'éveil sont additifs. En ce sens, chez l'animal, des données suggèrent aussi que l'intégration des signaux circadiens et homéostatiques se trouve en aval de la source de chacun des mécanismes de régulation (Deboer et al., 2004).

6. Conclusion

Notre projet de recherche se démarque par son originalité d'avoir utilisé le modèle des chronotypes pour étudier la régulation normale du sommeil et de l'éveil chez l'humain. Malgré l'observation d'une première série de résultats contradictoires et à première vue décevants, les résultats de nos travaux conduisent finalement à une meilleure compréhension de l'origine de la préférence pour un horaire de sommeil ainsi que de l'interaction entre les grands mécanismes régulateurs du sommeil. En particulier, nos résultats démontrent que des différences dans la régulation homéostatique du sommeil peuvent être à la source de la typologie circadienne. Il semble effectivement que les personnes ayant une dynamique amplifiée d'accumulation et de dissipation de la pression homéostatique pendant l'éveil et le sommeil préfèrent des horaires de sommeil plus hâtifs que la population générale, tel qu'identifié par le questionnaire de chronotype. De façon intéressante, nos résultats soutiennent aussi que les processus circadien et homéostatique de régulation du sommeil et de la vigilance fonctionnent de manière indépendante.

Dans ce projet, la force d'avoir obtenu deux populations distinctes de chronotypes se transforme en faiblesse quant au nombre de sujets comparés. En effet, le projet se proposait de comparer au départ 2 groupes de 12 sujets tandis que les analyses à l'intérieur des chronotypes extrêmes et intermédiaires réduisent ce nombre de moitié. Par conséquent, l'importance de répliquer nos résultats dans une nouvelle population de chronotypes est capitale. D'autant plus que nos résultats sont les premiers à montrer des différences dans la dynamique du processus homéostatique, ce qui constituera le premier de nos résultats à répliquer. (Ajout p. 192b) Aussi, le nombre d'analyses effectuées dans le cadre du ce présent conduit malheureusement à une hausse du risque d'obtenir de faux positifs. De plus, bien que nous ayons obtenu des différences entre chronotypes avec une augmentation relativement modeste de la durée de l'éveil à l'aide de la fragmentation du sommeil, il sera important de vérifier l'effet d'une augmentation plus considérable de la durée de l'éveil sur la différence entre chronotype. L'idéal, au cours des projets futurs, serait d'augmenter le nombre de sujets étudiés, de faire varier la quantité d'éveil et d'investiguer sur plusieurs continents de manière à vérifier les différences homéostatiques à l'intérieur de populations génétiquement hétérogènes. De plus, tel qu'exprimé à l'intérieur de certains des articles de cette thèse, les critères de sélection des chronotypes étudiés auraient peut-être avantage à

Une limite expérimentale se retrouve aussi dans l'inclusion de chronotypes féminins utilisant des contraceptifs hormonaux. Bien que l'utilisation de contraceptifs ne semble pas affecter la cyclicité circadienne des femmes (Wright et Badia, 1999; Baker et al., 2001b), leur prise semble modifier l'amplitude des marqueurs circadiens ainsi que l'architecture du sommeil (Wright et Badia, 1999; Baker et al., 2001a, b; Burdick et al., 2002). Cependant, puisque le nombre de femmes utilisant des contraceptifs hormonaux est relativement semblable chez les deux groupes de chronotypes (3 Types-M et 2 Types-S), l'influence de ce facteur nous semble avoir été minimisé.

être modifiés. Par exemple, il pourrait être pertinent de ne pas exclure les chronotypes avec des durées de sommeil extrême (< 7 ou > 9 heures de sommeil par nuit) puisque certains indices laissent croire à un lien entre la dynamique homéostatique et la durée de sommeil.

Ce programme de recherche avait pour but d'accroître nos connaissances sur la régulation du sommeil de manière à augmenter la compréhension de plusieurs désordres circadiens. Or, les résultats de nos recherches indiquent que des différences homéostatiques pourraient être déterminantes dans la genèse de certains troubles dits "circadiens". Par exemple, nos résultats sont applicables à la physiopathologie des syndromes de sommeil en avance et en délai de phase tel que détaillé à la section précédante. Aussi, nos résultats pourraient aider à comprendre l'origine possible d'une mauvaise tolérance à un angle de phase anormal chez les travailleurs de nuit ou les cas de grande sensibilité au décalage horaire. En ce sens, un angle de phase plus court, comme observé chez les Types-M avec des phases intermédiaires, pourrait engendrer une adaptation du système à un épisode de sommeil se terminant dès l'augmentation de la propension circadienne à l'éveil. Une telle adaptation pourrait rendre impossible le maintien du sommeil à un moment circadien où la propension circadienne à l'éveil serait très élevée. De plus, nos résultats peuvent s'appliquer à la compréhension des modifications du sommeil au cours du vieillissement. Effectivement, il semble y avoir des modifications circadiennes ainsi qu'homéostatiques avec l'âge et une modification de l'angle de phase a également été observée (Duffy et al., 1998, 1999).

Les découvertes scientifiques génèrent toujours beaucoup de questionnements. La première interrogation qui mène directement à un nombre invraisemblable de possibilités d'études neurophysiologiques, morphologiques, génétiques, moléculaires et autres serait : mais d'où vient donc cette variabilité homéostatique ? Une avenue prometteuse serait d'évaluer rigoureusement la réponse homéostatique chez des personnes ayant des variations dans certains gènes ciblés pour leur probable implication dans le processus S, ce qui a déjà été débuté par quelques groupes de recherche. Il sera également pertinent d'exploiter les techniques d'imagerie en combinaison avec la privation ou la restriction de sommeil chez les chronotypes pour identifier les zones d'intérêt au niveau du système nerveux central. Finalement, des études animales combinant enregistrements électroencéphalographiques et enregistrements de l'activité neuronale ou gliale seront aussi manifestement nécessaires pour élucider la ou les sources de la variabilité interindividuelle homéostatique.

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Institution :	Faculté de médecine, Université de Montréal

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Institution :	Faculté de médecine, Université de Montréal

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University of Montréal
Tel : 514-338-2222 ext 2246
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6555, boul. Gouin Ouest
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Téléphone: (514) 338-2222
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Université de Montréal

Marie Dumont, PhD
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University of Montréal
Tel : 514-338-2222 ext 2246
Fax : 514-338-2531
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L'expérimentation de cette étude a été réalisée par Suzie Lavoie, une étudiante à la maîtrise dirigée par Marie Dumont ainsi que par moi-même. Le Dr Brahim Selmaoui a effectué la majorité des dosages de la mélatonine dans les échantillons de salive. Le Dr Paquet, agissant à titre de statisticien, a collaboré avec moi pour les analyses statistiques ainsi qu'à la création de la figure 1 de l'article. J'ai également été la rédactrice principale de l'article. Marie Dumont a agit comme chercheure principale de ce projet de recherche.

Article 2 :

Mongrain V, Carrier J, Dumont M. Chronotype and sex effects on sleep architecture and quantitative sleep EEG in healthy young adults. *Sleep*, 28 : 819-827, 2005.

Mon rôle dans cette étude a été à la fois de procéder à la gestion de l'expérimentation, de préparer les plans d'analyse, d'exécuter la totalité des analyses, d'apporter une contribution originale aux idées développées dans l'introduction ainsi que dans les interprétations discutées et d'écrire le manuscrit. Julie Carrier, co-directrice du Laboratoire de Chronobiologie, a révisé le manuscrit et y a apporté quelques modifications. Marie Dumont a agit comme chercheure principale de ce programme de recherche.

Article 3 :

Mongrain V, Carrier J, Dumont M. Differences in sleep regulation between morning and evening circadian types as indexed by antero-posterior analyses of the sleep EEG. *European Journal of Neuroscience*, 23 : 497-504, 2006.

Mon rôle dans cette étude a été de procéder à la gestion de l'expérimentation, de préparer les plans d'analyse, d'exécuter l'ensemble des analyses, d'apporter une contribution majoritaire aux idées élaborées dans l'introduction ainsi que dans la discussion et d'écrire le manuscrit. Julie Carrier a révisé le manuscrit et y a apporté quelques changements. Marie Dumont a agit comme chercheure principale de ce programme de recherche.

Article 4 :

Mongrain V, Carrier J, Dumont M. Circadian and homeostatic sleep regulation in morningness-eveningness. *Journal of Sleep Research*, 15 : 162-166, 2006.

Dans cette étude, j'ai procédé à la gestion de l'expérimentation, j'ai préparé les plans d'analyse et exécuté l'ensemble des analyses, j'ai contribué de façon majoritaire aux idées élaborées dans l'introduction ainsi que dans la discussion et j'ai écrit le manuscrit. Julie Carrier a révisé le manuscrit et y a apporté quelques modifications. Marie Dumont a agit comme chercheure principale de cette étude.

Article 5 :

Mongrain V, Dumont M. Increased homeostatic response to behavioral sleep fragmentation in morning compared to evening types. *Sleep*, soumis.

Dans cette étude, j'ai procédé à la gestion de l'expérimentation, j'ai préparé les plans d'analyse et exécuté l'ensemble des analyses, j'ai contribué de façon majoritaire aux idées élaborées dans l'introduction ainsi que dans la discussion et j'ai écrit le manuscrit. Marie Dumont a agit comme chercheure principale de ce programme de recherche.

Article 6 :

Mongrain V, Noujaim J, Blais H, Dumont M. Daytime alertness in chronotypes: diurnal variations and effects of behavioral sleep fragmentation. *Behavioural Brain Research*, en préparation.

Dans cette étude, j'ai procédé à la gestion de l'expérimentation, j'ai préparé les plans d'analyse et exécuté une partie des analyses, j'ai contribué de façon majoritaire aux idées élaborées dans l'introduction et dans la discussion et j'ai écrit le manuscrit. Jonathan Noujaim, stagiaire de recherche de Marie Dumont, a effectué plusieurs séries d'analyse et une revue de littérature préliminaire sur la vigilance. Hélène Blais, assistante de recherche de Marie Dumont, a effectué le rejet d'artéfact sur la totalité des enregistrements électroencéphalographiques à l'éveil et a effectué une partie des analyses de ces données. Marie Dumont a agit comme chercheure principale de ce programme de recherche.

8.3 Curriculum Vitae

(version abrégée de 2 pages)

VALÉRIE MONGRAIN

3970 boul. Lévesque Ouest
Laval, QC, H7W 2N9
Tél : 450-973-1916,
Courriel : mongrain@distributel.net
Date de naissance : 8 octobre 1977

Laboratoire de Chronobiologie
Hôpital du Sacré-Cœur de Montréal
5400 boul. Gouin ouest, Mtl, H4J 1C5
514-338-2222 poste 3747 ou 3768
valerie.mongrain@umontreal.ca

FORMATION

Doctorat en philosophie, Science Neurologiques, Université de Montréal **2003-**
Maîtrise en Sciences, Sciences Neurologiques, Université de Montréal **2002-2002**
Baccalauréat en Sciences, Sciences biomédicales, Université de Montréal **1998-2001**

DISTINCTIONS

Prix Bernard-Belleau de l'ACFAS (5 000 $), Montréal, Canada **2006**
- Accordé à un étudiant au doctorat pour l'excellence de son dossier de recherche et la qualité de son projet de recherche.

Prix de la Société Canadienne du Sommeil (200 $), Montréal, Canada **2006**
- Accordé à un étudiant pour la qualité d'un résumé de recherche.

Bourse de voyagement pour mérite scientifique (175$ U.S.), Floride, USA **2006**
- Accordée aux auteurs des meilleurs résumés soumis à la Society for Research on Biological Rhythms.

Prix d'excellence étudiant (200 $), Montréal, Canada **2005**
- De l'axe sommeil et rythmes biologiques (FRSQ) obtenu pour l'article : Mongrain et al., 2004.

Bourse de voyagement (165$ U.S.), Whistler, Canada **2004**
- Accordée aux auteurs des résumés soumis à la Society for Research on Biological Rhythms.

Bourse d'excellence (300 $), Québec, Canada **2004**
- Accordée aux auteurs des 10 meilleurs résumés soumis à la Société Canadienne du Sommeil.

Prix d'excellence Bayer (500 $), Montréal, Canada **2002**
- Catégorie sciences biomédicales, biochimie et microbiologie, reçu pour une présentation orale.

BOURSES

Bourses de formation post doctorale (60 000 $), FRSQ **2006-2008**
Bourses d'études supérieures BESC-D (70 000 $), CRSNG **2005-2007**
Bourse de formation au doctorat (60 000 $, déclinée), FRSQ **2005-2008**
Bourse d'excellence (6 000 $), FES, Université de Montréal **2005**
Bourse d'excellence, départ. de physiologie (3 035 $), Université de Montréal **2005**
Bourse d'excellence (5 000 $), FES, Université de Montréal **2004**
Bourse d'excellence, départ. de physiologie (3 968 $), Université de Montréal **2004**
Bourse d'études supérieures ES-A (42 000 $), CRSNG **2003-2005**
Bourse d'excellence, départ. de physiologie (3 706 $), Université de Montréal **2003**
Bourse de congé de maternité (1 500 $), FES, Université de Montréal **2003**
Bourse de maîtrise J.A. DeSève (10 000 $), Centre de recherche, HSCM **2002-2003**
Bourse de maîtrise (10 000 $), Marie Dumont et axe sommeil du RSMQ **2002-2003**
Bourse de stage d'été (3 600 $), Départ. de psychiatrie, Université de Montréal **2001**
Bourse de stage d'été (3 400 $), Départ. de psychiatrie, Université de Montréal **2000**

EXPÉRIENCE DE RECHERCHE

Assistante de recherche, Chronobiologie, Hôpital du Sacré-Cœur de Montréal **2003-**
Assistante de recherche, Chronobiologie, Hôpital du Sacré-Cœur de Montréal **2001**
Stagiaire de recherche, Chronobiologie, Hôpital du Sacré-Cœur de Montréal **2001**
Stagiaire de recherche, Laboratoire de cytogénétique, Université de Montréal **2000**

ACTIVITÉS SCIENTIFIQUES

Juge des présentations par affiches au colloque sur les rythmes circadiens (C-110) dans le cadre du 74e Congrès de l'ACFAS, Université McGill, Montréal, 18 mai 2006.
Juge à la finale régionale rive-nord de l'Expo-Sciences Bell, Laval, 10 mars 2006.

PUBLICATIONS SÉLECTIONNÉES

Articles publiés :

Valérie Mongrain, Jean Paquet et Marie Dumont. Contribution of the photoperiod at birth to the association between season of birth and diurnal preference. Neurosci Lett 406 : *113-116*.

Valérie Mongrain, Julie Carrier et Marie Dumont (2006b) Circadian and homeostatic sleep regulation in morningness-eveningness. J Sleep Res 15 : *162-166*.

Valérie Mongrain, Julie Carrier et Marie Dumont (2006a) Difference in sleep regulation between morning and evening circadian types as indexed by antero-posterior analyses of the sleep EEG. Eur J Neurosci 23 : *497-504*.

Valérie Mongrain, Julie Carrier et Marie Dumont (2005) Chronotype and sex effects on sleep architecture and quantitative sleep EEG in healthy young adults. Sleep 28(7): *819-827*.

Valérie Mongrain, Suzie Lavoie, Brahim Selmaoui, Jean Paquet et Marie Dumont (2004) Phase relationships between sleep-wake cycle and underlying circadian rhythms in morningness-eveningness. J Biol Rhythms 19(3): *248-257*.

Article soumis :

Valérie Mongrain et Marie Dumont. Increased homeostatic response to behavioral sleep fragmentation in morning compared to evening types. Sleep (septembre 2006).

Geneviève Goulet, Valérie Mongrain, Jean Paquet et Marie Dumont. Daily light exposure in morning-type and evening-type individuals. J Biol Rhythms (août 2006).

Résumés de communication publiés :

Valérie Mongrain et Marie Dumont (2006) Effect of sleep fragmentation on spectral activity of sleep EEG in morning-type and evening-type individuals. 10th Meeting of Society for Research on Biological Rhythms (Floride), page 56.

Valérie Mongrain et Marie Dumont (2006) Effets de la fragmentation du sommeil sur l'activité à ondes lentes de l'EEG des types matinaux et vespéraux. colloque C-110 (rythmes circadiens) du 74e Congrès de l'ACFAS, Université McGill, Montréal (Québec), page 14.

Valérie Mongrain and Marie Dumont (2005) Rate of decay of slow-wave activity along the antero-posterior axis of the sleep EEG in morning-type and evening-type individuals. 35th Annual Meeting of Society for Neuroscience (Washington DC, USA), #700.14.

Valérie Mongrain et Marie Dumont (2005) Topographic variations of quantitative sleep EEG in morning-type and evening-type individuals. Associated Professional Sleep Societies 19th Annual Meeting (Denver, USA), Sleep, 28 Abstract Supplement, #0167, page A56.

8.4 Article complémentaire

Contribution of the photoperiod at birth to the association between season of birth and diurnal preference.

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Contribution of the photoperiod at birth to the association between season of birth and diurnal preference

Valérie MONGRAIN M.Sc.[1,2], Jean PAQUET Ph.D.[1] and Marie DUMONT Ph.D.[1,3]

[1]Chronobiology Laboratory, Sacré-Cœur Hospital of Montréal, Québec, Canada, H4J 1C5;

[2]Department of Neurosciences and [3]Department of Psychiatry, Université de Montréal, Québec, Canada, H3C 3J7.

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Corresponding author: Marie Dumont, Ph.D.
Chronobiology Laboratory
Sacré-Cœur Hospital
5400 blvd Gouin W.
Montréal (Québec) Canada, H4J 1C5
Tel: (514) 338-2222 ext. 2246
Fax: (514) 338-2531
Email: marie.dumont@umontreal.ca

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ABSTRACT

The aim of the present study was to evaluate the influence of season of birth and photoperiod at birth on diurnal preference in young adults. Participants were 1591 volunteers aged 17 to 35 years (69% women). The scores obtained on the Morningness-Eveningness Questionnaire (MEQ) of Horne and Östberg were first assessed as a function of gender and season of birth by a 2-way ANOVA. Results revealed a higher MEQ score (reflecting more morningness) in women than in men, and in individuals born during autumn than in those born during spring. There was no gender-by-season interaction. The MEQ scores of 4 categories of photoperiod at birth were also compared. Individuals born during short photoperiods of 8 to 10 hours showed stronger morningness than individuals born during photoperiods of 12 to 14 hours. The highest morningness scores were observed in subjects born during 8-10 h photoperiods in the autumn. By contrast, diurnal preference of subjects born during 8-10 h photoperiods in the winter did not differ from diurnal preference of subjects born during longer photoperiods. Our results add support to previous reports showing an association between season of birth and morningness-eveningness, but this association cannot be explained entirely by the length of the photoperiod at birth. We suggest that the association between season of birth and diurnal preference reflects an influence of light intensity and/or variations in photoperiod during early development on the characteristics of the circadian system.

Key Words: morningness-eveningness, circadian rhythms, human, season of birth, photoperiod, light exposure, development.

INTRODUCTION

Diurnal preference refers to the preferred timing for activity and sleep. Morning-type individuals go to bed early and wake up early, whereas evening types fall asleep later and wake up later. This interindividual difference, also designated as chronotype, is associated with the entrained circadian phase assessed with melatonin and temperature rhythms [3,9,16]. Determinant factors for human chronotype are still largely unknown. The length of the endogenous circadian period seems to be of importance [10] as well as individual parameters of homeostatic sleep regulation [15,23]. In the past ten years, strong evidence has accumulated showing that diurnal preference could be genetically determined [2,14,19,24]. However, as observed in many traits and diseases, a given chronotype probably results from the combination of genetic and environmental factors.

In animals, it has been shown that postnatal light exposure influences the development of circadian rhythms. Rats and mice reared in dim light or in darkness show a greater sensitivity to light, both for light-induced phase shifts and for acute effects of light on sleep-wake behavior [6,20]. In rats, the suprachiasmatic nucleus itself shows plasticity in response to light as the number of neurons and glial cells vary as a function of lighting conditions during weaning [5,13]. It is therefore likely that light exposure at birth or in the early stages of development influences the future response of the circadian timing system to the entraining properties of the light-dark cycle and, consequently, modulates the phase of the sleep-wake cycle in the adult individual.

In humans, this possibility has been indirectly investigated in three studies that found an association between season of birth and chronotype in adults and teenagers [4,17,18]. Individuals born during spring/summer show a greater tendency to be classified as evening types whereas individuals born during autumn/winter are more likely to become morning types. In the studies conducted with Italian and Spanish populations, this association was stronger in males than in females or present only in male subjects [17,18]. The authors interpreted their results mainly in terms of photoperiod effects during the gestational or postnatal period on the development of circadian rhythms. However, they did not analyze their results directly in relation to photoperiod at birth.

In this paper, our aim was to confirm the association between chronotype and season of birth in a different population and to evaluate more directly the hypothesis of an association between photoperiod at birth and adult chronotype.

METHODS

A French translation of the Morningness-Eveningness Questionnaire (MEQ) of Horne and Östberg (1976) was completed by 1591 volunteers aged 17 to 35 years, in Montreal, Canada (45º 31' N). The questionnaires were distributed mostly in undergraduate classes at the University of Montreal, over a 4-year period. A cover page was added to the MEQ, asking for gender and date of birth. All of the students were invited to fill out the questionnaire and an optional section asked for personal contact information for those interested in participating to a sleep study. Some of the questionnaires (less than 20%) were completed during laboratory screening procedures by volunteers recruited by advertisements posted at the university or published in local newspapers.

As the exact dates for the determination of seasons vary by a few days depending on the year, the specific positions of solstices and equinoxes were determined for each questionnaire using an algorithm available on the internet [12]. The exact place of birth of the participants was not indicated on the questionnaires. Since more than 95% of undergraduate students at the University of Montreal come from the province of Québec, mostly from the Montreal area, the photoperiod (day length from sunrise to sunset) associated with each date of birth was estimated using the photoperiod for Montreal City. Contributions of season of birth and gender to the MEQ scores were assessed using a Season-by-Gender analysis of variance (ANOVA). Effects of the photoperiod at birth were analyzed with ANOVAs conducted on four categories of photoperiod length (8-10 h, 10-12 h, 12-14 h, 14-16 h). Data are reported as mean ± SEM.

RESULTS

Subjects included 1102 women (69.3%) and 489 men (30.7%), with a mean age of 22.6 (± 0.1) years. MEQ scores ranged from 18 to 78 and approximated a normal distribution with a mean of 48.8 (± 0.3). According to Horne and Östberg's criteria [11], individuals with a MEQ score lower than 42 are classified as evening types, those with a MEQ score higher

than 58 are morning types, and subjects with scores from 42 to 58 are designated as neither types. Using this classification, the MEQ identified 316 morning types (19.9 %), 854 neither types (53.7 %) and 421 evening types (26.4 %). Seasons of birth were distributed all across the year, with 464 subjects (29.2%) born during spring, 379 subjects (23.8%) born during summer, 369 (23.2%) born during autumn and 379 (23.8%) during winter. Significantly more subjects were born during spring than during the other three seasons (chi-square = 14.9, $p < 0.01$). The proportion of women was similar among subjects born during each season (between 66.4 % and 71.5 %).

There was no Season-by-Gender interaction on MEQ scores ($F_{3, 1583} = 0.1$, $p = 0.9$). However, there was a main effect of Gender, the scores being higher in women than in men (49.4 ± 0.3 vs. 47.4 ± 0.5; $F_{1, 1583} = 10.5$, $p < 0.01$). A significant main effect of Season of birth was also found ($F_{3, 1583} = 2.8$, $p < 0.05$) and is illustrated in Figure 1. Post-hoc Tukey tests showed that MEQ scores of subjects born during autumn were significantly higher (stronger morningness) than the scores of subjects born during spring (50.1 ± 0.5 vs. 47.9 ± 0.5; $p = 0.01$). There was no difference between the subjects born during summer and those born during winter. The ANOVA on the 4 categories of photoperiod showed a significant effect of photoperiod on MEQ scores ($F_{3, 1587} = 2.7$, $p < 0.05$). As illustrated in Figure 2, individuals born during short photoperiods ranging between 8 and 10 hours had significantly higher MEQ scores than individuals born during photoperiods ranging between 12 and 14 hours (50.0 ± 0.5 vs. 47.8 ± 0.6, respectively; $p < 0.05$, post-hoc Tukey tests). Since seasonal differences were significant only between autumn and spring, an ANOVA was also conducted separately on MEQ scores of subjects born during these two seasons (photoperiods from 8.7 to 15.7 h). Results showed a significant effect of photoperiod ($F_{3, 829} = 3.8$, $p < 0.01$), with MEQ scores being higher in subjects born during autumnal 8-10 h photoperiods (51.1 ± 0.7) than in subjects born during spring 12-14 h (47.6 ± 0.8; $p < 0.01$) and 14-16 h (48.4 ± 0.6, $p < 0.05$) photoperiods. When computed on MEQ scores of subjects born during summer or winter seasons (photoperiods also from 8.7 to 15.7 h), the ANOVA failed to show any significant effect of photoperiod ($F_{3, 754} = 0.3$, $p = 0.8$). Those results are illustrated in Figure 2.

DISCUSSION

Our results add further support to the presence of an association between season of birth and diurnal preference. Individuals born during autumn had higher MEQ scores (showing more morningness) than individuals born during spring. Our results are consistent with those reported in Italian and Spanish populations where more morning types were found for birth dates between October and March than from April to September [18]. Our results showed significant differences specifically between autumn (late September to late December) and spring (late March to late June), similar to the results of a recent study conducted in French adolescents on a smaller sample [4]. That study found a peak of morningness during September/October and a through in March/April. We found significantly more morningness in women than in men, which is in accordance with previous studies conducted on large samples of young subjects [1,7]. However, the effect of season of birth was the same in both genders. This suggests that the interaction between gender and season of birth reported in Italian and Spanish populations [17,18] could be related to some other influences on chronotype. Those other influences, such as socio-cultural or climatic differences, might also explain why the association between MEQ scores and seasons of birth was not as robust in our sample (difference of 2 MEQ units, $p < 0.05$) as it was in the study of Natale and Adan [17] (difference of 4 MEQ units with $p < 0.00001$).

It is clear from our results that the photoperiod at the date of birth cannot by itself explain all of the effect of season of birth on chronotype. The significant effect of the photoperiod at birth on chronotype was limited to subjects born during spring or autumn, and was totally absent when assessed in subjects born in summer or winter. As illustrated in Figure 2, MEQ scores were especially high in subjects born during the shortest photoperiods in autumn. Since those short photoperiods characterize both autumn and winter, other factors causing differences in light exposure between these two seasons should be considered.

One factor could be the average hours of sunshine. In Montreal, the number of monthly hours of sunshine is smaller during autumn (101 h) than during winter (128 h), particularly in November (84 h) and December (80 h). Since the photoperiod is the shortest during those two months, the low availability of natural bright light may add to the short

photoperiod to reduce levels of light exposure during the first post-natal weeks in individuals born during late autumn in Montreal. It has been shown in animals that there is a critical window of sensitivity to light in the post-natal days when differences in light exposure can modify the future circadian response to light [6,20]. If the same principle applies to humans, very low light exposure during early development may increase light sensitivity, which is analogous to increasing the strength of the light-dark cycle as an entrainment signal (*zeitgeber*). It is known that the phase of entrainment is affected by the strength of the zeitgeber [21]. Since most humans have an endogenous period longer than 24 hours [8], an increased strength of the zeitgeber would advance the circadian phase of entrainment and therefore favor morningness in diurnal preference. However, this interpretation remains speculative at this time as, to our knowledge, circadian light sensitivity has not been investigated yet in relation to season of birth or in relation to morningness-eveningness.

Another difference in light exposure between autumn and winter is the direction of photoperiod changes. During autumn, photoperiod shortens from the equinox to the winter solstice (12.2 h to 8.7 h) whereas during winter, it increases from the solstice to the spring equinox (8.7 h to 12.2 h). In animals, it is known that early light exposure can modify structurally and functionally the SCN itself [5,13]. In hamsters, an early exposure to the light-dark cycle has been found to lengthen the endogenous circadian period compared to animals kept in constant darkness [25]. Since the human SCN is still in development after birth [22] and is sensitive to changes in photoperiod length [26], it might also be very sensitive to increasing or decreasing day length during critical periods of development. According to our results, we hypothesize that a shortening of day length from the equinox to a photoperiod shorter than 12 hours, typical of the autumn season, may favor the development of a shorter endogenous period, as found in morning types [10]. Conversely, increasing day length from the equinox to a photoperiod longer than 12 hours, typical of the spring season, may favor the development of a longer endogenous periodicity as observed in evening types. To our knowledge, the effects on circadian periodicity of lengthening or shortening of the photoperiod during development have not been studied in humans or animals.

In the present study, the photoperiod at birth was approximated because the exact place of birth was not specifically asked to the participants. This information would certainly be important to collect in future studies. However, in spite of this lack of precision, a significant relation between photoperiod and morningness-eveningness was found and our results add new evidence in support of an association between season of birth and morningness-eveningness. It is remarkable that similar findings were found in 4 different countries and it suggests that further replications in countries located at other latitudes would be of interest. It is expected that the association between diurnal preference and season of birth would be stronger in more extreme latitudes where seasonal variations in photoperiod length are larger. This association is intriguing and its interpretation is quite speculative in the present state of knowledge. It cannot be excluded that other seasonal factors, related to (e.g., changes in light-dark contrast or in wavelength) or independent from light exposure, might play a role in the association between season of birth and diurnal preference. Animal studies strongly suggest that early light exposure can influence the developing human SCN. It is therefore likely that this environmental factor interacts with genetic predispositions to lead to the phenotypic expression of morningness-eveningness. Previous studies on the effects of early light exposure on the development of the circadian timing system addressed essentially the effects of continuous darkness (DD) or continuous light exposure (LL). The pattern of association that we found between photoperiod at birth and chronotype suggests that other parameters of early light exposure, such as light intensity and variations in photoperiod, might also have a critical influence on the development of adult circadian rhythms. This is an avenue worth pursuing in future animal and human studies.

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FIGURE LEGENDS

Figure 1: Averaged (+ SEM) scores on the morningness-eveningness questionnaire (MEQ) for subjects born during each of the four seasons.

Figure 2: Averaged (+ SEM) scores on the morningness-eveningness questionnaire (MEQ) in subjects classified according to four categories of photoperiod at birth, illustrated for all subjects (total) and for subjects born during each of the four seasons.

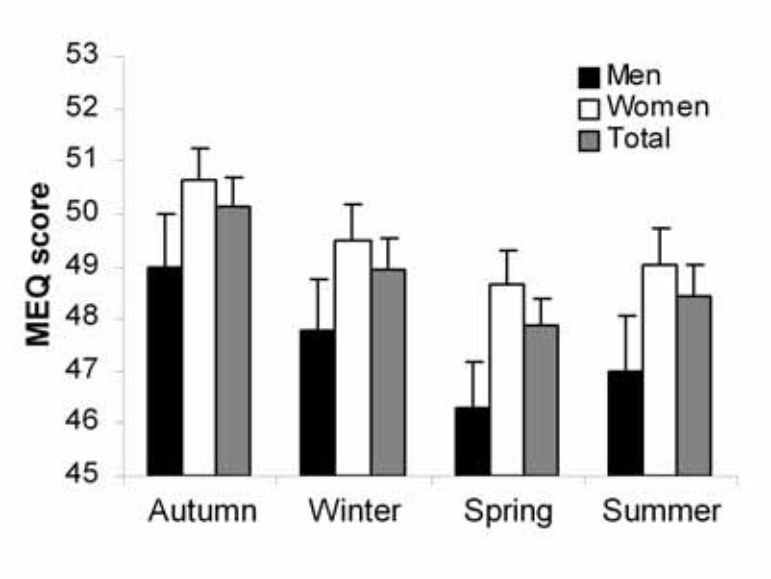

Figure 1

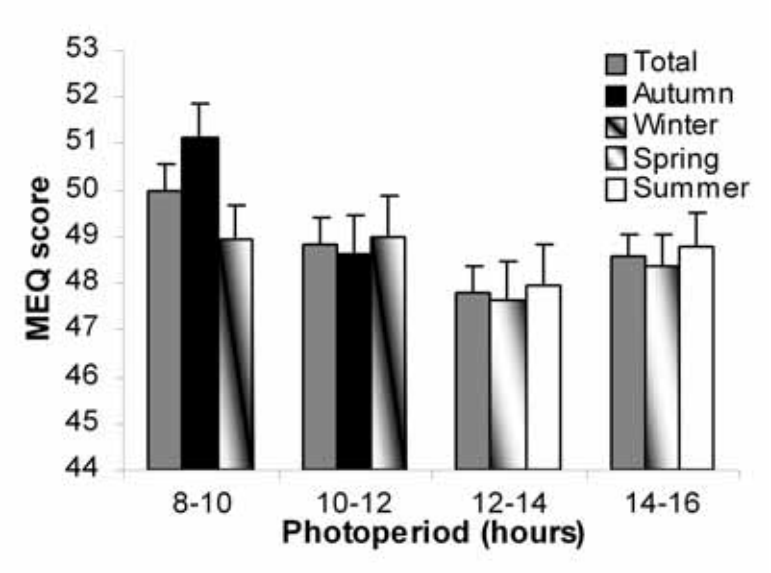

Figure 2

Accord des coauteurs : Article complémentaire

1. Identification de l'étudiant et du programme :

Nom de l'étudiant :	Valérie Mongrain
Programme :	Ph.D. en sciences neurologiques
Institution :	Faculté de médecine, Université de Montréal

2. Description de l'article :

Mongrain V, Paquet J, Dumont M. Contribution of the photoperiod at birth to the association between season of birth and diurnal preference. *Neuroscience Letters*, 406 : 113-116, 2006.

3. Déclaration de tous les coauteurs autres que l'étudiant :

À titre de coauteur de l'article identifié ci-dessus, je suis d'accord pour que **Valérie Mongrain** inclue cet article dans sa thèse de doctorat intitulée : *Rythmes circadiens et mécanismes homéostatiques de récupération chez des personnes de type matinal ou vespéral*.

Jean Paquet

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